

**CONTRIBUTION OF SWEETPOTATO VIRUSES TO CULTIVAR DECLINE IN
UGANDA**

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DECLARATION

I declare that this thesis is my original work, except where otherwise identified by references and I have not plagiarized the work of others. This thesis has not been presented for a degree award in any other university and is not concurrently submitted for any degree other than that of Doctor of Philosophy (PhD) of Makerere University.

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DEDICATION

This work is dedicated to millions of mothers and children in Africa whose livelihoods directly depend on Agriculture

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Success does not come in one day but it's a continuous process with the support of many individuals. I therefore take this opportunity to thank the LORD ALMIGHTY GOD for bringing many resourceful persons at the time this study was conducted. Special thanks go to my supervisors Dr. Settumba B. Mukasa and Dr. Richard W. Gibson for their enthusiasm, constructive criticism and continuous guidance and support throughout the study. Appreciation to my doctoral committee particularly Dr. Geoffrey Tusiime, and Dr. Denis Mpairwe for their encouragement, guidance and support offered to me during the study. Special thanks to Dr. Robert Mwanga and Dr. Jan Low of the International Potato Center (CIP) for providing technical guidance and financial support during the study. Also, my sincere thanks are due to Mr. Segundo Fuentes of CIP, Lima Peru for training me on ELISA diagnosis and not forgetting Dr. Jan Kreuze of CIP Lima Peru for accepting to review the first manuscript published out of this study.

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ABSTRACT

Sweetpotato is a vegetatively propagated crop where vine cuttings from previous crops or sproutslips from roots are used as planting material. This practice can lead to the accumulation of systemic pathogens, especially viruses when the planting material used are not virus tested. The contribution of this practice to degeneration of sweetpotato cultivars in Uganda has been only speculative, and with different views in high and low sweetpotato virus disease pressure zones of central and eastern Uganda. The overall objective of this study was to establish the importance of viruses in mediating cultivar decline in Uganda. Four sweetpotato cultivars of 'Beauregard', 'Dimbuka', 'Ejumula' and 'NASPOT 1' were planted in a series of field trials in central (Kabanyolo; MUARIK) and eastern (Serere; NaSARRI) Uganda over five generations (G1, G2, G3, G4 and G5). The trials started with virus-free planting material (G0). Each succeeding trials retained planting material from the previous one, plus G0 material. Virus symptoms were observed 1 month after planting in all the generations, with *Sweet potato feathery mottle virus* (SPFMV) and *Sweet potato chlorotic stunt virus* (SPCSV) being the most frequently detected viruses. The cultivars 'Beauregard' and 'Ejumula' had highest disease incidence and severity, and degenerated after a single season in both locations. Storage root yields and numbers were greatest in G1 and gradually reduced in subsequent generations (G2, G3, G4 and G5) for each cultivar in both locations. Field plots were planted with vines of cultivars Beauregard, Dimbuka, Ejumula, Kabode and NASPOT 1 graft inoculated with SPFMV and or SPCSV along side G0 as control at both Kabanyolo and Serere. SPFMV spread rapidly to control plots at Kabanyolo, and these plots had similar yields to those singly infected with SPFMV. At Serere where SPFMV spread slowly, plots infected with SPFMV yielded 40% less than the control. Recovery and reversion from SPFMV infection were more frequent at Serere than at Kabanyolo. Infection by SPCSV alone resulted in yield losses of 14–60% and no reversion was observed, while mixed infections of SPFMV and SPCSV resulted in yield losses of 60–95% in both locations depending on the cultivar. SPCSV and SPFMV were found to translocate from infected vines to roots and then to sprouts. When roots obtained from Kabanyolo and Serere fields were evaluated for possible virus accumulation, over 70 % of the roots from Kabanyolo tested positive for both SPFMV and SPCSV, and their sprouts had visible virus symptoms. Roots obtained from Serere fields did not show symptoms on sprout and only 25% tested positive for SPFMV. Roots from graft inoculated plants (either with SPCSV or SPFMV alone) under screen house condition produced both

infected and non-infected roots as well as sprouts. Sprouts infected with SPFMV alone reverted to healthy status while those infected by SPCSV only showed recovery, and none infected by both viruses even showed recovery. This study has proved that viruses rapidly accumulate in sweetpotato through vines and roots which are used for propagation resulting into cultivar decline. Both single and dual infection by SPFMV and SPCSV contributed to the observed cultivar decline in both locations. Susceptible cultivars like Bearegard and Ejumula degenerated within one season when grown in high disease pressure zone. Cultivars Dimbuka and NASPOT 1 (and also Ejumula at Serere) gradually declined in yield and can be grown for up to three seasons in central and up to five seasons in eastern Uganda before replacing with virus indexed planting material. Virus indexing services and rapid multiplication of clean planting material need to be establish to support sustainable production of sweetpotato in Uganda.

PUBLICATIONS FROM THIS THESIS

Part of the work under this thesis has been prepared into papers (published) and manuscripts as shown below:

Adikini, S., Mukasa, S.B., Mwanga, R.O.M. and Gibson, R.W. 2015. Sweetpotato cultivar degeneration rate under high and low sweetpotato virus disease pressure zones in Uganda. *Canadian Journal of Plant Pathology*, 37:1, 136-147, DOI:10.1080/07060661.2015.1004111

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CHAPTER ONE

INTRODUCTION

1.1 Morphology, origin and distribution of sweetpotatoes

Sweetpotato is a dicotyledonous perennial plant which is normally grown as an annual for its vines and storage roots (Woolfe, 1992). It belongs to a single species *Ipomoea batatas* (L.) Lam in the morning glory family (*Convolvulaceae*) (Austin, 1988; Austin and Huaman, 1996) and it is the only plant of economic importance as food in over 500 species of plants in this family (Woolfe, 1992). The crop is a highly heterozygous hexaploid with $2n=6X=90$ chromosomes although some plants morphologically similar to *I. batatas* with $2n=4X=60$ have been described and named, they are considered synonyms of this species. The crop exhibits great phenotypic and genotypic diversity and this is reflected by the skin or flesh colour of the root, the size and shape of the roots, leaves, branches, the depth of rooting, time to maturity, resistance to pest and diseases, and even the flavour and texture of cooked roots.

The origin of this crop is not very clear but it is believed to have been domesticated over 5000 years ago in tropical America (O' Brien, 1972; Srisuwan *et al.*, 2006; Rännäli *et al.*, 2008). Based on the morphological analysis of sweetpotato and the wild *Ipomoea*, Austin (1988) proposed that sweetpotato originated between Yucatan peninsula of Mexico and the Orinoco River in Venezuela. Evidence from molecular marker studies showed the highest diversity to be in central America and therefore could be the primary centre of diversity as well as origin of sweetpotato (Huang and Sun, 2000). The exact date of arrival of sweetpotato on the African continent is not known but evidence indicates that slave traders brought it into the region. It is believed that Columbus introduced the crop to Western Europe around 1492. Shortly thereafter, Portuguese explorers took sweetpotato to India, South East Asia, East Indies, and Africa (Austin, 1988; Zhang *et al.*, 2004; Srisuwan *et al.*, 2006). The crop was introduced to East Africa during the 16th century by slave traders (Woolfe, 1992; Allemann *et al.*, 2004). Since then, sweetpotato is now grown the World over in tropical, subtropical, and warm temperate regions between 40°N and 32°S latitude and at elevations up to 2,500 m (Woolfe, 1992; He *et al.*, 1995). Sweetpotato may have reached Uganda via trade routes from both east and west and is now widely grown in all regions of the country.

1.2 Importance of sweetpotato

Sweetpotato is the third most important root crop in the world after potato and cassava and is the fifth most important food crop on a fresh-weight basis after rice, wheat, maize and cassava in developing countries (CIP, 1999). The crop is cultivated in over 100 developing countries and annual world production is estimated at more than 106 million tons (FAO stat, 2012). Asia is the leading producer with over 80% of world production followed by Africa with 15% and the rest of the world produces only 5%. Out of the 90 million tons of sweetpotato produced annually in Asia, 85 million tons is produced by China alone hence the world leading producer. In Africa, sweetpotato is one of the most widely grown root crops covering around 2.9 million hectares with an estimated production of 14.2 million tons of roots (FAO, 2012). The crop is particularly important in countries surrounding the great lakes in the eastern and central Africa countries, Malawi, Angola, Mozambique, and Madagascar in Southern Africa, and Nigeria in West Africa (Woolfe, 1992). Uganda is the leading producer of sweetpotato in Africa and 2nd in the world after China with yield estimate of 2.83 Metric ton in 2010 (FAO, 2012). The crop is grown in almost all districts in Uganda with the major producing districts found around Lake Victoria.

Sweetpotato plays a big role in household food security and as a source of income for many farmers, especially women (Bashaasha *et al.*, 1995). It is a good source of carbohydrates and vitamins, and both the storage root and foliage are nutritious. Orange-fleshed sweet potatoes (OFSP) are particularly nutritious, ranking highest in nutrient content of all vegetables for vitamins A and C, folate, iron, copper, calcium and fibre (Woolfe, 1992; Mwanga *et al.*, 2002). The major nutritional element of OFSP is β -carotene, a vitamin A precursor (Woolfe, 1992; Allemann *et al.*, 2004), which is essential for the proper functioning of the immune system, retina and mucous membranes. A deficiency in vitamin A is the principle cause of childhood blindness in developing countries (Ziegler, 2001). OFSP has been recognized as a potential means for saving thousands of children in Africa from vitamin A deficiency-related diseases such as night blindness (Van Jaarsveld *et al.*, 2005; Kapinga *et al.*, 2009).

The crop can grow in a wide range of environments ranging from sea level up to 2500m above sea level. It requires few inputs and can perform well in marginal soils, making it appropriate for subsistence farmers with limited resources. It has a short growing season that allows it to fit into many different cropping systems. Its roots can be harvested piecemeal to

provide fresh daily food for the family (Bashaasha *et al.*, 1995). Sweetpotato peelings and foliage are also used as animal feeds.

1.3 Constraints to sweetpotato production in Uganda

Despite the importance of sweetpotato, its productivity has remained very low, with an average yield of 4.5 t/ha reported in Uganda (CGIAR, 2005; Low *et al.*, 2009). The increased production observed has been more due to an increase in area under cultivation rather than from increased yield. The low yield is a result of several interacting factors including biotic (pests and diseases), abiotic (drought, poor soils) and socio-economic (poor yielding varieties of low nutritive values, shortage of high quality planting materials, poor postharvest handling, and poor infrastructural facilities for production and distribution of quality planting materials) constraints (Namanda *et al.*, 2011; Ngailo *et al.*, 2013).

Limited availability of quality planting materials has been considered as one of the most important factors hindering production of sweetpotatoes in most of the African countries. This has been attributed to lack of a formal seed system for sweetpotatoes; farmers recycle planting materials obtained from their own field or from their neighbours (Bashaasha *et al.*, 1995). Some of these planting materials from farmers' fields are of low yielding varieties while others may be infected by viruses which reduce their quality and quantity. Hall *et al.* (1998) reported that varieties planted by farmers do not necessarily indicate their assessments of which varieties are best suited for a given area but might reflect what is available in the form of vines for vegetative propagation at the beginning of the planting season. Lack of quality planting material is also threatened by prolonged drought in many areas (Namanda *et al.*, 2011). Farmers in areas prone to drought have tried to address this by planting vines in swamps or by leaving un-harvested roots to sprout when the rain starts. This however, remains a challenge itself in that, farmers have to wait for roots to sprout so delaying planting. Insect pests facing their own food shortages during the dry season also concentrate with the first rains in vine multiplication plots. This result in loss of planting material due to defoliators, and early virus infection transmitted by virus vectors such as aphids and whiteflies (Low *et al.*, 2009). To address the challenge of limited planting materials, there is a need to have a functional seed system that will ensure timely availability of enough quality planting materials to the farmers so that they are able to renew the planting stocks whenever there is degeneration.

Sweetpotato production is further limited by pests, and sweet potato weevils (*Cylas* spp) have been ranked as number one pest worldwide (Chalfant *et al.*, 1990; Bashaasha *et al.*, 1995; Smit, 1997; Nottingham and Kays, 2002). The weevil tunnels and feeds on vines and storage roots thereby reducing the quality and yield of the crop. According to Stanthiers *et al.* (1999), yield loss from weevil infestation can be as high as 100%. The yield loss is mainly caused by three major species of weevils; *Cylas puncticollis*, *C. brunneus* mainly found in Africa and *C. formicarius* which is found in the United States, Asia and the Caribbean. Sweetpotato weevils also render the storage roots unfit for human and animal consumption since the affected roots become bitter, often coupled with a bad smell (Akazawa *et al.*, 1960; Uritani *et al.*, 1975; Sato *et al.*, 1981).

After weevils, viruses are the 2nd major biotic constraint to sweetpotato production in the world. Over 30 viruses have been reported to occur worldwide but only 12 are recognized by the International Committee of Virus Taxonomy (Clark *et al.*, 2012). Out of the known viruses, only six including *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato chlorotic stunt virus* (SPCSV), *Sweet potato mild mottle virus* (SPMMV), *Sweet potato chlorotic flecks virus* (SPCFV), *Sweet potato caulimovirus* (SPCaLV) and *Sweetpotato leaf curl uganda virus* (SPLCUV) have been reported in Uganda (Gibson *et al.*, 1998; Mukasa *et al.*, 2003; Aritua *et al.*, 2007; Wasswa *et al.*, 2011). Single infection of these viruses causes mild or no symptoms and consequently little or no yield loss in sweetpotato (Milgram *et al.*, 1996; Kareija *et al.*, 1998b; Mukasa *et al.*, 2006). Due to lack of symptoms and difficulty in detecting them directly in sweetpotato, most single virus infections are not recognized by farmers who continuously spread them through propagation of infected vines. This results into virus accumulation and cultivar degeneration over time. However, the synergistic interaction of SPFMV and SPCSV results in severe symptoms referred to as sweet potato virus disease (SPVD) and yield loss of up to 90% has been reported (Kareija *et al.*, 1998a). SPVD has also been reported to result in extinction of elite high yielding but susceptible varieties (Gibson *et al.*, 1998; Mukasa *et al.*, 2006). This study therefore aimed at determining the importance of sweet potato viruses to cultivar decline in Uganda. This will help in the development of appropriate disease management practices that will promote production and productivity of the crop.

1.4 Problem statement

Sweetpotato virus disease (SPVD) caused by synergistic interaction of *Sweet potato feathery mottle virus* and *Sweet potato chlorotic stunt virus* is the most important disease of sweetpotato in Uganda causing up to 90% yield loss in infected susceptible cultivars (Aritua *et al.*, 2000; Karyeija *et al.* 1998a; Mukiibi, 1977). Single infection by either SPFMV or SPCSV may not show symptom in most Ugandan sweetpotato cultivars (Untiveros *et al.*, 2007) and their specific effect on yield under field condition is not known. The effect of SPVD has been escalated by lack of a proper seed system that has made farmers to continuously recycle vines from their own or neighbours mature crop fields or to use sprouts from roots that survive in soil after a long dry spell. This practice of recycling planting material may lead to virus accumulation and perpetuation since the virus status of such material is not known and this may result in cultivar degeneration. No study has been done to establish the effect of recycling planting material given that the initial material is virus tested. It is also not known how long such materials can be grown field exposed in different agroecologies without getting infected with the viruses and or degenerating. In addition little or no information is available on whether sweetpotato storage roots can be infected with the viruses and whether the viruses can even show symptoms on the sprout to enable farmers rogue out. Sweetpotato degeneration in other countries have been associated with mutation and accumulation of viruses in propagating planting materials (Bryan *et al.*, 2003; Clark, 2007). In Uganda, seed stocks of high value cultivars like Ejumula are speculated by farmers to decline in yield with time. Landraces and some research station-bred cultivars have been grown exposed in farmers' fields for decades and seem not to degenerate and those infected with SPFMV are also reported to revert to healthy status (Gibson *et al.*, 1997). There is thus apparently contradictory evidence for and against cultivar decline being important in Uganda and the extent to which virus infection contributes to yield decline if at all, and how often the cultivar should be replaced with new clean planting material.

1.5 Justification of the study

Despite sweetpotato being important in life of many farmers, the average yield attained on farm has remained low. Several efforts have been made to improve yield through breeding for high yielding varieties like NASPOT series (Mwanga *et al.*, 2003, 2007 and 2011) unfortunately, after few years of production, their yield seem to decline (Kapinga *et al.*, 2005). Use of virus indexed material have been reported to improve such yield but this has not been exploited in Uganda due to lack of information on cultivar degeneration.

Therefore, understanding how sweetpotato cultivars degenerate under field conditions, and in different agroecologies would strengthen the relevancy on the use of virus indexed material in Uganda. It would also provide information on the frequency of replacing a given cultivar with new clean planting material.

Also little attention has been given to the effect of single infection by SPFMV or SPCSV yet they are reported to be wide spread compared to dual infection (Mukasa *et al.* 2003; Tairo *et al.*, 2005, Aritua *et al.* 2007). Single virus infection causes mild or no symptoms (Karyeija *et al.*, 1998a) and may go unrecognized by both researchers and farmers. This lack of symptoms implies that singly infected materials are continuously recycled by farmers and may be the invisible contributors of yield decline in Uganda. Studies conducted so far on the effect of single virus infection on sweetpotato yield are contradictory, some have reported no effect on yield (Milgram *et al.*, 1996, Clark and Hoy, 2006), others have reported better yield performance when infected material is planted (Gutiérrez *et al.*, 2003), while others have reported significant yield loss (Gibson *et al.*, 1997; Njeru *et al.*, 2004; Mukasa, 2004; Domola *et al.*, 2008; Clark and Hoy, 2006). In countries such as China, USA and South Africa, sweetpotato cultivar decline due to viruses has been reported in commercial varieties. This has led to expensive eradication programs and continual release of virus-free planting stocks (Fuglie *et al.*, 1999). In Uganda, some landraces and research station bred varieties have been grown exposed in fields for a long time and have been speculated not to decline and when infected by these viruses, they can revert to healthy (Gibson *et al.*, 1997; Aritua *et al.*, 1998b; Gasura *et al.*, 2009; Wasswa, 2012). On the other hand some varieties have been abandoned due to poor yield in both areas with high and low SPVD. Due to this contradiction, there is a need to further study the effect of SPFMV and SPCSV on the yield of sweetpotato cultivars grown in Uganda comparing them in different agro-ecologies. This will provide better understanding of the impact of these viruses on yield and quality of planting material so that appropriate management practices can be designed and recommended for implementation.

In Uganda, sweetpotato propagation is mainly done using cuttings from mature fields of previous season but this method is not sustainable especially in areas with long dry spells as most of the established fields tend to dry out leading to lack of planting materials in the subsequent season. Farmers in these areas tend to wait for surviving roots to sprout and use that as a planting material; this results in late planting. Also the health status of such

materials in terms of pests and diseases are not clear. To address this challenge, method of root storage in sand and sprouting during the dry season has been developed (Namanda, 2012) and is currently under promotion by CIP in Uganda, Tanzania and Malawi. The limitation with this method is lack of information on virus accumulation in the roots and whether the roots from infected plants can produce symptomatic sprouts to enable farmers rogue. In USA, sweetpotato root sprouts have been reported to accumulate mutation and viruses resulting in to cultivar decline (Bryan *et al.*, 2003, Clark and Hoy, 2006). Infected roots of some cultivars like Beauregard normally show external cracking and internal corkiness and the sprout produce visible virus symptom that allow rouging (Clark and Moyer 1988; Gutierrez *et al.*, 2003). This study sought to understand the viral movement and accumulation into the roots of sweetpotatoes and whether infected root sprouts can show symptoms or revert to healthy as in the case of vine cutting. Understanding the epidemiology of sweetpotato virus in roots will strengthen the current technology of using storage roots as source of planting material in drought prone areas thus ensuring sufficient availability of clean planting material to farmers at onset of rain.

1.6 Objectives

The overall objective of this study was to establish the relative importance of viruses in mediating sweetpotato cultivar decline in Uganda. The information generated will help in designing long lasting virus management strategies and improve on the production of clean planting material for farmers hence boosting their production and ability to satisfy the increasing market demand for sweetpotato.

Specific objectives

1. To determine the rate of degeneration of sweetpotato cultivars in high and low SPVD pressure zones of Uganda.
2. To determine the effect of SPFMV and SPCSV on the yield of sweetpotato cultivars grown under field condition in two agro-ecologies in Uganda.
3. To determine virus movement from infected vines to storage roots and potential of symptom expression on the sprouts.

1.7 Hypotheses

1. The rate of degeneration of sweet potatoes due to viruses does not vary among cultivars grown in different SPVD pressure zones.
2. Single virus infection has no effect on the yield of sweetpotatoes in all the agro-ecologies.
3. Sweetpotato storage roots are not infected by viruses hence does not show symptom on the sprouts.

CHAPTER TWO

LITERATURE REVIEW

2.1 Sweet potato viruses and their effects on yield

More than 30 viruses have been described infecting sweetpotato worldwide (Salazar and Fuentes, 2000; Loebenstein *et al.*, 2003; Clark *et al.*, 2012). These viruses have been assigned to 9 families including *Bromoviridae*, *Bunyaviridae*, *Caulimoviridae*, *Closteroviridae*, *Comoviridae*, *Flexiviridae*, *Geminiviridae*, *Luteoviridae*, and *Potyviridae* (Table 1). Half of them are recently described DNA viruses belonging to families *Geminiviridae* and *Caulimoviridae*. Most of these viruses are associated with symptomless infections in sweetpotato and in some cases even in the indicator plant *Ipomoea setosa* (Clark *et al.*, 2012). Although new, improved generic virus detection methods based on new DNA sequencing technologies (Mardis, 2011) and the use of rolling circle amplification (RCA) are advancing the process of virus characterization at the molecular level, the biological characterization and validation of newly identified isolates in terms of their effect on yield and reaction on host plants is not documented. Among the viruses so far reported, only six including *sweet potato chlorotic stunt virus* (SPCSV), *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato mild mottle ipomovirus* (SPMMV), *Sweet potato chlorotic fleck virus* (SPCFV), *Sweet potato caulimo-like virus* (SPCaLV) and *sweetpotato virus G* (SPVG) (Aritua *et al.*, 2000; Mukasa *et al.*, 2003; Aritua *et al.*, 2006; Ndunguru *et al.*, 2009) have been reported in Uganda. In addition, sweetpotato begomovirus (*Sweet potato leaf curl Uganda virus*) was also reported to occur in Uganda (Wasswa *et al.*, 2011). The effects of some of these viruses on production has not been studied; for others their effect ranges from minimal to completely devastating depending on the infecting virus, virus complex and sweetpotato cultivar involved (Kikkinos and Clark, 2006b) as discussed below.

Table 1: Some of the sweetpotato viruses by Family and Genus that have been reported, and their distribution worldwide

Virus	Family	Genus	Distribution	References
<i>Cucumber mosaic virus (CMV)</i>	<i>Bromoviridae</i>	<i>Cucumovirus</i>	Israel, Egypt, Kenya	Cohen and Loebenstein, 1991.
<i>Tomato spotted wilt virus (TSWV)</i>	<i>Bunyaviridae</i>	<i>Tospovirus</i>	U.S.A, Canada	Clark and Hoy, 2007
Sweet potato C-3 virus	<i>Bunyaviridae</i>	<i>Phlebovirus</i>	Brazil	Fuentes and Salazar, 1989; Loebenstein, <i>et al.</i> , 2009
Sweet potato pakakuy virus (SPPV) (synonyms Sweet potato badnavirus A and B)	<i>Caulimoviridae</i>	<i>Badnavirus</i>	Worldwide	Kreuze <i>et al.</i> , 2009; Mbanzibwa, <i>et al.</i> , 2011 Clark <i>et al.</i> , 2012
Sweet potato collusive Virus (SPCV) (synonym Sweet potato caulimo-like virus)	<i>Caulimoviridae</i>	<i>Cavemovirus</i>	Madeira, New Zealand, Papua New Guinea, China, Central America, and East Africa	Atkey and Brunt, 1987 Loebenstein <i>et al.</i> , 2009 Cuellar, <i>et al.</i> , 2011; De Souza, J., and Cuellar, W. J. 2011.
Sweet potato vein clearing virus (SPVCV)	<i>Caulimoviridae</i>	<i>Solendovirus</i>	Central America and the Caribbean islands	Cuellar <i>et al.</i> , 2011
Sweet potato chlorotic stunt virus (SPCSV)	<i>Closteroviridae</i>	<i>Criinivirus</i>	Worldwide	Cohen <i>et al.</i> , 1992; Winter <i>et al.</i> , 1992; Hoyer <i>et al.</i> , 1996; Gibson <i>et al.</i> , 1998; Alicai <i>et al.</i> , 1999b.
Sweet potato ringspot virus	<i>Comoviridae</i>	<i>Nepovirus</i>	Papua New Guinea (21)	Brown <i>et al.</i> , 1988
Sweet potato chlorotic fleck Virus (SPCFV)	<i>Flexiviridae</i>	<i>Carlavirus</i>	South and Central America, Asia, East Africa, Australia and French Polynesia	Aritua <i>et al.</i> , 2009; Loebenstein <i>et al.</i> , 2009 Mukasa <i>et al.</i> , 2003; Rännäli <i>et al.</i> , 2009
Sweet potato C-6 virus	<i>Flexiviridae</i>	<i>Carlavirus</i>	Cuba, Mexico, Peru, and USA	Loebenstein <i>et al.</i> , 2009
Ipomoea crinkle leaf curl virus (ICLCV)	<i>Geminiviridae</i>	<i>Begomovirus</i>	USA 10	Cohen <i>et al.</i> , 1997

<i>Ipomoea yellow vein virus</i> (IYVV)	Geminiviridae	Begomovirus	Spain	Li <i>et al.</i> , 2004; Lozano <i>et al.</i> , 2009
Sweet potato Golden vein associated virus (SPGVAV)	Geminiviridae	Begomovirus	Brazil	Paprotka <i>et al.</i> , 2010
Sweet potato symptomless virus 1 (SPSMV-1)	Geminiviridae	Mastrevirus	Peru, Tanzania Central America and Asia	Kreuze <i>et al.</i> , 2009; Mbaziwa <i>et al.</i> , 2011 Clark <i>et al.</i> , 2012
Sweet potato mosaic associated virus (SPMAV)	Geminiviridae	Begomovirus	Brazil	Paprotka <i>et al.</i> , 2010
Sweet potato leaf curl virus (SPLCV)	Geminiviridae	Begomovirus	U.S.A, Sicily, Kenya, China, Brazil, India, Italy, Japan	Lotrakul <i>et al.</i> , 1998; Miano <i>et al.</i> , 2006.
Sweet potato leaf curl Canary virus (SPLCCaV)	Geminiviridae	Begomovirus	Canary Island	Lozano <i>et al.</i> , 2009
Sweet potato leaf curl China virus (SPLCV-CN)	Geminiviridae	Begomovirus	China	Luan <i>et al.</i> , 2007
Sweet potato leaf curl Georgia virus (SPLCGV)	Geminiviridae	Begomovirus	U.S.A	Lotrakul <i>et al.</i> , 2003
Sweet potato leaf curl Lanzarote virus (SPLCLaV)	Geminiviridae	Begomovirus	Spain	Lozano <i>et al.</i> , 2009
Sweet potato leaf curl Spain virus (SPLCESV)	Geminiviridae	Begomovirus	Spain	Lozano <i>et al.</i> , 2009
Sweet potato leaf curl South Carolina virus (SPLCSCV)	Geminiviridae	Begomovirus	U.S.A	Ling <i>et al.</i> , 2010,
Sweet potato leaf curl Uganda virus (SPLCUV)	Geminiviridae	Begomovirus	Uganda	Masswa <i>et al.</i> , 2011
Sweet potato leaf speckling Virus (SPLSV)	Luteoviridae	Polerovirus	Peru and Cuba	Loebenstein <i>et al.</i> , 2009
Sweet potato mild mottle virus (SPMMV)	Polyviridae	Ipomovirus	East Africa	Hollings <i>et al.</i> , 1976; Colinet <i>et al.</i> , 1996.
Sweet potato mild speckling virus (SPMSV)	Polyviridae	Polyvirus	Argentina, Peru, Brazil	Kwak <i>et al.</i> , 2007
Sweet potato feathery	Polyviridae	Polyvirus	Worldwide	Abad <i>et al.</i> , 1992; Colinet and Kummerl,

<i>mottle virus</i> (SPFMV)				1993; Sakai <i>et al.</i> , 1997; Krcuze <i>et al.</i> , 2000; Abad <i>et al.</i> , 2007.
<i>Sweet potato latent virus</i> (SPLV)	<i>Potyviridae</i>	<i>Potyvirus</i>	Taiwan, Peru, China, Japan	Colinet <i>et al.</i> , 1998., Kwak <i>et al.</i> , 2007; Wang, <i>et al.</i> , 2007
<i>Sweet potato vein mosaic virus</i> (SPVMMV)	<i>Potyviridae</i>		Argentina	Nome. 1973
<i>Sweet potato virus 2</i> (SPV2)	<i>Potyviridae</i>	<i>Potyvirus</i>	Taiwan, China, USA, South Africa, Australia, and Barbados	Ateka <i>et al.</i> , 2004; Jones and Dwyer 2007; Kokkinos and Clark, 2006; Ling <i>et al.</i> , 2010; Perez <i>et al.</i> , 2009
<i>Sweet potato virus C</i> (SPVC)	<i>Potyviridae</i>	<i>Potyvirus</i>	USA	Kokkinos and Clark, 2006b
<i>Sweet potato virus G</i> (SPVG)	<i>Potyviridae</i>	<i>Potyvirus</i>	China, Egypt, U.S.A	Colinet <i>et al.</i> , 1998; Kokkinos and Clark, 2006; Kwak <i>et al.</i> , 2007; Ling <i>et al.</i> , 2010
<i>Sweet potato yellow dwarf virus</i> (SPYDV)	<i>Potyviridae</i>	<i>Ipomovirus</i>	Taiwan	Liao <i>et al.</i> , 1979

2.1.1 *Sweet potato feathery mottle virus*

Sweet potato feathery mottle virus (SPFMV) is the most common and widely distributed virus. It occurs wherever sweetpotatoes are grown (Tairo *et al.*, 2005). Surveys carried out in East Africa have reported SPFMV as the most predominant virus infecting sweetpotato, with over 50% of the plants showing virus symptoms being reported to have single infections with the virus (Mukasa *et al.*, 2003; Tairo *et al.*, 2005; Aritua *et al.*, 2006). SPFMV belongs to the genus *Potyvirus* and family *Potyviridae*. The virus is single stranded with positive sense RNA genome of about 10.6kb and it's composed of flexuous filamentous particles measuring 830-850nm in length (Schaefer and Terry, 1976). SPFMV is transmitted in a non-persistent manner by several aphid species including *Aphis gossypii*, *A. craccivora*, *Lipaphis erysimi* and *Myzus persicae* (Schaefer and Terry, 1976; Palomar *et al.*, 2000). These aphids however do not colonize sweetpotato and therefore itinerant alate aphids might be the means of transmission (Byamukama *et al.*, 2004; Aritua *et al.*, 1998a). In addition to insect transmission, this virus can be transmitted through vegetative propagation using infected cuttings obtained from farmers' fields season after season and mechanically by grafting to *Ipomoea* species such as *I. batatas*, *I. setosa*, *I. nil*, *I. incarnata* and *I. purpurea*, and some strains to *Nicotiana benthamiana*, *N. clevelandii*, *Chenopodium amaranticolor* and *C. quinoa* (Cadena-Hinojosa and Campbell, 1981; Brunt *et al.*, 1996, Palomar *et al.*, 2000). Leaf symptoms in sweetpotato are generally mild and transient, but may include vein clearing, vein feathering, and chlorotic spots, particularly on older leaves. Symptoms on roots may include external cracking and internal necrosis, depending on cultivar and virus strain (Clark and Moyer 1988; Gutierrez *et al.*, 2003). Root symptoms have not been reported or observed among Ugandan cultivars and it is not known if storage roots from Ugandan cultivar can get infected with this virus. It has been reported that most cultivars grown in Uganda are resistant and some can revert to normal state when infected with SPFMV (Gibson *et al.*, 1998; Wasswa, 2012) but whether this is true for root sprouts is not known. Symptoms, host range, and serology have been used to group SPFMV isolates into two strains, the common strain (C) and the more severe russet crack (RC) strain (Moyer and Kennedy, 1978; Moyer *et al.*, 1980; Cali and Moyer, 1981; Usugi *et al.*, 1994). However, based on phylogenetic analysis of 3' nt sequences of an extensive number of isolates it is now clear that SPFMV can be distinguished in four phylogenetic lineages RC (rosette crack), O (ordinary), EA (East Africa), and C (common) strain (Kreuze *et al.*, 2000).

Effects of SPFMV on sweetpotato cannot easily be recognized either by farmers or researchers since single infection causes mild or no symptoms in most cultivars (Gutiérrez *et al.*, 2003). As a result, most yield research in East Africa has focused on SPVD and little data have been reported on yield loss due to SPFMV. Studies conducted in USA and Israel on single infection with SPFMV in comparison with virus tested healthy material found no significant yield difference (Loebenstein *et al.*, 2009; Clark and Hoy, 2006; Milgram *et al.*, 1996). The economic damage was associated with external cracking and internal corkiness of roots in areas where the rosette crack strain of SPFMV occurred making tuberous roots unmarketable (Clark and Hoy, 2006; Domola *et al.*, 2008). In Peru, SPFMV infected plants even produced much higher yield than the healthy controls (Gutierrez *et al.*, 2003). In South Africa, a study conducted by Domola *et al.* (2008) reported yield reduction due to SPFMV infection. In Uganda, Gibson *et al.* (1997) reported that virus-tested healthy plants yielded twice as much as those inoculated with SPFMV in screen house trials. Studies in New Zealand and Venezuela revealed similar results (Over de Linden and Elliott, 1971; Pozzer *et al.*, 1994). There is thus contradictory evidence on the effect of SPFMV on yield and degeneration of sweetpotato though overall these studies suggest SPFMV alone can cause significant decline in yields, and therefore economic losses in some cultivars. In Uganda, the effect of SPFMV on yield under field condition is not clear though over the years some of the released varieties have shown some trend of yield decline and some have been completely abandoned by farmers (Aritua *et al.*, 1999; Gasura *et al.*, 2009). It's not clear whether the cultivar decline is due to viruses, therefore it is important to determine the effect of SPFMV under field conditions in order to establish its contribution to the cultivar decline so as to develop appropriate management strategies. The major impact of SPFMV is largely seen when co-infected with SPCSV, resulting in SPVD which is very devastating to the production of sweetpotato in Uganda and Africa at large.

2.1.2 Sweet potato chlorotic stunt virus

Sweet potato chlorotic stunt virus (SPCSV) is another important virus of sweetpotato worldwide due to its ability to mediate severe synergistic disease interaction with several other viruses including potyviruses, cucomoviruses, and carlarviruses (Karyeija *et al.*, 2000; Kikkinos and Clark, 2006b; Untiveros *et al.*, 2007). SPCSV belongs to the genus *Crinivirus* of the family *Closteroviridae* (Schaefer, and Terry, 1976). This virus consists of flexuous, filamentous particles ranging from 850 to 950nm long and 12nm wide (Schaefer, and Terry, 1976). The coat protein size is 33KDa and is similar to other criniviruses (Schaefer, and

Terry, 1976; Cohen *et al.*, 1992; Kreuze, 2002). The complete genome of SPCSV was sequenced by Kreuze (2002) and consists of two RNA molecules. With a total length of 17630 nucleotides, SPCSV is the second largest single-stranded positive-sense RNA virus infecting plants after *Citrus tristeza virus* (CTV, genus *Closterovirus*). RNA1 has a total length of 9407 nucleotides and contains five putative open reading frames (ORFs). RNA2 contains 8223 nucleotides and has seven putative ORFs (Kreuze, 2002). SPCSV is transmitted by whiteflies in a semi persistent, non circulative manner and is not mechanically transmissible (Schaefers and Terry, 1976; Cohen *et al.*, 1992; Wisler *et al.*, 1998; Sim *et al.*, 2000). Like in SPFMV, SPCSV has a host range limited mainly to the family *Convolvulaceae* and genus *Ipomoea* although *Nicotiana* spp and *Amaranthus palmeri* are reportedly susceptible (Cohen *et al.*, 1992). SPCSV can be serologically divided in two major serotypes; one (designated serotype East Africa) occurs only in East Africa and Peru, while the other serotype is found in United States, South America, West Africa, and Egypt (Hoyer *et al.*, 1996; Alicai *et al.*, 1999b; Kreuze, 2002; Gutierrez *et al.*, 2003; IsHak *et al.*, 2003; Abad *et al.*, 2007).

Sweetpotato plants singly infected by SPCSV showed no symptoms in some cultivars or mild symptoms which are easily confused with nutritional deficiency (Gibson *et al.*, 1998; Mukasa *et al.*, 2006). As such, farmers have been using infected planting material for generations. Research on the effects of SPCSV alone on yield of sweetpotato grown under field condition in Uganda and Africa at large is limited, however the virus has been reported to cause up to 30% yield reduction in Israel (Milgram *et al.*, 1996). In Kenya, Njeru *et al.* (2004) reported yield loss of between 15% to 75% depending on the cultivar while in Peru, a yield loss of 28% to 44% was reported (Gutierrez, *et al.*, 2003, Untiveros, *et al.*, 2007). In a screen house experiment conducted in Uganda, SPCSV was reported to cause yield loss of 44% (Mukasa, 2004) and 88% (Gibson *et al.*, 1998) in cultivar Tanzania. Based on these results, SPCSV alone can cause significant yield reduction and there is therefore a need to investigate its effect on yield of sweetpotatoes grown under field conditions in order to ensure proper management of the disease. The major economic impact of SPCSV is when there is co-infection with other potyviruses like SPFMV resulting in severe symptom referred to as SPVD which is associated with greater yield loss. This has been the focus of most research in Africa paying less attention to single virus infection that could be a major factor in cultivar decline.

2.1.3 *Sweet potato mild mottle virus (SPMMV)*

Sweet potato mild mottle virus (SPMMV) belongs to the family *Potyviridae*, in the genus *Ipomovirus* (Colinet *et al.*, 1996). The virus was first reported in East Africa in 1970s from sweetpotato showing leaf mottling, vein chlorosis, dwarfing and poor growth (Sheffield, 1957; Hollings *et al.*, 1976; Brunt *et al.*, 1996). The virus has since been reported to occur in West Africa, South Africa, Indonesia, China, Philippines, Papua New Guinea, India, New Zealand, and Egypt (Hollings *et al.*, 1976; Carey *et al.*, 1997; Fletcher *et al.*, 2000; Domola *et al.*, 2008). SPMMV has similar morphology to other potyviruses in terms of virion size, cytoplasmic ‘pin wheel’ inclusions and the viral genome organisation (Hollings *et al.*, 1976; Colinet *et al.*, 1998). The virions of SPMMV are made up of flexuous rod shaped particles, 800-950nm in length. It contains a positive single-stranded RNA genome of 10.8kb and a coat protein of 37.7kDa (Loebenstein *et al.*, 2009). Although SPMMV was originally described as being transmitted by whiteflies in a persistent manner (Hollings *et al.*, 1976), later studies have failed to confirm its whitefly transmissibility (Tugume *et al.*, 2010). The virus can also be transmitted by grafting and mechanical inoculation but not by seed, pollen or by contact between plants (Brunt *et al.*, 1996; Loebenstein *et al.*, 2003). In comparison to other sweetpotato-infecting viruses, SPMMV has a wide host range, including species in 14 different families (Hollings *et al.*, 1976; Brunt *et al.*, 1996). *Chenopodium quinoa*, *I. setosa*, *N. tabacum*, *N. benthamiana*, *N. glutinosa* and *N. clevelandii* are good experimental hosts for SPMMV. Symptoms of SPMMV- infected *I. setosa* are similar to those induced by SPFMV, which include vein clearing and distortion (Hollings *et al.*, 1976; Clark and Moyer, 1988). Data on the effect of SPMMV on yield of sweetpotato is limited. However, Njeru *et al.*, (2004) reported significant yield losses ranging between 17- 95% due to SPMMV among the Kenyan varieties while in Uganda Mukasa (2004) observed no differences between the yields of SPMMV infected plants and healthy controls under greenhouse conditions.

2.1.4 *Sweet potato caulimo-like virus (SPCaLV)*

Sweet potato caulimo-like virus (SPCaLV) also called Sweetpotato caulimovirus (SPCV) is a possible member of the genus *Caulimovirus* in the family *Caulimoviridae* (Brunt *et al.*, 1996). This virus consist of isometric particles with a diameter of 50-52nm and has a double-stranded DNA genome with coat protein of 42-44kDa (Brunt *et al.*, 1996). The virus has been found in widely scattered geographical locations: Madeira, New Zealand, Papua New Guinea, Solomon Islands (Atkey and Brunt, 1987), China (Gao *et al.*, 2000), Uganda (Aritua *et al.*, 2006), Kenya, Nigeria, Egypt, and Puerto Rico (Kreuze and Fuentes, 2008; Loebenstein *et*

al., 2009). No diagnostic symptoms on sweetpotato have been shown to be associated with SPCaLV. Early symptoms on *I. setosa* include chlorotic flecks along the minor veins and interveinal chlorotic spots which may develop into general chlorosis, wilting and premature death of leaves (Clark and Moyer, 1988). The only known natural host of SPCaLV is *I. batatas*. In the laboratory, SPCaLV can be made to infect *I. setosa* and *N. megalosiphon*. SPCaLV is known only to be transmitted by grafting. There are no known vectors for the virus neither is the virus transmitted by mechanical inoculation, nor contact between plants and seed (Clark and Moyer, 1988; Brunt *et al.*, 1996). Future characterization studies will allow for the elucidation of additional viral characteristics and subsequent definitive classification (Clark and Moyer, 1988). Also the economic impact of this virus on yield is not known and therefore requires future research

2.1.5 Sweet potato chlorotic fleck virus (SPCFV)

SPCFV is the member of the genus *Carlavirus* in the family *Flexiviridae* (Aritua *et al.*, 2007, 2009). This virus is also called sweetpotato symptomless virus in Japan due to its symptomless nature of infection in local genotypes and as C-2 (Aritua *et al.*, 2009). The virus consists of filamentous particles of 750 to 800 by 12 nm in size and capsid protein size of 33.5kDa (Fuentes and Salazar, 1992, Aritua *et al.*, 2009). SPCFV has single stranded positive sense RNA genome consisting of 9104 nucleotides containing six putative open reading frames and sharing the highest nucleotide and amino acid sequence similarities with the viruses of the genus *Carlavirus* in the family *Flexiviridae* (Aritua *et al.*, 2007). The virus is widely distributed occurring in Brazil, Bolivia, China, Colombia, Cuba, Indonesia, Japan, Panama, Peru, Philippines, Kenya, Tanzania, and Uganda (Usugi *et al.*, 1991; Fuentes and Salazar, 1992; Fletcher *et al.*, 2000; Aritua *et al.*, 2007; Jones and Dwyer, 2007). The virus is the fourth most common in Uganda infecting about 5% to 8% of diseased plants (Mukasa *et al.*, 2003, Aritua, *et al.*, 2007) but its economic impact on yield of sweetpotato is not known. The virus has a narrow host range restricted to few members of the family *Convolvulaceae* and *Chenopodia* and is transmitted mechanically but not by seed or aphids (Loebenstein *et al.*, 2009, Aritua *et al.*, 2009).

2.1.6 Sweet potato begomoviruses

Geminiviruses (family *Geminiviridae*) are plant viruses with a circular single strand DNA genome encapsidated within twinned isometric particles. Based on their genome organization, host range and insect transmission, geminiviruses are classified into four

genera: *Mastrevirus*, *Curtovirus*, *Topocuvirus* and *Begomovirus* (Brown *et al.* 2012). Begomoviruses represent major plant pathogens in tropical, subtropical and, to a more limited extent, temperate regions (Morales, 2001). Begomoviruses are transmitted by whiteflies (*Bemisia tabaci* Genn.) to dicotyledonous plants (Fauquet *et al.*, 2003; Simmons *et al.*, 2009). Most of the species in this genus have bipartite genomes consisting of two ssDNA molecules, referred to as DNA-A and DNA-B, but there are some monopartite species, found within the Old World, that have only one genome component similar to DNA-A. *Ipomoea* infecting begomoviruses are monopartite viruses. Phylogenetic analysis showed that these viruses, for which the name of sweepoviruses has been proposed, are grouped in a monophyletic cluster, separated from the main begomovirus branches, the Old and New World groups (Lozano *et al.*, 2009; Fauquet and Stanley, 2003; Briddon *et al.*, 2005). *Sweet potato leaf curl virus* (SPLCV) has wide distribution and has been reported to occur in U.S.A, China, Japan, Spain, Argentina and Kenya (Lotrakul *et al.*, 1998; Luan *et al.*, 2007; Lozano *et al.*, 2009).

In 2011, *sweet potato leaf curl Uganda virus* (SPLCUV) was reported in Uganda and it has been suggested as the 3rd most widely distributed virus after SPFMV and SPCSV (Wasswa *et al.*, 2011). Most infected sweetpotato cultivars do not show symptoms and therefore its effect on yield is not known in Uganda. The potential importance of begomoviruses is indicated in part by the study of Clark and Hoy (2006) in which they found that yields of sweetpotato cultivar 'Beauregard' were reduced by 25-30% by SPLCV despite the fact that no symptoms were observed on the plants. Selecting symptomless vine cuttings for propagating material from genotypes that have virus resistance (Gibson *et al.* 1997), and/or roguing symptomatic plants from production fields can be used as effective means of managing SPVD (Gibson *et al.*, 2004). However, Begomoviruses have the potential to compromise these efforts as they are capable of causing significant yield losses without inducing apparent symptoms. There is a need therefore, to incorporate specific begomovirus test procedures into sweetpotato virus indexing protocols and to determine the prevalence and effects of this group of viruses in those regions where begomoviruses have recently been reported.

2.1.7 Sweetpotato virus disease complexes and their effect on yield

SPCSV interacts synergistically with SPFMV, enhancing its accumulation by approximately 600 fold (Karyeija *et al.*, 2000; Kokkinos and Clark, 2006b). Yield losses of up to 100% have been reported in individual plants of susceptible cultivars in Africa under very severe infection with SPVD (Karyeija *et al.*, 1998b). In Uganda yield losses of 66-90% have been

reported due to SPVD under experimental conditions (Mukiibi, 1977; Aritua *et al.*, 2000). The variation in yield loss is influenced by the type of cultivar, the nature of the farming system and the abundance of the vector population (Aritua *et al.*, 1998a). In addition to yield reduction, SPVD has made many farmers in Uganda abandon susceptible but high yielding and nutritious sweetpotato cultivars (Gibson *et al.*, 1998; Mukasa *et al.*, 2006). Due to the effect of SPVD, sweetpotato research in Uganda has put much emphasis on breeding for resistant varieties and several have been released and distributed to farmers (Mwanga *et al.*, 2003, Mwanga *et al.*, 2007). However little effort has been put in maintaining virus free seed stocks of such varieties from which farmers can get healthy planting materials. As a consequence some of these released varieties have succumbed to viruses and degenerated over time. Understanding how viruses accumulate in planting material over time will help in designing appropriate seed systems that will ensure production of virus free planting materials.

In addition to SPVD, other sweetpotato virus complexes so far reported involve SPCSV that enhances replication of other viruses resulting into higher titre. Mukasa *et al.* (2006) showed that SPMNV is also enhanced by SPCSV, with virus titres increased approximately 1000-fold. The infected sweetpotato was severely diseased resulting into storage root yield loss of 80% when compared with healthy plants under screenhouse conditions. Due to severe symptoms caused, the name sweetpotato severe mosaic virus disease was proposed (Mukasa *et al.*, 2006). Synergistic interaction was also observed between SPCSV and carla- and cucomo- viruses resulting in yield loss of 40% and 50% respectively (Untiveros *et al.*, 2007).

2.2 Sweetpotato cultivar decline in Uganda

Cultivar decline/ degeneration refer to a phenomenon where the yield and or quality of a cultivar gradually declines in a broadly accumulative manner over succeeding growing seasons or years. Information on sweetpotato cultivar degeneration is limited in Africa. A few studies have been conducted in USA and New Zealand on the cultivar Beauregard and it was found that yield decline increases with increasing numbers of field exposure (Bryan *et al.*, 2003; Lewthwaite *et al.*, 2011). In Uganda many high yielding varieties were released and distributed to farmers; however, after 3 to 4 cycles of planting materials that originated from clean foundation seed, there is reduced performance of the stock (Kapinga *et al.*, 2005; Gasura *et al.*, 2009). In fact some of these varieties have been abandoned and farmers have resorted to production of landraces that are low yielding and only produce acceptable yield

after a very long growth period (Gibson *et al.*, 2004; Gasura *et al.*, 2009). Yield decline has been observed to occur in both high SPVD pressure regions of central and western Uganda and in low SPVD pressure zones in north eastern Uganda (Gasura *et al.*, 2009). Despite the speculation that some Ugandan sweetpotato varieties decline, no studies have been done to confirm this. Sweetpotato degeneration may either be due to physiological causes or pathogens that appear to accumulate within the propagating material. Physiological degeneration can be recovered through proper crop management (Rahman *et al.*, 2010). Sweetpotato viral infection and mutation are considered significant contributors to cultivar decline as both induce detrimental effects that are proliferated by the vegetative propagation system (Bryan, *et al.*, 2003; Clark and Hoy, 2006; Lewthwaite *et al.*, 2011). This often gives the impression that a new variety (carrying few viruses/mutations) yields much better than traditional varieties, when in fact it may be no better after a year or two when viruses have accumulated.

2.3 Virus re-infection of virus indexed sweetpotato vines planted in the field

Virus free sweetpotato cuttings once established in the field have been observed to quickly get infected with sweetpotato viruses (Pozzer *et al.*, 1994; Milgram *et al.*, 1996). This is because sweetpotato viruses are vectored mainly by aphids and whiteflies and as such virus reinfection can occur rapidly during the growing season provided there is a source of inoculum nearby (Pozzer *et al.*, 1994; Milgram *et al.*, 1996; Bryan, 2002). The common inoculum sources include saved seed roots or cuttings of sweetpotato that become infected with virus during the growing season, un-harvested roots, discarded foliage and peelings that re-establish themselves as plants especially when the land is fallowed. (Karyeija *et al.*, 1998a; Bryan *et al.*, 2003).

According to Pozzer *et al.* (1994) while examining reinfection rates of SPFMV in healthy sweetpotato planted in plot that previously had sweetpotato and in plots that had never been planted with sweetpotato found that SPFMV had re-infected 80% of sweetpotato in the first plot and only 20% in an isolated field. In another study in Israel, Milgram *et al.* (1996) found that reinfection rates of SPFMV in healthy plants was greater in fields located near virus infected fields and almost no infection occurred in an isolated fields. While in USA, Bryan (2002) reported 100% reinfection in sweetpotato fields with SPFMV by the end of the growing season with the cultivar Beauregard. In all these studies, it's clear that virus indexed material once grown in the field is rapidly infected by viruses. Use of cuttings from such field

over generations leads to gradual decline in yield and quality of planting material due to presence and accumulation of viruses (Bryan *et al.*, 2003; Kapinga *et al.*, 2005; Lewthwaite *et al.*, 2011). Keeping planting stock free from virus reinfection is important in maintaining high yield and quality of sweetpotato planting material. In the Ugandan farming system, new sweetpotato fields are established near the old ones yet it has been reported that most vines from such farmers' fields are virus free (Gibson *et al.*, 1997). No studies have been conducted to establish rate of virus reinfection of healthy sweetpotato planting material in Uganda and to what extent this affects yield over successive cropping cycles. Such study will provide information on how fast healthy sweetpotato planting materials degenerate if at all and the frequency of replacement with clean planting material.

2.4 Sources of sweetpotato planting material

In Uganda there are no formal seed systems through which farmers can access clean sweetpotato planting material implying all the materials used are sourced from farmers' fields or neighbours and on very rare occasions from extension workers especially during on farm multi-location trials. Two major sources of sweetpotato planting materials are used worldwide: i) vine cuttings from mature crops and ii) sprouts from storage roots, with the former commonly used in tropical areas and the latter in temperate areas (Gibson *et al.*, 2009). However, other methods like tissue culture and macro propagation can be used to rejuvenate and maintain sweetpotato planting materials from the two sources for various uses.

2.4.1 Vine cuttings from a mature crop

Usually farmers use symptomless apical cuttings of about 30cm length from a mature crop to plant a new field (Gibson *et al.*, 1997). When the vines are limited, farmers may use the middle and lower part of the vine and yet virus distribution in vines is not yet well understood. In fact, whether the 30cm apical cuttings recommended to farmers has no or low virus load is not clear and this would be important when selecting for planting materials. Various studies have shown that the distribution of SPFMV along the length of sweetpotato vines is irregular, particularly in terms of titres or presence or absence in different parts (Green *et al.*, 1988; Abad and Moyer, 1992; Gibb and Padovan, 1993; Aritua *et al.*, 1998b, Miano, 2008). In some parts of Uganda that experience long dry seasons, farmers maintain vines in valley bottoms with residual moisture or leave roots in their fields to re-sprout when the rains return (Gibson *et al.*, 2009, Barker *et al.*, 2009). The status of such vines in terms of pests and diseases is not known. Also insect pests facing their own food shortages during the

dry season often concentrate in the first rains in vine multiplication plots with resultant high losses of planting material due to defoliators and early virus infection due to virus transmitters (Low *et al.*, 2009).

2.4.2 Use of roots as planting material

Although the use of shoots from root sprouts has largely been a method of conserving sweetpotato planting materials in high latitude areas like N. America and China where the cold winter kills the outdoor crop, the method has been thought to be useful in areas with long drought seasons (Gibson *et al.*, 2009). Most farmers in drought prone areas in sub-Saharan Africa have indirectly been using root sprouts from their field as a source of planting material (Gibson *et al.*, 2009). The roots used are those that are accidentally left in the field after harvest or those from plots that were deliberately not harvested for purpose of obtaining sprouts. The limitation with this is that, sprouting of these roots starts at the onset of rain and therefore valuable time is lost while waiting to obtain sufficient vines. The virus status of the root sprouts is also not known and this could seriously affect the final yield. Due to these limitations, root storage in sand and sprouting has been developed in order to ensure timely supply of planting materials in drought prone areas (Namanda, 2012). This method is currently under promotion in Uganda, Tanzania and Malawi by the International Potato Centre (CIP). To ensure that this technology works well, there is a need to understand virus epidemiology in roots. Most sweetpotato viruses whether singly or in multiple infections does not show symptoms in the roots. There is no documented evidence on the virus status of SPFMV-infected or SPCSV infected mother or whether the symptoms of even SPFMV alone perhaps manifest on the foliage. This makes it very difficult for farmers to select for storage roots to be saved as source of planting material. For this conservation system to be useful there is a need to determine whether viruses are translocated to and accumulate in the roots and whether that sprout produced from such roots are virus free. This will facilitate selection for healthy roots as sources of planting material.

2.5 Detection of viruses in sweetpotato plant

For any effective implementation of virus control measures, there is a need for an accurate diagnosis of a virus disease and mapping of its geographical and temporal distribution in an area or crop. Many methods have been developed for the detection and identification of plant viruses. A single diagnostic test or assay may provide adequate information on the identity of a virus, but a combination of methods is generally needed for unequivocal diagnosis. Selected

methods for detection of plant viruses must be sensitive, specific, rapid (be completed within a relatively short period of time) and affordable. Recent advances in techniques for the detection of proteins and nucleic acids have provided an opportunity to develop methods with these qualities for the diagnosis of plant virus diseases. The type of diagnostic test used ultimately depends on resources, facilities, availability of reagents, level of specificity and sensitivity, required expertise and skills available to carry out these assays, type and number of samples to be tested, and the amount of information available on the virus to be detected. In sweetpotato, detection methods range from symptomology, electron microscopy, serological tests, and nucleic acid analysis (Torrance and Jones, 1981; Torrance, 2005; Lievens and Thomma, 2005; James *et al.*, 2006).

2.5.1 Visual symptoms

This is one of the first methods deployed when diagnosing virus diseases in plants and it involves visual observation of the symptoms. When susceptible plants are infected, they may show very clear virus symptom which may include among others stunted growth, distorted leaf shape, mosaic, vein clearing, chlorosis, curled or wrinkled leaves which can easily be differentiated from apparently healthy ones (Green 1971; Agrios, 2005). These symptoms may vary with crop species or varieties, season, pathogen strain and whether the plant is infected with one or more viruses (Matthews 1980; Moyer and Salazar, 1989; Mukasa *et al.*, 2003). Nonetheless, in latent infections, plants can be considered virus free making it difficult for farmers, extension workers or even researchers to select for virus free plants (Hollings, 1965). Plants can also exhibit virus-like symptoms as a response to unfavourable weather conditions, soil mineral/nutrient imbalances, infection by non-viral pathogens, damage caused by pests, air pollution, and pesticides (particularly herbicides). In addition, different viruses can produce similar symptoms or different strains of a virus cause distinct symptoms in the same host. While symptoms provide vital information on virus diseases, adequate field experience is required when making a decision based on symptomology alone. Usually, it is necessary that visual inspection for symptoms in the field is done in conjunction with other confirmatory tests to ensure accurate diagnosis of virus infection (Bock, 1982).

2.5.2 Indicator plants

Indicator plants are plants that react to certain viruses by producing specific symptoms that can be used for detection and identification of such viruses (Agrios, 2005). Commonly used indicator plants include *Gomphrena globosa*, *Phaseolus vulgaris*, *C. quinoa*, *C.*

amaranticolor, *Petunia hybrida*, *N. debneyi*, *N. benthamiana*, *N. tabacum*, *Vicia faba*, *Vigna unguiculata*, and *Ipomoea* species (Lister, 1959; De Bokx, 1970; Green 1971; Allen and Matteoni, 1991). For sweetpotato virus detection, *I. setosa* and *I. nil* or *Chenopodium quinoa* are widely used. *I. setosa* is considered a near-universal indicator plant for sweetpotato viruses (Clark and Moyer, 1988). Each indicator plant has the optimal growth stage at which inoculation is done in order to obtain a clear plant response to virus infection; for the case of *I. setosa*, it is best done at two leaf growth stage.

Indicator plants can be inoculated using several methods including use of vectors, sap transmission or grafting (Lister, 1959; Carraro *et al.*, 2008). Insect (vector) inoculation involves first rearing the virus free insect vectors on confirmed healthy plants in cages for some time after which the insect is starved for a period of 3-4 minutes and then fed on diseased plants for periods ranging from a few minutes to several days depending on the virus-vector relationship. The relationship is either non-persistent, semi-persistent or persistent (Bedford *et al.*, 1994; Wisler *et al.*, 1998; Sim *et al.*, 2000; Johnson *et al.*, 2002). The viruliferous vectors are then transferred to healthy indicator plants in cages for inoculation feeding for 3-4 hours (Cohen and Duffuss, 1989; Escriu *et al.*, 2000). This method is advantageous in that it helps determine/confirm the vector of a plant virus, allow assay of viruses which are not mechanically transmitted and help obtain information about the mode of transmission of a given virus. The challenge however, is that it is cumbersome rearing the insect and sometimes the insect may fail to transmit the virus to the indicator plant.

Sap or mechanical inoculation on the other hand, involves the application of virus containing plant extracts (i.e. inoculum) to the abraded leaf surface of healthy plants (Green, 1971; Garnsey, 1974). The mechanical application of the virus is done using a spray gun, soft brush or stroking the plant with fingers. In this method, the indicator plants react by forming localized lesions on the inoculated leaves or by showing systemic symptoms on new leaves within a week or so (Lister, 1959; Garnsey, 1974).

In addition to the vector and mechanical inoculation, some indicator plants are best inoculated using the graft method. This method allows vascular union to form between the stock and the scion hence facilitating virus movement from the test plant into the indicator plant. This method has successfully been used in virus indexing of many plants including

sweetpotato. However, grafting is time consuming but is less laborious for a few plants and more sensitive than some techniques like enzyme linked immunosorbent assay (ELISA). Although the technique is in most cases limited to closely related plants, some distantly related species, for example, of solanaceous plants like potato, tomato and thorn apple are graft compatible (Nauriyal *et al.*, 1958; Ashkennazi, 1974; Schneider, 1978). Overall, inoculation of indicator plant as a method of virus detection is slow, requiring a long time period for symptoms to show. In addition, symptom expression is influenced by factors such as environment and virus strains (Lister, 1959; Garnsey, 1974) and it is important to keep the plants long enough to allow the symptoms to appear. However, it can be very sensitive and indicator plants are often used as the first of a series of tests.

2.5.3 Electron Microscopy

Electron microscopy (EM) provides very useful information on the morphology of the virus particles and is commonly used for virus detection when EM facilities are readily available (Baker *et al.*, 1985; Milne, 1993). Many plant viruses induce distinctive intracellular inclusions, or develop large crystalline accumulations of virus particles, and their detection by EM can provide a simple and rapid test to confirm viral infection (Edwardson *et al.*, 1993). Because of the uniqueness of inclusions produced as a result of infection by some viruses, unknown viruses can sometimes be identified to the genus level. Plant virus inclusion technology, however, requires extensive hands-on experience and is seldom used by the novice for routine viral disease identification. Viruses that occur in low concentrations in plant sap are not easily seen unless the virus in the test material is concentrated before visualization. The efficiency of virus visualization can be improved in combination with serology. Also EM is labour intensive and expensive; therefore it cannot often be used for the rapid processing of multiple samples. Many agricultural research institutions cannot afford to have an electron microscope due to the prohibitively high costs involved in installation and maintenance of the facility.

2.5.4 Serology

Serological techniques, which include enzyme-linked immunosorbent assay (ELISA) (Clark and Adams, 1977), tissue blot immunosorbent assay (TBIA) (Lin *et al.*, 1990) and lateral flow devices (Tsuda *et al.*, 1992), are powerful tools for the detection of plant viruses. These techniques are based on an antigen-antibody binding reaction between epitopes on the surface of virus particles and the binding sites of specific antiviral antibodies (Van Regenmortel,

1982). An antigen, which usually consists of purified virus nucleoprotein particles, is injected into an appropriate animal for example, a rabbit or mouse, to induce the production of antibodies (Bazin, 1982; Nikolaeva *et al.*, 1995; Abouzid *et al.*, 2002; Meng *et al.*, 2003). Two types of antibodies can be made: polyclonal antibodies that consist of a population of antibodies that bind to different regions of the antigen protein (Ball, 1974), and monoclonal antibodies that consist of one type of antibody that binds to one specific region on the antigen protein (Jordan and Hammond, 1991).

Serology provides a quick method and the procedure is relatively simple. However, serological techniques are limited mainly to detecting virus coat protein, and coat proteins are prone to degradation by proteases in sample material when not kept under special conditions (-80°C) for long periods (Torrance and Jones, 1981). Viroids consist of naked RNA unencapsidated by a protein coat (Diener, 1971; Tsagris *et al.*, 2008) and cannot be detected serologically. Again if the virus titre is very low, a serological technique may give false negative results (Owens and Diener, 1981; Esbenschade and Moyer, 1982; Candresse *et al.*, 2000). Furthermore, serology is less effective where changes such as recombination occur in the coat protein of viruses e.g. in geminiviruses (Garrido-Ramirez *et al.*, 2000; Pita *et al.*, 2001) and cannot be used to identify previously un-described pathogens. The commonly used serological techniques are discussed below

2.5.4.1 Immunosorbent electron microscopy (ISEM)

This technique was introduced by Derrick (1973) as “serologically specific electron microscopy” and has become widely used in plant virology (Milne, 1991). ISEM combines the specificity of serological assays with the visualization capabilities of electron microscopy. Virus particles are selectively “trapped” on antibody-coated grids with little contaminating host-plant material. Hence, the technique is more sensitive for detecting viruses and can also be used to estimate the degree of serological relationship between viruses. ISEM has been used in detecting sweetpotato viruses. For example, Zhao *et al.* (1995) used ISEM to detect SPFMV while Yang *et al.* (1998) used both of these methods to detect SPFMV, SPLV and TMV. Although ISEM is a sensitive technique, it has the same drawbacks as electron microscopy. Nonetheless, it is ideal for confirmatory tests using small number of samples, if the EM facility and specific antisera are available.

2.5.4.2 Enzyme linked immunosorbent assay

Enzyme linked immunosorbent assay (ELISA) involves use of nitrocellulose membrane or micro titre plate as support for the reagents used in serological reaction. The method is rapid, simple (all steps are or can be done at room temperature), economical in use of antisera and relatively sensitive as it can detect as little as 10 ng of virus. The technique is suited to testing large numbers of samples (Hammond *et al.*, 1992) and can be used simultaneously to detect and quantify the virus by observing and measuring the intensity of colour development. By using different antibodies in separate micro titre plates, it is possible to simultaneously test plants for different viruses (Hammond *et al.*, 1992; Ndowora and Lockhart, 2000; James *et al.*, 2006). Also, using a mixture of specific antibodies, several viruses can be detected (but not distinguished) at the same time using the same membrane or micro titre plate for example, for phytosanitary purposes. However, ELISA has got some limitations. Antibodies that can detect SPFMV, SPMMV and SPCSV are readily available but antibodies for the detection of some viruses like SPLCV are not yet developed. The technique is less sensitive than some techniques such as PCR (Hu, 1995) especially if the virus titre in the plants is low. ELISA also requires polyclonal or monoclonal antibodies specific for each virus of interest that do not cross react with plant proteins (Hammond *et al.*, 1992; Abouzid *et al.*, 2002). For sweetpotato viruses, three different ELISA methods i.e. nitrocellulose membrane (NCM), double antibody sandwich (DAS) and triple antibody sandwich (TAS) ELISA have been mostly used and are discussed below.

Nitrocellulose membrane (NCM) ELISA. This is a direct immune enzymatic test that uses nitrocellulose membrane as support for the reagents used in serology. This method uses two antibodies; one to find the virus and another, tagged with an enzyme, to detect the virus. In NCM-ELISA, sap or semi-purified virus is first spotted onto the nitro-cellulose membrane and allowed to dry for 30 minutes. The blotted membrane can then be kept at room temperature for future analysis allowing samples to be taken to different laboratories to continue the detection. Remaining absorption sites are then blocked with a high protein solution. The membrane is then, following CIP protocol, probed with the first virus specific antibody (IgG) for reacting with the antigen (Priou, 2001) followed by anti-rabbit antibody which finds the first antibody. Its sensitivity is comparable to that of DAS-ELISA yet it is simpler to perform than DAS-ELISA and TAS-ELISA (Banyamandhub-Munbodh, 1997). Unlike with DAS- and TAS-ELISA, after adding the substrate, NCM-ELISA positive reactions stored at room temperature remain stable for years. For positive reactions, colour

development is proportional to the amount of virus in the sample thus allowing some virus quantification (Priou, 2001). However, NCM-ELISA is generally less reliable than DAS- and TAS-ELISA for both detection and quantification of the virus because colour intensity is measured by visual observation. So far CIP has developed NCM ELISA for detecting only 10 common viruses including SPFMV, SPMMV, SPLV, SPCFV SPMSV, C-6 virus, SPCSV, SPCaLV, *Sweet potato virus G* (SPVG), and *Cucumber mosaic virus* (CMV) and yet over 35 viruses are known to infect sweetpotato implying that many viruses go undetected.

Double antibody sandwich (DAS) ELISA. This method uses micro-titre plates as a support for reagents instead of a nitrocellulose membrane. DAS-ELISA first involves the use of polyclonal IgG to coat the plate, and then the test samples are applied. This is followed by the anti-virus conjugate (IgG-AP) diluted in a high protein conjugate buffer and then by an appropriate substrate for colour development. Results are assessed by visual inspection and spectrophotometric measurement of absorbance (Clark and Adams, 1977).

Triple antibody sandwich (TAS) ELISA. Like DAS ELISA, it uses micro-titre plates as a support for reagents instead of a nitrocellulose membrane. This method uses three antibodies: one to trap, one to find the virus and another tagged with an enzyme to detect the virus. With TAS-ELISA, polyclonal IgG coating antibody is used to coat the plate followed by the test samples. However, this is followed by a monoclonal IgG antibody (detecting antibody diluted in a high protein conjugate buffer) prepared in a mouse against the antigen (virus). This is followed by an antibody against the mouse conjugated with alkaline phosphatase raised in a mouse or a goat (IgG-AP diluted in a high protein conjugate buffer) that 'finds' the anti-antigen mouse antibody (or Mab above) (Clark and Adams, 1977). The substrate is added and, like the DAS-ELISA, results are assessed by spectrophotometric measurement of absorbance (Clark and Adams, 1977). The results are semi-quantitative. This method is more sensitive than NCM and DAS ELISA (Hobbs *et al.*, 1987; Hammond *et al.*, 1992; Stipkovits *et al.*, 1993) but has only been developed for a few viruses.

2.5.5 Nucleic acid method for detecting plant viruses

This method targets either DNA or RNA for virus detection. The advantage is that, any region of the viral genome can be targeted to develop the diagnostic test. In addition, this method can be used to detect viroid and satellite RNA that lack coat protein and therefore cannot be detected using serology. Techniques such as molecular hybridization, polymerase

chain reaction (PCR), quantitative real-time PCR (qPCR), rolling-circle amplification (RCA) and deep-sequencing of small RNAs (siRNA) have emerged as powerful methods in the identification and characterization of viruses infecting sweetpotato (Colinet *et al.*, 1998; Valverde *et al.*, 2004; Clark *et al.*, 2012). These techniques show great promise and may circumvent the problems associated with serological and biological indexing (Colinet *et al.*, 1998) but the limitation is that they are expensive in terms of equipment and reagents used.

2.5.5.1 Molecular hybridization

In these assays, a single-stranded complementary nucleic acid (either DNA or RNA), which has been “labelled” with a reporter molecule is used as a probe to hybridise with the target nucleic acid. The double-stranded probe-target hybrid molecules are then detected by several methods, depending on the reporter molecule used. The dot- or spot-blot hybridization assay is a commonly used technique in plant virus diagnostics (Maule *et al.*, 1983; Garger *et al.*, 1983; Rosner *et al.*, 1986; Baulcombe and Fernandez-Northcote, 1988). Nucleic acid spot hybridization involves immobilizing DNA or complementary DNA (cDNA) on a nylon membrane. The nucleic acid is then probed with a labelled sequence by molecular hybridization (Cox and Singer, 2004), different methods being used afterwards depending on the label which can either be DNA or RNA probes. Unlike DNA probes, single-stranded RNA probes can hybridize with the target sequence without re-annealing and RNA-RNA hybrids are more stable than DNA-RNA hybrids. However, the potential risk of degradation of RNA probes due to RNAase contamination during hybridization and high costs of generating such probes make the use of DNA probes more common in virus detection assays (Cox and Singer, 2004).

Nucleic acid hybridization, procedures have been developed for detection of several sweetpotato viruses. Probes consist of labelled viral DNA or RNA with radioactive or non-radioactive labels. A method using a SPLCV-specific probe direct-labelled with horseradish peroxidase and detected with chemiluminescence, has been tested with DNA samples from *I. batatas* and other *Ipomoea* species (Valverde *et al.*, 2004b). Strong signals were obtained when tests were conducted using DNA from field (sweetpotato) and greenhouse (*Ipomoea* spp.) grown plants that were infected with SPLCV. Abad and Moyer (1992) developed *in-vitro* transcribed RNA probes for SPFMV detection. The probes were developed using cDNA containing the 3'-terminal region of the capsid protein cistron. The riboprobe was more sensitive than immunological assays because it could overcome interference with host factors that compromise the reliability of immunodiagnostic assays.

2.5.5.2 Polymerase chain reaction (PCR)

PCR is an *in-vitro* method for amplifying target nucleic acid sequences. PCR starts with nucleic acid extraction from the plant tissue by methods such as CTAB (cetyl trimethyl ammonium bromide; Lodhi *et al.*, 1994) and TRizol (Stewart and Via, 1993). These methods are designed to extract DNA and/or RNA. Also, commercial kits for nucleic acid extraction are used commonly for PCR-based detection (Cuellar *et al.*, 2011; Cuellar *et al.*, 2008; Kreuze *et al.*, 2009). However, extraction kits are too expensive for use in large-scale processing of samples. For RNA viruses including SPFMV and SPCSV, a cDNA strand complementary to the virus has to be synthesized first using a reverse transcriptase (RT) enzyme thus the name reverse transcription polymerase chain reaction (RT-PCR). After extraction of the nucleic acid, PCR is carried out in an automated thermal cycler and three steps are involved: (i) denaturation at high temperature (usually 94–95 °C) to separate the two complementary strands of the double-stranded DNA, (ii) annealing of two oligonucleotide primers to their complementary sequences in the opposite strands of the target DNA (annealing temperature depends on the nucleotide composition and length of the primer, usually anywhere between 35 and 65 °C), and (iii) extension of each primer through the target region (usually at 72 °C) using a thermostable DNA polymerase (e.g. Taq polymerase). Each DNA strand made in one cycle will serve as a template for synthesis of a new DNA strand in the next cycle. This results in an exponential increase in PCR product as a function of cycle number. Final results are obtained at the end of the reaction by using a gel to separate the different components, staining using staining reagents like ethidium bromide or gel red and finally visualizing using ultraviolet (UV) light.

The sensitivity of PCR is an advantage for detection of viruses that are unevenly distributed and are present at very low levels in infected plants. For example, SPLCV has been detected from both indicator hosts and sweetpotato by PCR using virus specific and degenerate primers (Onuki and Hanada, 1998; Lotrakul, 2000; Li *et al.* 2004). Also, RT-PCR assay utilizing degenerate primers corresponding to the border sequences of the NIb and the coat protein genes has been used for detection of sweetpotato potyviruses (Colinet *et al.* 1998; Souto *et al.* 2003). Sequencing of PCR products has been useful to determine the taxonomic status of these viruses (Colinet *et al.*, 1998; Mukasa *et al.*, 2003; Souto *et al.*, 2003). Degenerate primers have been developed that can be used to amplify a portion of the gene that encodes the heat shock protein 70 homologue (HSP70) present in all known members of the family *Closteroviridae* (Alicai *et al.*, 1999b). SPCSV has been detected by reverse

transcription PCR (RT-PCR) using HSP70 primers (Alicai *et al.*, 1999b; Sim *et al.*, 2000). PCR with virus-specific primers for the reverse transcriptase encoding region of SPCV and SPVCV allowed rapid detection of caulimovirids (Cuellar *et al.*, 2011).

Accumulation of virus sequence information has enhanced the design of virus taxon-specific primers and probes, specific detection of viruses, verification of the results (Colinet *et al.*, 1998; Souto *et al.*, 2003; Mukasa *et al.*, 2003), and has significantly improved resolution of partially characterized viruses, e.g., SPV2, SPCFV, and SPVC. RT-PCR/RFLP has been used to detect and distinguish strains of SPFMV and SPV2 (Ryu *et al.*, 2002; Tairo *et al.*, 2006; Kwak *et al.*, 2007; Yamasaki *et al.*, 2009; Tugume *et al.*, 2010). Simultaneous detection of SPFMV, SPCSV, and SPMMV is possible by multiplex RT-PCR in a one-step or two-step reaction procedure (Rukarwa *et al.*, 2010; Opiyo *et al.*, 2010). Although PCR-based methods can amplify viruses present in low titres, false negative reactions with known infected plants have been found with potyviruses (Souto *et al.*, 2003) and sweepoviruses (Li *et al.*, 2004; Kokkinos and Clark, 2006b; Wasswa *et al.*, 2011). Sweetpotato contains inhibitory compounds that can affect sensitivity and reliability of virus detection by PCR (Fenby *et al.*, 1998). Thus, an internal standard is needed to ensure the quality of the nucleic acid extracts used in PCR assays. In addition, PCR requires prior knowledge of the viral genome of at least two sections of about 25 nucleotides upon which forward and reverse primers are designed (Gibbs and Mackenzie, 1997).

2.5.5.3 Real time quantitative polymerase chain reaction (qPCR)

Quantitative real-time PCR (qPCR) is a sensitive method for the detection of viruses in plant tissues whereby the amplification of mRNA or coding sequence of a host gene is included as an internal control in the detection of RNA and DNA viruses, respectively (Clark *et al.*, 2012). Sweetpotato genes including the 18S and 26S rRNA genes, the cytochrome oxidase gene, and plant Mitochondrial NADH dehydrogenase (Nad 5) gene have been used as internal control in the qPCR assay to normalize differences in RNA and DNA concentrations between samples. This method has been used in the detection and quantification of SPCSV, SPFMV, SPMMV, SPVG, SPV2, and SPLCV directly from infected sweetpotato plants (Kokkinos and Clark, 2006a; Mukasa *et al.*, 2006; McGregor *et al.*, 2009; Perez-Egusquiza *et al.*, 2009). qPCR has been shown to be more efficient and sensitive in detecting sweetpotato viruses than conventional PCR (Kokkinos and Clark, 2006a). The method has also been improved and modified to enable the detection of three potyviruses (SPFMV, SPVG and

PV2) in a multiplex assay by mixing primers and using probes labeled with distinct fluorophores and also developed generic primers and TaqMan probes to detect all sweetpotato viruses whose sequences were available on GenBank (Ling *et al.*, 2010). qPCR offers a high degree of detection sensitivity and can reduce time, money, and labour expenditure. However, its use in routine virus detection may be restricted by the sequence specificity of the TaqMan probes and primers, as well as the expensive reagents and instruments (Clark *et al.*, 2012).

2.6 Management of sweetpotato viruses

Although there are virtually no antiviral compounds available to cure plants with viral diseases, efficient control measures can greatly mitigate or prevent viral diseases from occurring. Virus identification is a mandatory first step in the management of a viral disease. The subsequent management strategy depends on the means by which a particular virus enters a crop, how the virus is transmitted between plants within a crop, and how the virus survives without the crop (Haddidi *et al.*, 1998). Preventative measures may include use of certified virus-free seed or vegetative stocks, elimination of the virus reservoirs in the surrounding wild vegetation, and modification of planting and harvesting practices. In sweetpotato, however, integrated management practices including chemical, cultural, biological and genetic have been applied to control virus diseases as discussed below.

2.6.1 Use of virus tested planting materials

Use of *in-vitro* techniques through meristem tip culture followed by virus indexing of the generated plantlets using *I. setosa*, and ELISA has been utilized with success to obtain disease and pest free sweetpotato seed stocks (Ying *et al.*, 2004). Virus indexed planting material has been reported to improve yields by about 30-60 % (Karyeija *et al.*, 1998a; Fuglie *et al.*, 1999; Gibson *et al.*, 2004; Clark and Hoy, 2006). For example in China, the cultivation of virus-free cultivars have significantly increased yield ranging from 10.3% to 101.9% with an average increase of 37.9% (Fuglie *et al.*, 1999). In addition to improved yield, farmers in Shandong province in China also reported an annual net benefit of about \$145million from using virus free sweetpotato seed tubers (Fuglie *et al.*, 1999). Use of virus tested planting material allows farmers to grow high yielding and yet susceptible varieties for example in USA, farmers have continued to grow Beauregard which is very susceptible to SPFMV-RC (Bryan, 2002; Clark and Hoy, 2006). Use of virus tested planting material by farmers in Africa particularly East Africa is still limited. Few countries like South Africa have exploited

the technique and their yield average is 40t/ha which is far higher than the yield obtained in East Africa where average yield ranges between 5-10t/ha (Low *et al.*, 2009). This implies that yield potential of sweetpotato in Uganda has not been achieved. Such low yield can be increased through access to clean planting material hence the need to establish virus-free seed stock of high yielding and farmer preferred varieties.

Several methods have been exploited in generating virus free planting materials in vegetatively propagated crops. These can be single or a combination of methods including meristem/shoot tip culture, thermotherapy, chemotherapy, cryotherapy, and tissue culture as discussed below.

2.6.1.1 Meristem tip culture

Sweetpotato was one of the first crops that was subjected to virus eradication with this technique (Loebenstein and Harpaz, 1960; Alconero *et al.*, 1975; Frison and Ng, 1981; Kuo *et al.*, 1985; Green and Lo, 1989) which is based on uneven distribution of viruses in the youngest tissues of the shoot apex. The efficiency of virus eradication depends on the size of shoot tips excised. The smaller the size of meristem excised the greater the likelihood of obtaining a virus-free plant. However, small meristems are more tedious to excise and they regenerate at a lower frequency (Faccioli and Marani, 1998). Furthermore, the distribution of viruses in shoot tips may vary depending on the virus-host combination. Therefore, various sizes of shoot tips must be excised and tested to determine the optimal size range supporting efficient virus elimination and also plant regeneration at a high rate. More robust methods with less dependency on the size of shoot tips would therefore enhance the efforts for virus elimination. Meristem tip culture when combined with thermotherapy increases the chances of virus elimination in many vegetatively propagated crops (Berg and Bustamante, 1973; Wu and Su, 1991; Wasswa *et al.*, 2010).

2.6.1.2 Thermotherapy

This involves keeping plants or more frequently a part of them at temperatures higher than the normal growth temperature but within the physiological tolerance limits of each plant (Panattoni *et al.*, 2013). In sweetpotato for example, the thermotherapy temperature is between 35°C to 40°C. Higher temperatures significantly reduce replication of many plant viruses by disrupting viral RNA synthesis. Thermotherapy can be done either on potted *in vivo* plants or *in vitro* plants before meristem tip excision (Zapata *et al.*, 1995; Cheema *et al.*,

1999). *In vivo* thermotherapy has been found to eliminate *Plum pox virus* (PPV) from plum trees using a temperature of 37°C for 2-3 weeks (Kegler., 1967, 1968). Combining thermotherapy with either meristem tip culture or *in vitro* chemotherapy have been reported to eliminate complex viruses of PPV, *Prunus necrotic ringspot virus* (PNRSV) and *Prune dwarf virus* (PDV) from four plum cultivars (Janečková, 1993; Manganaris, *et al.*, 2003). *In vitro* thermotherapy alone is also sufficient for elimination of some viruses; *African cassava mosaic virus* (ACMV) was eradicated from cassava when *in vitro* plants were kept at 37°C for 6 weeks under a 16 hours light and 8 hours dark period (Kaiser and Teemba, 1989). Similarly, *Cassava brown streak virus* (CBSV) was eradicated from meristem tip derived plantlets of *in vitro* cassava plants heat treated at 36°C for 8 hours darkness and 40°C for 16 hours light for a period of 4 weeks (Wasswa *et al.*, 2010). In sweetpotato, thermotherapy at temperature of 32°C for 8 hours of darkness and 36°C for 16 hours of light for period of four weeks has been used to eliminate SPFMV and SPMNV (Panta *et al.*, 2007; Rukarwa *et al.*, 2010) but not SPCSV.

2.6.1.3 Cryotherapy

Cryopreservation is used for long-term preservation of plant genetic resources. Recently, cryotherapy of shoot tips has also been described as an efficient method for elimination of viruses (Brison *et al.*, 1997; Wang *et al.*, 2003, Wang and Perl, 2006; Wang *et al.*, 2008). In this method, shoot tips are excised, frozen in liquid nitrogen, and regenerated to plants following thawing and post culture. When used for cryotherapy, conditions are selected to allow survival of only a limited number of the least differentiated cells and eliminate a large proportion of virus-infected tissues. Therefore, cryotherapy can result in virus-free regenerants with much greater frequency than what is obtained typically with the conventional meristem tip culture (Wang *et al.*, 2008). The shoot tips used for cryotherapy can be relatively large without compromising successful virus elimination, which makes preparation of the materials more feasible (Brison *et al.*, 1997; Wang *et al.*, 2003). To date, cryopreservation has been widely applied to plant species from tropical and temperate regions (Engelmann, 1997; Wang and Perl, 2006). This method has successfully been applied in elimination of nine viruses (Wang *et al.*, 2009; Wang and Valkonen, 2009) including *Plum pox virus* from an interspecific prunus root stocks (Brison *et al.*, 1997), *Cucumber mosaic virus* (Helliot, 2002), *Banana streak virus* from *Musa* spp. shoot tips, *Grape vine virus A* (Wang *et al.*, 2003; Bayati *et al.*, 2011), SPFMV and SPCSV (Wang and Valkonen, 2008b, Wang and Valkonen, 2009), *Potato leaf roll virus*, *Potato virus Y* (Wang and Perl, 2006), and

Raspberry bushy dwarf virus (Wang *et al.*, 2008). This method has also been used to eliminate sweetpotato little leaf phytoplasma (Wang and Valkonen, 2008a) and Huanglongbing bacterium causing “citrus greening” (Ding *et al.*, 2008).

2.6.1.4 Electrotherapy

Electrotherapy involves the application of electrical pulses to eliminate viruses from either *in vivo* or *in vitro* plants. Electrotherapy has been applied to eradicate *Almond mosaic virus* from almond trees (Quacquarelli *et al.*, 1980), *Potato virus X* (PVX), *Potato virus A* and *Potato virus Y* from potato (Lozoya-Saldana *et al.*, 1996; Meybodi *et al.*, 2011) and also *Grape vine virus A* in grape vine (Bayati *et al.*, 2011). Electrotherapy has also been used in eliminating viruses in the genera Potyvirus, Luteovirus and Carlavirus from garlic, sugarcane, potato, and *Araceae* crops, respectively (Hernandez *et al.*, 1997). The efficiency of electrotherapy has previously been investigated on *Banana streak virus* (BSV)-infected banana plants and 40-80% of plants were sanitized (Hernandez *et al.*, 2002). This method has not been explored in elimination of sweetpotato viruses hence need for research in this area.

2.6.1.5 Chemotherapy

Chemotherapy involves use of antimetabolite chemicals such as ribavirin, 5-Azacytidine, and 3-Deazauridine that block the virus nucleic acid synthesis/replication. Chemical treatment is either done directly by spraying *in vivo* plants or indirectly by adding these chemicals to media of *in vitro* plants (Bittner *et al.*, 1987; De Fazio, 1987; Toussaint *et al.*, 1993). Ribavirin has been successfully used to eliminate viruses belonging to 9 families including *Closteroviridae*; *Comoviridae*; *Flexiviridae*, *Betaflexiviridae*, *Bromoviridae*, *Potyviridae*, *Secoviridae*, *Alphaflexiviridae*, and *Geminiviridae* from crops like grapevine, apple, plum, bamboo, black currant, citrus, fragaria, potato, orchid, tobacco and many others (Panattoni *et al.*, 2013). In sweetpotato however, not much has been achieved by this method to eliminate sweetpotato viruses hence need for further investigation.

2.6.2 Selection and use of symptomless sweetpotato planting material

Farmers in Uganda and East Africa at large have got the ability to select and grow varieties that have mild or no virus symptoms. Both landraces and station bred varieties like the NASPOT series are grown in farmers' fields (Mwanga *et al.*, 2003, Mwanga *et al.*, 2007; Mwanga *et al.*, 2011). Symptomless vine tips measuring about 30 cm obtained from old crop fields are used to establish new fields. This practice has been supported by the fact that most

of the symptomless sweetpotatoes growing in farmers' fields are actually virus free (Gibson *et al.*, 1997; Mukasa *et al.*, 2003; Ateka *et al.*, 2004; Aritua *et al.*, 2006) and those infected have ability to revert to healthy status (Gibson *et al.*, 1997; Wasswa, 2012). Unfortunately, this approach can lead to the perpetuation of plants with high incidence of unrecognized single infections (Mukasa *et al.*, 2003; Rukarwa *et al.*, 2010) with viruses such as SPCSV and SPFMV that then serve as sources of inocula leading to SPVD development. In addition viruses like *Sweet potato leaf curl virus* has been reported in Uganda yet most cultivars do not show symptoms (Wasswa *et al.*, 2011); this could be one of the contributors of low yield. The abundance of alternative weed hosts around sweetpotato fields also poses a challenge for maintaining healthy seed stock in farmers' fields.

2.6.3 Phytosanitation practices

This aims at preventing introduction of new infections and or reducing sources of virus inocula among which includes, isolation which can be achieved by planting new field at least 15-20 meters away from the old one (Aritua *et al.*, 2003, Gibson *et al.*, 2004). However, such practice may be difficult to introduce because of the tradition of growing crops in small plots around homesteads (Karyeija *et al.*, 1998a). The alternative means of isolation is by planting non host barrier crops like maize around the field (Aritua *et al.*, 2003; Gibson *et al.*, 2004).

Another phytosanitation practice is by reducing virus sources. This can be achieved through roguing of infected plants 1 month after planting, eliminating wild *Ipomoea species* and by destroying crop residues. Since, spread of SPVD is short distant and dependent on the amount of inocula in the surroundings (Aritua *et al.*, 1999), roguing is very advantageous in areas where small sweetpotato plots are planted close to each other, a common practice in central Uganda. For this method to be effective, infected plants should be removed from the field within one month after planting (Gibson *et al.*, 2004).

2.6.4 Breeding for resistance and use of resistant cultivars

Resistant cultivars have provided one of the most successful approaches to the control of pathogens of many crops, especially those which cannot be controlled by other means. However, continuous plant breeding programs are often necessary to overcome the effects of and or the development of new races/strains of pathogens and breakdown of host resistance. Growers therefore need to be aware of the availability of new cultivars and be able to evaluate their potential in relation to other agronomic characteristics. In sweetpotato,

resistance genes can be introduced through conventional breeding or by genetic transformation. However, several characteristics of sweetpotato make it difficult to breed for resistance (Mwanga *et al.*, 2002; Mcharo *et al.*, 2005). The crop is a highly heterozygous allohexaploid ($2n = 90$) with complex segregation ratios and many traits are inherited quantitatively. Self-incompatibility also makes it time consuming to introgress desirable traits.

Breeding for SPVD resistance was initially focused on developing cultivars resistant to SPFMV, given its universal distribution. However, in Uganda many sweetpotato cultivars especially landraces are naturally resistant to SPFMV, showing no or only mild initial symptoms, from which they usually recover, and containing very low virus titres (Esbenshade and Moyer, 1982; Green *et al.*, 1988; Abad and Moyer, 1992; Gibson *et al.* 1998; Mwanga *et al.* 2002; Kokkinos and Clark, 2006b). These landraces are low yielding and can only produce acceptable yield after a very long period of growth in the field and they have other undesirable traits (Gibson *et al.*, 2000). In addition, their resistance is lost when the varieties are co-infected with SPCSV (Karyeija *et al.*, 1998a; Mwanga *et al.*, 2002). Due to the ability of SPCSV to break down resistance and also to cause significant yield decline by itself (Gutierrez *et al.*, 2003), Kreuze, (2000) recommended breeding for SPVD resistance be directed towards developing varieties resistant to this virus.

Two separate recessive genes that control resistance for SPFMV and SPCSV were reported and their heritability was moderate to high (Mwanga *et al.*, 2002). With this knowledge, resistant cultivars like NASPOT 1 and NASPOT 11 with high yield and other desirable attributes have been bred through participatory breeding in Uganda and farmers have adopted them (Mwanga *et al.*, 2003; Mwanga *et al.*, 2011). Some of the local landraces that are high yielding and resistant to viruses have also been released (Mwanga *et al.*, 2003; Mwanga *et al.*, 2007). These resistant cultivars are not totally immune to infection but are less frequently infected and the symptom severity, virus titres and yield loss are low and when infected they can revert to a healthy state (Gibson *et al.*, 1997). However, these varieties may be resistant to one or a few viruses and susceptible to others and therefore, breeding for virus resistance might require genes from several source plants hence delaying and complicating the process. Breeding for resistance may also eventually give rise to more severe virus isolates (Van den Bosch *et al.*, 2006; van den Bosch *et al.*, 2007).

Other sources of resistant genes have been reported in wild *Ipomoea* species, which in the future can be used to improve resistance to SPVD and other virus strains. Wild *Ipomoea* species that are resistant to single infection by SPFMV, SPCSV and combination of the two viruses were identified (Karyeija *et al.*, 1998b). However, accessions of these wild species need to be studied to verify whether or not this was an aspect of non-host resistance (Heath, 2000). In addition, most wild species are diploid whereas sweetpotato is a hexaploid complicating the process of incorporating resistance from the wild species through cross breeding (Diaz *et al.*, 1996). Wild plants also contain numerous undesirable traits. Apart from all that, conventional breeding is laborious and requires a lot of time (Jennings, 1957). Each generation takes a minimum of whole sweetpotato growing season and a series of backcrosses are needed to remove the undesirable characteristics (Wilson *et al.*, 1989; Morales, 2001).

2.6.4.1 Sweetpotato recovery and reversion to healthy from virus infection

Some vegetatively propagated crops have the ability to suppress virus multiplication or inhibit their spread resulting into symptomless plants a phenomenon known as recovery. The cutting from a recovered portion may grow without virus and this is called reversion. Recovery and reversion were observed in cassava when symptomatic plants infected with *African cassava mosaic virus* started to produce new leaves without symptoms (Storey and Nichols, 1938). In sweetpotato, it would be expected that asymptomatic viruses like SPFMV or SPCSV and or other viruses should accumulate to infect 100% of field plants given: (i) field plants are continuously subjected to inoculation by vectors like aphids and whiteflies and, (ii) most single virus infections are largely asymptomatic in sweetpotato and selection by farmers of diseased free material cannot select against such viruses. However, this is not the case in sweetpotato because some varieties possess the capacity to free themselves from virus infection. Ugandan cultivars like Tanzania and New Kawogo have been reported to recover from SPFMV infection (Aritua *et al.*, 1998b; Wasswa, 2012) while some landraces like Munyeera can also recover from SPVD infection (Gasura *et al.*, 2009). In all the recovery/reversion studies done so far, the focus has been on SPFMV and is limited only to vines materials. It's not clear whether plants infected by SPCSV alone can revert to healthy. This requires further investigation. Also whether sprouts from infected root can recover and or revert from infection is not known. In addition, recovery from virus infection seems to be influenced by environment; in this study, recovery was thus compared in two agro-ecologies.

2.6.4.2 Genetic engineering as a means of introducing resistance in sweetpotato

Due to difficulty of breeding for virus resistance in sweetpotato through conventional means, genetic engineering has been sought as an alternative. This method has exploited several genes including virus coat protein genes (Baulcombe, 1996; Beachy, 1997; Fraser, 1998; Savenkov and Valkonen, 2001; Cipriani *et al.*, 2001; Okada *et al.*, 2001), Rice cysteine proteinase inhibitors (Gutierrez-Campos *et al.*, 1999), and RNA silencing (Kreuze *et al.*, 2008). One of the attempts of genetic transformation involved East African cultivars with the coat protein gene from the rosette crack strain to provide cross protection against the East African strain of SPFMV. When finally evaluated in the field, the resistance observed in the green house broke down, probably because the transgene was not from a locally prevalent SPFMV strain and/or because the plants became infected with SPCSV. Coat protein mediated resistance to SPFMV was also achieved by Okada *et al.* (2001), although durability of the resistance in the field was not reported.

Because SPCSV can cause yield loss on its own, and because it synergizes with most other sweetpotato viruses, it has been suggested that efforts to develop SPVD resistance in sweetpotato should target SPCSV (Tairo *et al.* 2005; Aritua *et al.* 2007). As a result, genetic engineering strategies to generate virus resistance in sweetpotato to both SPFMV and SPCSV have been tested. Rice cystein proteinase inhibitor (*OCI*) mediated resistance to potyviruses is thought to act through inhibiting the viral cystein proteinase NIa that processes the potyviral polyprotein (Gutierrez-Campos *et al.*, 1999). Closteroviruses also encode cystein proteinases to modify some of their proteins, and it was considered that expression of *OCI* in transgenic plants might confer resistance to both SPFMV and SPCSV. Cipriani *et al.* (2001) reported increased resistance to SPFMV in plants of cv. Jonathan transformed with *OCI*, but the resistance was not efficient and typical symptoms of SPVD developed in plants infected with SPCSV and SPFMV. RNA silencing has also been used to target both SPFMV and SPCSV simultaneously. A construct was designed to produce transcripts that generated a double-stranded RNA structure homologous to the polymerase genes of each virus, thereby efficiently inducing RNA silencing defence system against both viruses (Kreuze *et al.*, 2008). Many transgenic lines accumulated only low titres of SPCSV and no symptoms developed. These results showed that sweetpotato could be protected against the disease caused by SPCSV using RNA silencing. Nevertheless, the low concentrations of SPCSV in the transgenic plants were still sufficient to break down the high levels of resistance to SPFMV, and SPVD developed.

2.6.5 Chemical control

No chemical substances (viricides) are yet available for controlling virus diseases of plants in the field, although some compounds, e.g., ribavirin, applied as a spray or injected into the plant reduce symptoms drastically and may eliminate the virus from the treated host plant (Agrios, 2005). Foliar application of certain growth-regulating substances, such as gibberellic acid, may also overcome the stunting induced by some viruses and may stimulate the growth of suppressed axillary buds in virus infected trees.

CHAPTER THREE

SWEETPOTATO CULTIVAR DEGENERATION RATE UNDER HIGH AND LOW SWEETPOTATO VIRUS DISEASE PRESSURE ZONES IN UGANDA

3.1 Introduction

Sweetpotato (*Ipomoea batatas* L.) is a vegetatively propagated crop. In tropical countries like Uganda, vine cuttings, 20-30 cm in length, from previous crop or volunteer plants, are usually used as source of planting material (Bashaasha *et al.*, 1995; Gibson *et al.*, 2009). With repeated propagation, crops can accumulate systemic pathogens especially viruses during field exposure to viruliferous vectors such as aphids and whiteflies. Some of these viruses such as *Sweet potato feathery mottle virus* (SPFMV: family *Potyviridae*; genus *Potyvirus*) induce mild symptoms so crops may appear healthy even when they are infected. Pathogen accumulation together with mutations if not checked, may over time result in cultivar degeneration (Bryan *et al.*, 2003; Clark and Hoy, 2006; Clark, 2007; Lewthwaite *et al.*, 2011). Many vegetatively propagated crops including perennial tree crops, ornamentals, and herbaceous field crops plants like potato can become completely virus infected and degenerate unless virus-tested seed schemes are introduced (Walkey, 1991). In countries like China, USA and South Africa, sweetpotato cultivar decline has been reported in commercial varieties. This has led to expensive cleaning programmes and continual release of cleaned stocks (Fuglie *et al.*, 1999).

In Uganda, some of the once elite high yielding varieties have been abandoned by farmers due to decline in yield (Gibson *et al.*, 1998; Mukasa *et al.*, 2006; Gasura *et al.*, 2009), and new varieties including NASPOT 1, Vita, Kabode and others have been bred and adopted by farmers (Mwanga *et al.*, 2003; Mwanga *et al.*, 2007, Mwanga *et al.*, 2011). However, these new released varieties may themselves degenerate and inturn some farmers in Uganda still prefer to grow landraces. These may be resistant and appear to revert to healthy status when infected (Gibson *et al.*, 1997). Unfortunately, these landraces are low yielding and are not early maturing (Gibson *et al.*, 2004).

Among the viruses so far reported in Uganda, SPFMV and *Sweet potato chlorotic stunt virus* (SPCSV: family *Closteroviridae*; genus *Crinivirus*) are the most prevalent (Aritua *et al.*, 2000; Mukasa *et al.*, 2003) and co-infection results in sweet potato virus disease (SPVD) (Gibson *et al.*, 1998). This causes over 90% yield loss in susceptible varieties (Mukiibi, 1977;

Karyeija *et al.*, 1998b; Aritua *et al.*, 2000). In Uganda, SPVD is more prevalent in areas around Lake Victoria and the rift valley in the west where rainfall is more evenly spread throughout the year and low incidence regions are found in the eastern and northern districts where there is a prolonged dry season (Mukasa *et al.*, 2003; Aritua *et al.*, 2007). There was thus a need to investigate whether sweetpotato degeneration could be faster in regions with more SPVD and slower in regions with less SPVD pressure since there is speculation that degeneration occurs in both regions. Other viruses that have been reported in Uganda include *Sweet potato mild mottle virus* (SPMMV: family *Potyviridae*; genus *Ipomovirus*), *Sweet potato caulimo like virus* (SPCalV: family *Caulimoviridae*; genus *Cavemovirus*), *Sweet potato chlorotic fleck virus* (SPCFV: family *Flexiviridae*; genus *Carlavirus*) (Aritua *et al.*, 2000; Mukasa *et al.*, 2003; Aritua *et al.*, 2007; Ndunguru *et al.*, 2009) and of recent *Sweet potato leaf curl Uganda virus* (SPLCUV: family *Geminiviridae*; genus *Begomovirus*) (Wasswa *et al.*, 2011). Single infection by any of these viruses causes mild or no symptoms in infected plants and therefore farmers cannot recognize and select against the affected plants as they do for SPVD. Infected material may hence be maintained and result in cultivar degeneration. This study was designed to determine the rates of degeneration due to natural virus infection of a landrace, research station bred and exotic cultivars under two agro-ecologies with different SPVD pressure.

3.2 Materials and methods

3.2.1 Planting material

Four sweetpotato cultivars were used in this study: i) Beauregard which is exotic cultivar from USA. Its roots are orange fleshed, and is very susceptible to SPVD. This cultivar has been used in most of the degeneration studies conducted so far (Bryan *et al.*, 2003; Clark and Hoy, 2006). ii) Dimbuka which is a land race, with cream root flesh and moderately susceptible to SPVD. This variety is grown in all sweetpotato growing regions of Uganda (Mwanga *et al.*, 2007). iii) Cv. Ejumula is a land race with orange root flesh and susceptible to SPVD. It is also widely grown in Uganda (Mwanga *et al.*, 2007). iv) NASPOT 1 is a research station bred cultivar with white flesh root and resistant to SPVD. It has been widely adopted by farmers in most sweetpotato growing areas of Uganda (Mwanga *et al.*, 2003).

Vine cuttings of symptomless sweetpotato plants of cultivars, Dimbuka, NASPOT 1 and Ejumula were collected from sweetpotato fields at the National Crops Resources Research

Institute (NaCRRI), Namulonge in Wakiso district, central Uganda and grafted onto plants of the nearly universal sweetpotato virus indicator species, *Ipomoea setosa*. The grafted plants were monitored for five weeks for virus symptoms. Virus infection status was further confirmed using nitrocellulose membrane enzyme link immuno-sorbent assay (NCM ELISA). Healthy scions were thus selected and grown in plastic pots containing a mixture of sterilized soil, sand and animal manure in equal volumes in an insect proof screenhouse at NaCRRI. Cultivar Buregad was obtained as virus indexed tissue culture plantlets from root crops research program at NaCRRI and potted as above. Cuttings for field planting were multiplied through repeated two node cuttings in the same screenhouse.

3.2.2 Study site and experimental design for the field trials

This study was conducted in two major sweetpotato growing regions in Uganda: at Makerere University Agricultural Research Institute Kabanyolo (MUARIK) located in Wakiso district in the central region representing an area with a high SPVD incidence, and at the National Semi Arid Resource Research Institute (NaSARRI), Serere district in the eastern region, representing an area with a low SPVD incidence. The two sites were selected based on earlier survey reports by Mukasa et al. (2003) and Aritua et al. (2007). Both surveys reported high SPVD incidence around Lake Victoria region of which MUARIK is part and low incidence in the eastern region of Uganda of which NaSARRI is part. Five and four field consecutive trials were conducted in MUARIK and NaSARRI respectively as shown in Table 2.

Table 2: Sweetpotato planting material used in each trial in different seasons at MUARIK and NaSARRI

Trial	Generation planted	Planting seasons in the different sites	
		MUARIK	NaSARRI
1	G1	2011A	2011A
2*	G1, G2	2011B	2012A
3	G1, G2, G3	2012A	2012B
4	G1,G3,G4	2012B	2013A
5	G1, G2, G4, G5	2013A	

G1 = Generation1, G2 = Generation2, G3= Generation3, G4= Generation4, G5= Generation5. *The difference in planting dates between NaSARRI and MUARIK from 2nd field trial is because one field trial was lost due to drought in NaSARRI and had to be repeated in the subsequent season and this changed the planting dates in the subsequent seasons. A= planting started in April, B= planting started in September

The first field trial consisted of plots of four virus-free sweetpotato cultivars Dimbuka, Ejumula, NASPOT 1 and Beauregard. In subsequent field trials, a farmer practice of obtaining symptomless planting material from the previous mature crop was used up to the fifth generation of planting as an additional treatment. Thus the second field trial consisted of cuttings from each original field (G2) and fresh virus free cuttings from the screenhouse (G1) as a healthy control. The third trial consisted of cuttings from G2 and G1 of second trial forming G3 and G2 respectively, and healthy cuttings from the screenhouse (G1). Trial 4 consisted of cuttings from G3 and G2 in the third trial forming G4 and G3, respectively, and this was planted along with G1 from the screenhouse while the fifth trial consisted of G1, G2, G4 and G5. The same cultivars were maintained throughout the series of experiments except for Beauregard which was dropped from the 2nd trial onwards because SPVD was so wide spread and severe that no asymptomatic planting material could be retained. The experiment was arranged in a randomized complete block design (RCBD), replicated three times. In each block, each of the above generations of each cultivar was planted on 10 mounds each 1 m² and 0.4 m high. Each mound was planted with three vine cuttings of about 30 cm long. New Kawogo, a virus resistant cultivar and free of known viruses was planted round the field and between blocks to act as a guard row. Weeding was done using a hand hoe two to three times depending on the intensity of weeds.

3.2.3 Rate of virus re-infection of sweetpotatoes under field condition

Data on virus incidence and severity were collected monthly for a period of four months starting one month after planting. Incidence was based on the number of cuttings showing virus symptoms in each plot while disease severity was based on visual estimation of diseased plants per mound as manifested by different symptoms on a scale of 1 to 5 where 1 = plants showing no symptoms; 2 = virus symptoms just starting to appear and this can be as mild chlorotic spots on the older leaves or mild vein clearing or mild purpling at the margin of mature leaves; 3 = the symptoms above enlarge and become more visible; 4 = infected plants showing severe disease symptoms including leaf purpling, leaf chlorosis and leaf shape starts to get distorted; and 5 = infected plants showing very severe virus disease symptoms including total distortion in leaf shape, stunted growth, mosaic, leaf chlorosis and sometimes complete death of infected plant (Hahn *et al.*, 1981).

3.2.4 Serological detection of viruses from field plants

Serological detection of virus was carried out by NCM- ELISA according to the protocol

obtained from CIP, Lima, Peru. Samples were collected from symptomatic leaves or a leaf from the basal, middle and upper part of symptomless plants. At least eight samples per generation of each cultivar per trial were tested for 10 viruses for which antibody were available i.e. SPFMV, *Sweet potato mild mottle virus* (SPMMV), *Sweet potato latent virus* (SPLV), SPCFV, *Sweet potato mild speckling virus* (SPMSV), C-6 virus, SPCSV, SPCaLV, *Sweet potato virus G* (SPVG), and *Cucumber mosaic virus* (CMV). The plants were tested at the end of each trial just before harvest. The negative and positive controls were provided in NCM ELISA kit from CIP-Lima, Peru. The presence of virus was judged on the visual intensity of the colour change on the membrane.

3.2.5 Effects of virus infection on sweetpotato storage root yield

Harvesting was done six months after planting using a hand hoe. Each mound per cultivar was harvested separately and its total yield, marketable yield, total storage root number and marketable root number were recorded. Storage roots ranging from 100 to 1,000g were graded as marketable. Small roots and those with defects like rotting were graded as unmarketable. Vine weight was determined by weighing the combined vines of a given generation of a cultivar per plot.

3.2.6 Statistical analysis

Statistical analyses were done using Genstat 13th Edition. Data on virus incidence, severity, storage root yield, and vine weight were subjected to Analysis of variance and the means separated using Fisher's protected least significant difference at 5% probability level. The means of disease incidence and severity of respective generations were obtained from five field trials for MUARIK and four field trials for NaSARRI.

3.3.0 Results

3.3.1 Effects of cultivars on disease symptom development in the two agro-ecologies

In all the field trials, foliar symptoms typical of viruses were observed in all cultivars during the growing season at both MUARIK and NaSARRI. The symptoms that were commonly observed included, chlorotic spots, vein chlorosis, purpling of mature and middle aged leaves, pale green mosaic, stunted growth, leaf narrowing and leaf distortion. One month after planting, few plants developed symptoms at MUARIK (Figure 1) and none developed symptoms at NaSARRI (Figure 1). More plants became symptomatic as time progressed and

by the 4th month after planting, all (100%) Beauregard plants were symptomatic (Figure 1) with disease severity score of 5; followed by Ejumula, least in Dimbuka and NASPOT 1 in both locations (Figure 1). The disease incidence and severity were higher at MUARIK than in NaSARRI (Figure 1).

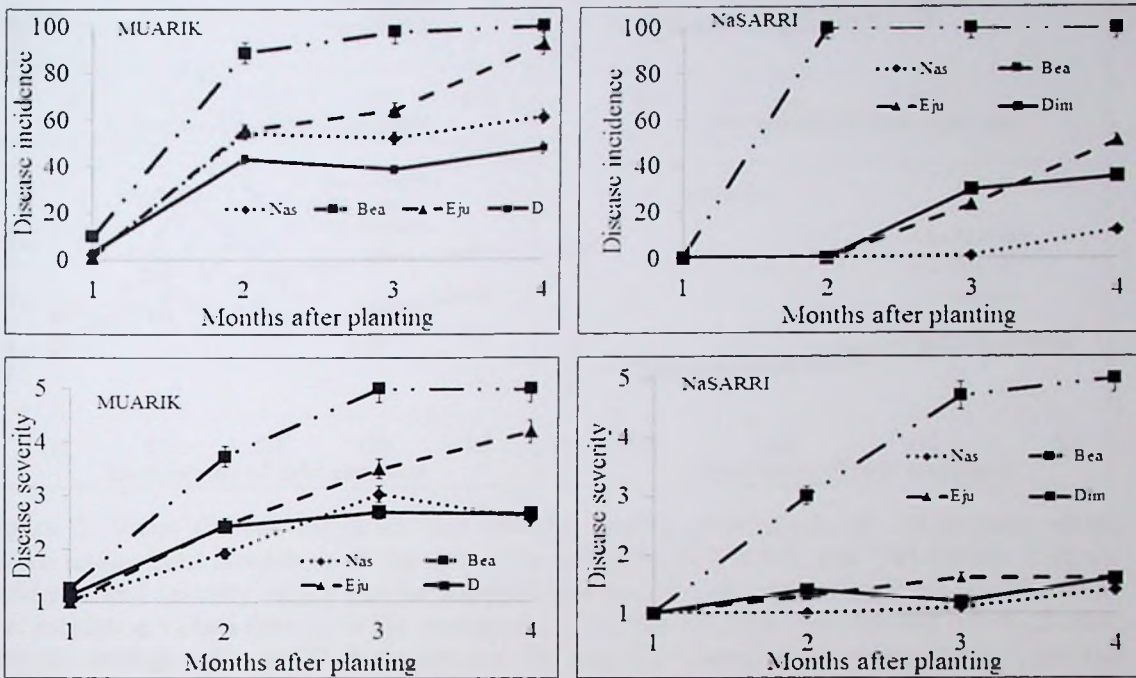


Figure 1: Virus disease incidence and severity with time among different sweetpotato cultivars grown under field condition in the two agro-ecologies (MUARIK and NaSARRI) Figure lengends, Nas= NASPOT 1, Bea= Beauregard, Eju=Ejumula, Dim=Dimbuka

3.3.2 Virus disease incidence and severity among generations of sweetpotato cultivars under two agro-ecologies

In both locations, G1 had less or no disease in the first month after planting as compared to the rest of the generations but as time progressed, incidence increased in all the generations in all cultivars. At MUARIK, cultivar Ejumula showed progressive increase in disease incidence and severity from G1 to G5 while for NASPOT 1 disease incidence and severity reduced in G3 and G4 and then increased in G5. For cultivar Dimbuka, the disease incidence and severity declined in G4 and G5 (Figure 2). At NaSARRI, significant differences ($P \leq 0.05$) were detected in disease incidence among generations with G1 having the least incidence while G4 had more plants showing disease symptoms in all the cultivars (Figure 2). Disease severity scores in NaSARRI were generally low and did not differ among generations (Figure 2).

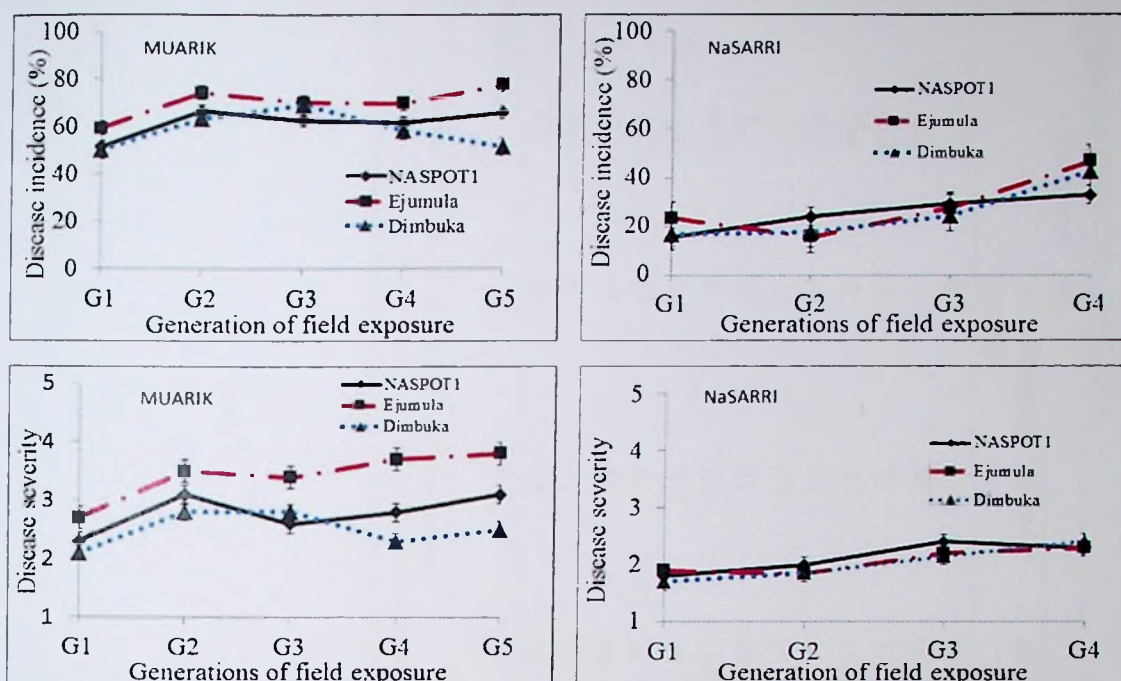


Figure 2: Virus disease incidence and severity among generations of sweetpotato plants grown under field conditions in the two agro-ecologies (MUARIK and NaSARRI). Disease incidence and severity values are the combined means of each generation in all the five trials (i.e. incidence values for G1, is the average of G1 in the 1st, 2nd, 3rd, 4th and 5th field trial, G2= the average value of G2 in the 2nd and 3rd trial, G3 = the average value of G3 in the 3rd and 4th trial, G4= the average value of G4 in the 4th and 5th trial and G5 is the average of G5 in the 5th trial).

3.3.3 Serological detection of viruses infecting sweetpotatoes under field conditions

NCM-ELISA results indicated that, SPCSV followed by SPFMV were the major viruses that infected sweetpotato in the field. Of 328 samples from MUARIK (Table 3), 50% tested positive for both SPFMV and SPCSV, components of SPVD while for NaSARRI trial (Table 4), of 268 samples tested, only 25% had both components. Also samples from NaSARRI that tested positive for both SPFMV and SPCSV did not show typical SPVD symptoms. There were more single infections of SPCSV than of SPFMV in both locations. Also fewer samples from G1 had viruses detected compared to those of G2, G3 and G4 across all cultivars indicating pathogen accumulation in vegetative material. Few samples mildly reacted positive for SPLV, SPMSV, C6, CMV and SPCaLV and were always found in combination with SPVD. None of the samples was positive for SPMMV or SPVG. Almost all the samples obtained from Beaugard at MUARIK had both SPFMV and SPCSV while Dimbuka and NASPOT 1 had the least virus infection detected (Tables 3 and 4).

Table 3: Number of samples and percentage infection by different viruses at different generations of sweetpotato planting material of cvs. NASPOT 1, Ejumula, Dimbuka and Bearegard at MUARIK

Cultivar	Generation	No. of Samples (n=328)	Percentage of plant samples that were positive for each virus					
			SPFMV	SPCSV	SPLV	C6	SPMSV	SPCV
NASPOT 1	G1	54	61	59	1	0	1	0
	G2	16	38	69	0	0	0	0
	G3	16	75	100	0	0	0	0
	G4	8	75	100	0	0	0	0
Ejumula	G1	54	68	61	1	0	0	3.7
	G2	16	69	75	0	6	0	6
	G3	16	81	94	0	6	0	6
	G4	8	88	100	0	0	0	0
Dimbuka	G1	54	30	46	0	0	0	1
	G2	16	44	31	0	6	0	18
	G3	16	50	94	0	0	0	25
	G4	8	75	86	0	0	0	0
Bearegard	G1	46	98	74	2	4	4	8
Total positive samples (%)			63	67	0.9	0.9	0.9	4
								50

G=generation, 1,2,3,4 number of cycles of field exposure of vine cuttings
 G1 samples for NASPOT 1, Dimbuka and Ejumula included samples obtained from 1st field trial, 3rd field trial and 4th field trial, G2 samples were from: 2nd and 3rd field trial, G3 samples were from 3rd and 4th field trial and G4 samples were from 4th field trial. n= the total number of samples tested

Table 4: Number of samples and percentage infection by different viruses at different generations of sweetpotato planting material of cvs. NASPOT 1, Ejumula, Dimbuka and Beauregard at NaSARRI

Cultivar	Generation	Number of samples (n=268)	Percentage of plant samples that were positive						
			SPFMV	SPCSV	CMV	C6	SPMSV	SPCV	SPFMV+SPCSV
NASPOT 1	G1	32	28	47	0	0	13	0	19
	G2	32	53	81	00	0	23	6	31
	G3	24	75	83	8	0	0	0	63
Ejumula	G1	32	41	38	0	0	9	3	13
	G2	32	19	44	0	0	0	0	13
	G3	16	13	44	6	0	0	0	12
Dimbuka	G1	32	28	44	0	0	0	0	22
	G2	32	34	44	0	0	6	0	34
	G3	16	31	44	6	0	19	0	12
Beauregard	G1	16	56	88	0	0	13	31	38
	Total positive		37	53	1	0	8	3	25

G=generation, 1,2,3,4 number of cycles of field exposure of vine cuttings

G1 samples for NASPOT 1, Dimbuka and Ejumula included samples obtained from 1st field trial, 2nd field trial and 3rd field trial, G2 samples were from 2nd and 3rd field trial, G3 samples were from 3rd field trial, n= the total number of samples tested

3.3.4 Effects of virus infection on root yield of cultivars

In the field trials at MUARIK and NaSARRI, differences ($P \leq 0.05$) were detected, among cultivars, and generations, for total yield, marketable yield, total number of storage roots and marketable number of storage roots (Tables 5 and 6). All trials at MUARIK had greater yield and storage root number than those at NaSARRI even though the later had less virus. At MUARIK, NASPOT 1 had greater total and marketable yield followed by Dimbuka, then Ejumula, and least in Beauregard (Table 6) except for the fifth trial where Dimbuka had greater yield than NASPOT 1. In addition, total number of storage roots was more in Dimbuka, followed by NASPOT 1, Ejumula and least in Beauregard while number of marketable storage roots was greater in NASPOT 1 followed by Dimbuka and Ejumula (Table 6). For trials at NaSARRI, total and marketable yield were greater in NASPOT 1 and least in Beauregard in the 1st field trial but in the 2nd, 3rd, and 4th field trials, Dimbuka had more yield than the other cultivars. Total and marketable numbers of storage roots were higher in Dimbuka, followed by Ejumula and least in NASPOT 1 except for the first trial where NASPOT 1 had more (Table 6).

Differences were detected ($P \leq 0.05$) among generations for yield and storage number of roots in both locations in the 3rd, 4th, and 5th trials (Table 5). Over all, G1 had greater total and marketable yield as well as total and marketable number of storage roots in all cultivars in both locations than the subsequent generations except for NASPOT 1 G2 and G4 of trial 1 and 5 at MUARIK that performed better than G1 although these results were not statistically different (Table 6). Based on the overall results for the five generations, there was initial significant yield decline in all the cultivars after the first generation and a relatively constant yield with slight increase from G2 to G4 in both locations as shown in Figure 3.

Table 5: ANOVA for differences in performance of different generations of sweetpotato cultivars grown at MUARIK and NaSARRI for five field trials

Source of variation	d.f	Mean Squares								
		MUARIK				NaSARRI				
		Total yield	Marketable yield	Total root number	Marketable root number	Total yield	Marketable yield	Total root number	Marketable root number	
1 st field trial	Block	2	201.30	130.20	720.8	127.8	13.50	26.50	16.3	49.0
	Cultivar	3	1969.50**	1656.40*	7788.9*	604.6*	154.20*	194.60	388.0*	1008.9*
	Error	6	85.60	61.03	570.3	463.0	8.201	9.215	21.33	30.89
	Total	11								
2 nd field trial	Block	2	98.40	96.54	299.1	606.1	5.056	0.72	5.627	67.4
	Cultivar	2	84.70	200.80*	2622.4*	466.7	32.70*	19.30*	920.2*	267.5
	Generations	1	30.70	35.10	102.7	288.0	15.12	8.68	256.9	107.5
	CXG	2	101.40	149.60	504.1	1122.2	3.50	1.26	183.7	8.2
	Error	10	27.10	34.50	429.4	271.9	4.80	4.40	138.4	76.5
	Total	17								
3 rd field trial	Block	2	32.86	61.00	817.4	227.4	0.58	0.45	105.6	9.1
	Cultivar	2	137.58*	193.4*	6603.4*	532.3*	5.78	2.50	87.2	27.1
	Generations	2	107.53*	90.78*	1302.3	744.7*	14.33*	6.12*	502.7*	95.1**
	CXG	4	12.219	14.72	181.9	25.5	1.44	0.13	76.5	3.59
	Error	16	27.72	25.03	390.8	139.3	1.687	0.63	88.1	6.33
	Total	26								
4 th field trial	Block	2	24.345	33.250	222.4	133.8	128.40	123.60	106.8	191.4
	Cultivar	2	220.18**	310.19**	4194.0**	923.1*	21.94	31.22	756.8*	499.0
	Generations	2	132.60**	216.08**	464.1	3417.4**	354.39**	359.48**	1265.3*	1454.8*
	CXG	4	4.13	4.73	843.4*	30.2	17.96	17.54	108.9	96.4
	Error	16	6.21	7.72	236.2	128.2	10.74	11.43	176.0	210.3
	total	26								
5 th field trial	Block	2	26.59	11.71	1513.4	126.78				
	Cultivar	2	128.72**	113.91**	4739.5**	1047.69**				
	Generation	3	38.13*	33.84*	1319.4*	630.74*				
	C X G	6	12.78	10.82	379.0	107.77				
	Residual	22	5.74	5.83	201.0	71.6				
	Total	35								

CxG= interaction between cultivar and generations, ** and *-significant at P= 0.0001 and 0.05, respectively.

Table 6: Sweetpotato cultivar yield for each field trial and generation in two SPVD pressure zones of MUARIK and NaSARRI

Trials	Cultivar	Generation	MUARIK					NaSARRI				
			Yield (t/ha)		Number of roots		Change in yield (%)	Yield (t/ha)		Number of roots		Change in yield (%)
			Total	Marketable	Total	Marketable		Total	Marketable	Total	Marketable	
1 st field trial	NASPOT 1	G1	72.2 ^a	61.2 ^a	212.0 ^a	123.7 ^a	0.0	20.2 ^a	19.17 ^a	45.7 ^a	44.0 ^a	0.0
	Beauregard	G1	10.7 ^c	4.7 ^c	102.0 ^b	17.3 ^b	0.0	3.0 ^c	0.00 ^c	24.3 ^b	0.0 ^c	0.0
	Ejumula	G1	31.2 ^b	23.5 ^b	188.7 ^a	88.0 ^a	0.0	9.3 ^b	6.00 ^b	42.3 ^a	20.3 ^b	0.0
	Dimbuka	G1	34.7 ^b	28.5 ^b	205.3 ^a	92.0 ^a	0.0	13 ^b	10.17 ^b	50.3 ^a	28.7 ^b	0.0
2 nd field trial	NASPOT 1	G1	19.2 ^a	16.5 ^a	51.3 ^a	36.3 ^a	0.0	7.7 ^a	6.17 ^a	39.0 ^a	25.0 ^a	0.0
		G2	25.8 ^a	24.7 ^a	67.7 ^a	57.3 ^a	-34.0	4.5 ^a	4.33 ^a	18.7 ^a	17.7 ^a	41.3
	Ejumula	G1	18.0 ^a	14.8 ^a	71.3 ^a	42.0 ^a	0.0	7.3 ^a	6.17 ^a	37.7 ^a	25.3 ^a	0.0
		G2	12.5 ^a	9.5 ^b	54.7 ^b ^a	30.3 ^a	30.0	7.2 ^a	5.83 ^a	36.3 ^a	22.7 ^a	2.2
	Dimbuka	G1	21.7 ^a	15.2 ^a	104.3 ^a	46.0 ^a	0.0	11.7 ^a	9.67 ^a	53.7 ^a	36.3 ^a	0.0
		G2	12.7 ^a	3.8 ^b	90.3 ^a	12.7 ^b	41.0	9.5 ^a	7.67 ^a	52.7 ^a	31.7 ^a	18.6
3 rd field trial	NASPOT 1	G1	24.7 ^a	22.3 ^a	69.0 ^a	50.3 ^a	0.0	3.2 ^a	2.7 ^a	18.0 ^a	10.0 ^a	0.0
		G2	13.5 ^b	11.7 ^b	51.3 ^a	33.0 ^a	45.3	1.5 ^a	1.3 ^b	7.0 ^b	5.3 ^b	53.1
		G3	16.8 ^{ab}	14.7 ^{ab}	63.7 ^a	38.7 ^a	32.0	1.5 ^a	1.5 ^{ab}	5.0 ^b	5.0 ^b	53.1
	Ejumula	G1	13.3 ^a	9.2 ^a	83.7 ^a	34.0 ^a	0.0	5.5 ^a	3.5 ^a	30.0 ^a	13.0 ^a	0.0
		G2	9.3 ^a	7.2 ^a	56.0 ^a	22.3 ^a	30.0	1.8 ^b	1.5 ^b	7.3 ^b	5.0 ^b	67.3
		G3	9.3 ^a	7.2 ^a	54.0 ^a	20.7 ^a	30.0	2.2 ^b	2.2 ^{ab}	8.7 ^b	8.7 ^{ab}	60.0
	Dimbuka	G1	15.8 ^a	11.8 ^a	124.7 ^a	43.3 ^a	0.0	4.5 ^a	3.7 ^a	18.7 ^a	13.7 ^a	0.0
		G2	11.2 ^b	6.2 ^a	108.0 ^a	21.3 ^b	29.1	2.8 ^b	2.2 ^b	13.7 ^a	7.3 ^b	37.8
		G3	12.5 ^b	7.8 ^a	96.7 ^a	26.0 ^{ab}	20.9	3.5 ^a	2.8 ^{ab}	14.0 ^a	9.7 ^{ab}	22.2
4 th field trial	NASPOT 1	G1	30.3 ^a	28.3 ^a	107.0 ^a	84.0 ^a	0.0	21.3 ^a	21.3 ^a	53.0 ^a	52.3 ^a	0.0
		G3	23.7 ^b	19.3 ^b	68.7 ^b	41.0 ^b	21.5	7.4 ^b	7.2 ^b	35.3 ^a	27.7 ^a	64.5
		G4	25.3 ^b	23.5 ^b	74.0 ^b	54.0 ^b	16.5	11.5 ^b	11.4 ^b	37.3 ^a	32.3 ^a	46.7

Ejumula	G1	23.0 ^a	20.2 ^a	97.0 ^a	62.0 ^a	0.0	16.3 ^a	15.8 ^a	64.0 ^a	50.7 ^a	0.0
	G3	13.0 ^b	8.7 ^b	75.3 ^a	27.0 ^b	43.5	9.4 ^b	8.8 ^b	49.7 ^a	35.7 ^b	42.3
	G4	15.8 ^b	11.5 ^b	76.3 ^a	33.3 ^b	31.3	7.6 ^b	6.7 ^b	49.3 ^a	37.0 ^b	53.6
	G5										
Dimbuka	G1	21.8 ^a	18.7 ^a	107.0 ^a	64.7 ^a	0.0	22.7 ^a	22.4 ^a	79.7 ^a	71.3 ^a	0.0
	G3	16.2 ^b	10.7 ^b	126.3 ^a	32.3 ^b	25.7	11.3 ^b	11.2 ^b	55.7 ^b	46.7 ^{ab}	50.2
	G4	17.8 ^b	11.7 ^b	128.0 ^a	34.7 ^b	18.3	8.3 ^b	8.3 ^b	44.0 ^b	37.3 ^b	63.2
	G5										
5 th field trial	NASPO T 1										
	G1	12.8 ^{ab}	12.5 ^a	54.3 ^a	41.7 ^a	0.0					
	G2	9.6 ^b	8.6 ^a	48.7 ^a	28.0 ^{ab}	25					
	G4	13.6 ^a	12.6 ^a	55.0 ^a	38.3 ^{ab}	-6.3					
Ejumula	G5	10.8 ^b	9.9 ^a	42.7 ^a	26.3 ^b	15.6					
	G1	12.3 ^a	10.5 ^a	77.3 ^a	37.7 ^a	0.0					
	G2	6.9 ^b	6.0 ^b	39.3 ^b	20.3 ^{ab}	43.9					
	G4	4.9 ^b	3.8 ^b	30.3 ^b	12.0 ^b	60.1					
Dimbuka	G5	5.5 ^b	4.9 ^b	34.3 ^b	20.3 ^{ab}	55.3					
	G1	15.9 ^a	14.1 ^a	97.7 ^a	54.0 ^a	0.0					
	G2	13.6 ^{ab}	11.9 ^{ab}	69.7 ^b	34.7 ^b	14.5					
	G4	15.4 ^a	13.8 ^a	89.3 ^{ab}	45.0 ^{ab}	3.1					
G1 to G5 represents number of cycles in field production. Column with the same letters among different generations for individual cultivar in each field trial is not significantly different. Change in yield was calculated as the difference in G1 from subsequent generation in a given trial divid by G1 of that trial then multiplieby 100.	G5	10.4 ^b	8.8 ^b	71.0 ^b	31.0 ^b	34.6					

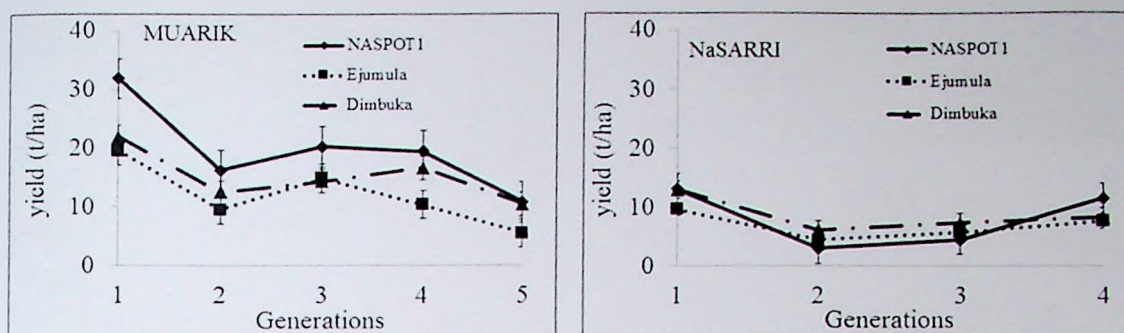


Figure 3: Effects of virus infection on yield of different generations of sweetpotato cultivars under two agro-ecologies.

3.3.5 Rate of sweet potato yield decline due to SPVD infection under natural condition in two agroecologies

In both location, sweetpotato root yield decline increased with time and was greater at MUARIK than NaSARRI (Figure 4). The yield decline at MUARIK ranged between 36%-65% for cv NASPOT 1, 47 %-72 % for cv Ejumula and 24 %-52 % for cultivar Dimbuka. At NaSARRI, the yield decline ranged between 11-60% for cv NASPOT 1, 20%-53% for Ejumula and 30%-50% for Dimbuka for all the generations. The substantive sweetpotato yield was achieved up to a point where the the yield degeneration curve meets the mean yield curve. Beyond this point, the rate of yield decline was greater than the actual yield obtained and it's at this point that the renewal of clean planting material is neccesary (Figure 4). Therefore at MUARIK, cvs. Ejumula can be grown up to at least three generations, cv NASPOT 1 up to and Dimbuka can be grown to four generations. At NaSARRI, cv Dimbuka can be grown upto 4 generations while cvs Ejumula and NASPOT 1 upto 6 generation (Figure 4).

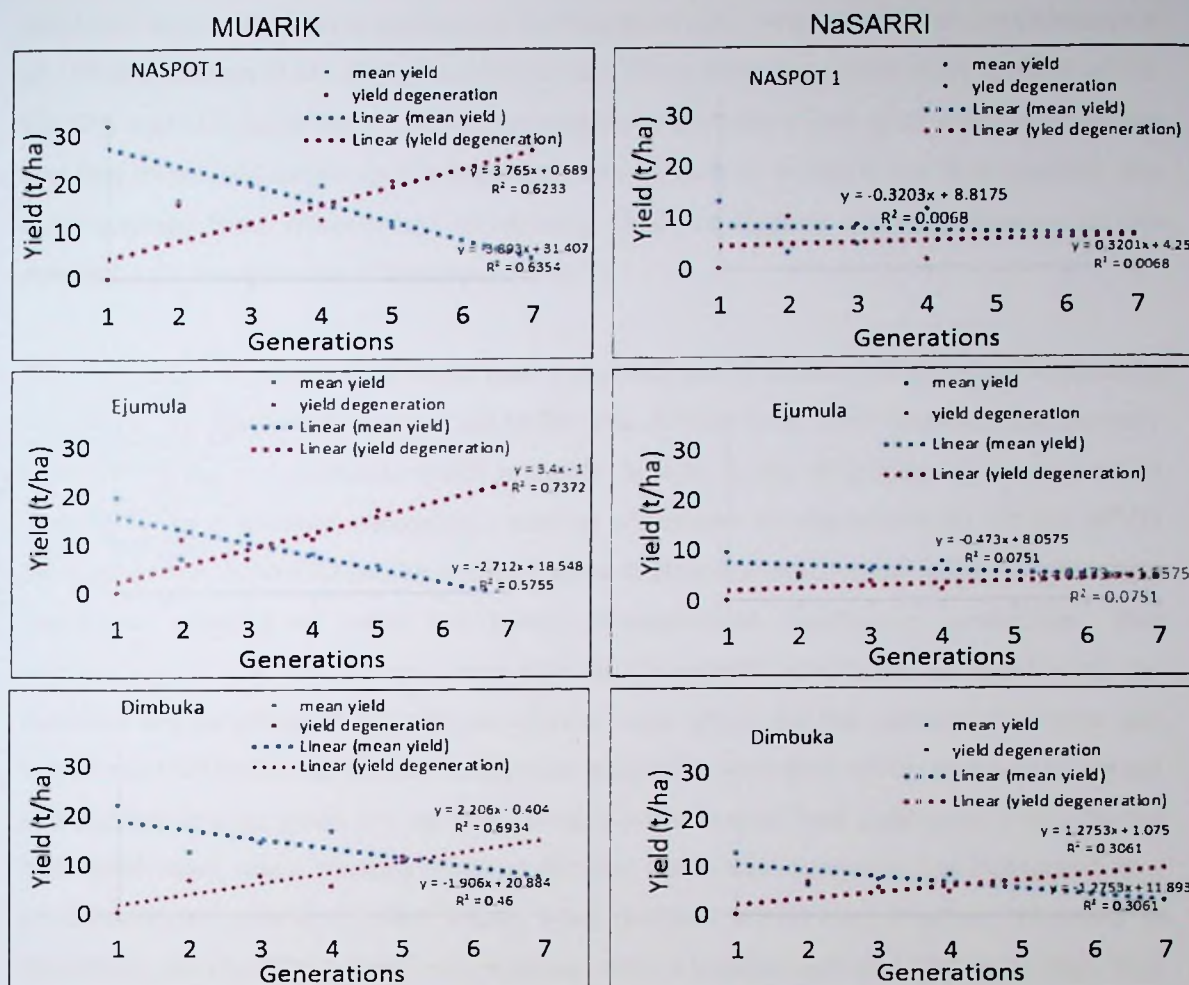


Figure 4: Rate of decline of sweetpotato cultivars in two agroecologies (MUARIK and NaSARRI). The mean yield values are the combine means of each generation in all the five trials. (i.e. mean yield values for G1, is the average of G1 in the 1st, 2nd, 3rd, 4th and 5th field trial, G2= the average value of G2 in the 2nd and 3rd trial, G3 = the average value of G3 in the 3rd and 4th trial, G4= the average value of G4 in the 4th and 5th trial and G5 is the average of G5 in the 5th trial). Yield degeneration values are the differences on the average mean yield of G1, G2, G3, G4 G5 from G1,

3.4 Discussion

This study has shown that virus indexed material planted in the field in Uganda quickly gets infected with viruses. Rapid virus spread in disease free sweetpotato plants has also been reported in Brazil (Pozzer *et al.*, 1994) and USA (Bryan *et al.*, 2003). G2, G3, G4 and G5 were also observed to have more infected plants than G1 within the first two months after planting in both locations, presumably because some of the planting materials selected for G2, G3, G4 and G5 were already infected despite being symptomless. Single virus infections

can cause very mild or no symptoms on the foliage of most sweetpotato cultivars (Karyeija *et al.*, 1998a; Mukasa *et al.*, 2006; Untiveros *et al.*, 2007). However, some of the cuttings of G2, G3, G4, and G5 plants did not develop symptoms within one month after planting, implying that they either had single virus infection or were virus free. In fact it has been reported that most cuttings from symptomless sweetpotato fields in Uganda are virus free or latently infected with a single virus (Gibson *et al.*, 1997).

The percentage of plants expressing virus symptoms and the extent of symptom expression were greater at MUARIK than at NaSARRI. The difference in virus incidence and severity observed in the two locations could probably be due to the difference in environmental factors, vector population abundance and/ or prevalence of alternate hosts for the SPVD causing viruses. In MUARIK, the rainfall regime is almost all-year-round and this encourages continuous planting of crops and hence all-year-round sweetpotato production. This overlapping of new and mature crops provides favourable conditions for rapid virus re-infection and perpetuation as there are always some plants for the survival of viruses and vectors. In NaSARRI, the rainfall is unimodal type with some parts of the year becoming hot and dry, hence most plants dry up thus killing the vectors and their pathogen. In fact for the NaSARRI trials, some planting material for this study was harvested and conserved in a screenhouse for planting the next season, otherwise they would have dried out. However in MUARIK, the planting material was obtained from a mature field and planted straight into the new field. Although virus incidence and severity was less at NaSARRI, some symptomless leaf samples tested positive for both SPFMV and SPCSV. This could probably be that the two viruses had just infected the plant or that factors other than the interaction of the two viruses may also control symptom development. Environmental factors like temperature have been observed to influence virus symptom development in some plants. However, no information is available on how environmental conditions affects SPVD development. This aspect needs to be investigated.

Among the cultivars tested, Beauregard was the most susceptible at both MUARIK and NaSARRI. It was completely infected with virus in G1 within the first trial and no planting materials could be obtained for the subsequent trials. This implies that for highly susceptible cultivars, virus free planting material is of little practical value as they are simply overwhelmed even in low virus pressure areas such as NaSARRI. Instead, breeding for resistance should be the focus. Ejumula was also susceptible at MUARIK with over 90% of

the plants diseased with average severity scores >4 by the end of each trial irrespective of the generation. Although in this study analysis of root yield degeneration indicated ejumula can be grown up to three generations, high incidence and severity of SPVD that affects vine quality limit its production to only one season in areas with extremely high SPVD pressure. This therefore requires use of virus free planting material every season of cultivation. Similar findings have been reported by Mwanga et al. (2007) in which Ejumula was found to be very susceptible to SPVD in a trial at Namulonge where SPVD inoculum pressure was high. In NaSARRI there was no cultivar difference in terms of virus incidence and severity except for Beauregard hence vine degeneration was low. This therefore implies that both moderately susceptible and resistant cultivars can be grown in this and other low SPVD pressure regions. This is further supported in this study in which the root yield degeneration was less and all the tested cultivars can be grown up to six generation, while at MUARIK cvs NASPOT 1 and Dimbuka can be grown for up to four generations (Figure 4).

A greater total yield, marketable yield and number of storage roots were generally observed in G1 as compared to G2, G3, G4 and G5 in both locations, presumably due to absence of viruses in the planting material. However, there were exceptions in that NASPOT 1 G2, trial 2, and G4 trial 5 had greater yield than the respective G1. Use of virus indexed or symptomless planting material have been reported to produce better yield than those infected with viruses (Milgram *et al.* 1996; Gibson *et al.*, 1998; Gutierrez *et al.*, 2003; Ling *et al.*, 2010). A single yield decline was observed after the first generation so that $G1 > G2 \leq G3 = G4$ (Figure 3) and this, may explain why farmers have been able to maintain some of these varieties for decades in the field. It also further supports the idea of using resistant varieties in areas with high SPVD and hence the need to breed for more resistant varieties. Despite the single decline in yield, there was slight yield increase at NaSARRI after three generations (Figure 3) probably due to ability of sweetpotato plants to recover or revert from virus infection. This is supported by low disease severity in this area although incidence remained relatively high. Plant recovery from virus infection is reported to be high in areas with hot weather conditions (Gibson and Otim-Nape, 1997) and this may be the case with sweetpotato plants in NaSARRI but this needs further investigation.

Although G1 performed better than most of the generations except for NASPOT 1 G2 and G4, planting G1 together with cuttings already exposed in the field may have enabled more rapid re-infection than if only G1 cuttings had been planted. This was supported by the fact

that the first field trial in this experiment which had only G1 produced greater yield than G1 in subsequent field trials that had both G1 and field exposed cuttings (Table 6). Therefore, farmers should be strongly advised when given clean planting material not to mix it with their old planting materials from their fields, if they must obtain the maximum benefit of using clean vine cuttings. Field exposed material, even if it may be symptomless, could act as a virus reservoir and when planted with susceptible cultivars could quickly spread and affect yield performance and quality of healthy vines leading to shortage of quality planting material in the subsequent seasons.

Despite less incidence and severity of SPVD in NaSARRI, the percentage yield loss was greater than that of MUARIK (Table 6). This may be because of un-identified symptomless viruses or new virus strains and other systemic pathogens. Wasswa et al. (2011) reported the asymptomatic presence of SPLCUV and suggested it could be the third most widespread virus in Uganda after SPFMV and SPCSV. There is a need to carry out survey and use appropriate technology to detect and identify as yet unknown viruses and determine their effect on the yield of sweetpotato. The high yield loss in NaSARRI could also be a result of site specific factors like drought, and soil fertility. The effect of these factors was not investigated in this study.

Overall, this study has proved that sweetpotato cultivar degeneration in terms of vines and storage root quality and quantity do occur in both high and low SPVD areas although at varying rate. Cultivation of cvs Ejumula, NASPOT 1 and Dimbuka at MUARIK is possible for maximum ranging from one to three generation and at NaSARRI, six generation is possible with all the cultivars. It was found that SPFMV and SPCSV were associated to observed degeneration. Therefore, it is important to determine how much each of these viruses contribute to yield decline under field condition and this formed the basis of the next study.

CHAPTER FOUR

EFFECTS OF SWEET POTATO FEATHERY MOTTLE VIRUS AND SWEETPOTATO CHLOROTIC STUNT VIRUS ON THE YIELD OF SWEETPOTATO IN UGANDA

4.1 Introduction

Sweetpotato is a vegetatively propagated crop and systemic pathogens like viruses can persist and spread over successive crop cycles (Bryan *et al.*, 2003). Over 30 viruses belonging to potyvirus, crinivirus, carlavirus, cucumovirus, ipomovirus, badina virus and begomovirus genera have been reported to infect sweetpotatoes worldwide (Mukasa *et al.*, 2006; Untiveros *et al.*, 2007; Valverde *et al.*, 2007; Clark *et al.*, 2012). Of these only six have been reported in Uganda, namely *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato chlorotic stunt virus* (SPCSV), *Sweet potato chlorotic flecks virus* (SPCFV), *Sweet potato caulimolike virus* (SPCV), *Sweet potato mild mottle virus* (SPMMV) and *Sweet potato leaf curl Uganda virus* (SPLCUV) (Gibson *et al.*, 1998; Mukasa *et al.*, 2003; Aritua *et al.*, 2007; Wasswa *et al.*, 2011). SPFMV and SPCSV are the most prevalent and when co-infecting sweetpotato result in severe symptoms described as sweetpotato virus disease (SPVD) (Gibson *et al.*, 1998; Karyeija *et al.*, 2000; Mukasa *et al.*, 2006) causing up to 90% yield losses in individual plants (Mukiibi, 1977; Karyeija *et al.*, 1998a; Aritua *et al.*, 2000).

Due to the severe yield loss caused by SPVD when individual plants are affected, most research in Africa including Uganda has concentrated on this, neglecting the occurrence as single infection with SPFMV or SPCSV. Plants affected by SPVD are easily recognizable by farmers due to the severe symptoms and may be controlled by rouging and or selecting symptomless planting material for the next crop (Aritua *et al.* 1999). On the other hand, the impact of individual viruses which are usually symptomless and are therefore difficult for farmers to manage, has not been well studied. In addition, no field study has so far been done in Uganda to determine the effect of single virus infections on yield of sweetpotatoes. Studies in other countries on the effect of SPFMV on yield of sweetpotato cultivars are contradictory. Some studies have reported no effects on yield of storage roots and vines in comparison with healthy plants (Milgram *et al.*, 1996; Clark *et al.*, 2006), while others have reported SPFMV infected plants producing slightly better yield than the healthy control (Gutiérrez *et al.*, 2003), and others have reported yield reduction of up to 46% (Gibson *et al.*, 1997; Njeru *et al.*,

2004; Mukasa, 2004; Domala *et al.*, 2008). Due to these conflicting reports, there is a need to further investigate the effect of SPFMV on Ugandan sweetpotato cultivars in order to design an effective management system in Uganda.

Also, single infection of SPCSV may produce no apparent symptoms in some cultivars and farmers often confuse its symptoms with purpling of mature leaves due to nutrient deficiencies in the soil or plant maturity (Gibson *et al.*, 1997; Mukasa *et al.*, 2003). As a result, cuttings from such plants are used for subsequent propagation. Data on effects of single infection of SPCSV under field conditions in Uganda are also limited. However, SPCSV alone has been reported to cause a 30% yield reduction in cultivar Costanero in Peru (Untiveros *et al.*, 2007). In Uganda, a yield reduction of 50% in cultivar Tanzania under screenhouse conditions was reported though the yield performances of both healthy plants and infected ones in this study were poor (Gibson *et al.*, 1998). Virus expression and its effect on a plant are influenced by the environment, and hence, there is a need to determine the effect of virus under field condition in contrasting agro-ecologies.

Continued use of symptomless but infected cuttings by farmers in Uganda could also be a 'silent' reason why the potential average yield has not been achieved. This study therefore aimed to determining the effects of single infection of SPFMV or SPCSV and their combination on the yield of four sweetpotato cultivars grown in Uganda under field conditions, the rate of recovery of sweetpotato cultivars from virus infection under two agro-ecologies.

4.2 Materials and Methods

These included sourcing for virus inoculum, multiplication of clean planting materials, Grafting clean planting material with virus (es), multiplication of inoculated plants in screen house, virus testing, setting up of field experiments, data collection and analysis as described below.

4.2.1 Virus testing

Three leaf samples (leaf discs of ~1 cm diameter) per plant were picked from the top, middle and bottom part of the plants and grounded in a polyvinyl bag using appropriate enzyme-linked immunosorbent assay (ELISA) extraction buffer (1 ml buffer per leaf disc). The plants tested were got from both screen house (section 4.2.2, 4.2.3, 4.2.4) and those grown in the

field (section 4.2.5, 4.2.7, 4.2.8). The leaf samples were tested either using NCM ELISA or DAS and TAS ELISA. Nitrocellulose membrane ELISA was carried out following the protocol obtained from CIP Lima, Peru to detect the presence of any of the ten viruses, that is SPFMV, SPMNV, *Sweet potato latent virus* (SPLV), SPCFV, *Sweet potato mild speckling virus* (SPMSV), C-6 virus, SPCSV, SPCV, *Sweet potato virus G* (SPVG) and *Cucumber mosaic virus* (CMV) for which antibodies were available. The presence of virus was judged on the visual intensity of the colour change on the membrane. Additional tests using DAS and TAS ELISA were specifically carried out to detect and estimate the virus titre for SPFMV or SPCSV, respectively, using the protocol by Clark and Adams (1977). The DAS-ELISA kit (containing coating antibody immunoglobulin G (IgG) and detecting antibody IgG-AP) against SPFMV and TAS ELISA kit containing (primary antibody, rabbit IgG, secondary mouse monoclonal antibody (Mab) and detection antibody, rabbit anti-mouse IgG-AP against SPCSV and respective positive controls were from Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Germany. The p-nitrophenyl phosphate substrate and microplates used were from Sigma Chemical Co. The absorbance was measured at 405 nm after one and half hours using a Bio-Rad microplate reader (model 680).

4.2.2 Sources of virus inoculum

Isolates of SPFMV and SPCSV were sourced by collecting suspected symptomatic cuttings from farmers' fields at Namulonge, Wakiso district. Cuttings were graft inoculated onto the nearly universal indicator plant, *Ipomoea setosa*, and left to grow to allow symptoms to express. The symptomatic plants were tested using NCM ELISA for ten viruses for which antibodies were available and those reacting positively for SPFMV and for SPCSV alone were selected and retested using DAS or TAS ELISA respectively. SPCSV was maintained in cultivar Kampala white, SPFMV was maintained in cv. Resisto and mixed infection of SPFMV + SPCSV was maintained in cv. Ejumula by grafting them with respective pure virus isolate(s) in a screenhouse at MUARIK. These cultivars were used for conserving virus isolates because they do not recover/ revert once infected with these viruses (Wasswa, 2012). The screenhouse was sprayed every two weeks with imidachloropid (locally known as confidor) to kill aphids and whiteflies. Virus presence in these cultivars was routinely checked by grafting to *I. setosa* plant and by serology using DAS or TAS ELISA.

4.2.3 Sources of healthy planting materials

Symptomless sweetpotato cuttings of cvs. Beauregard, Dimbuka, NASPOT 1 and Ejumula, were collected from sweetpotato fields at the National Crops Resource Research Institute (NaCRRI), Namulonge in Wakiso district, while cv. Kabode was obtained from farmers' fields in Soroti district. The cuttings were grafted on *I. setosa* and the grafted plants were monitored weekly for absence of virus symptoms by visual observation for five weeks. Their virus free status was further confirmed using NCM ELISA. Scions that tested negative for viruses, here referred to as healthy scions were multiplied in plastic pots containing a sterilized mixture of soil, sand and animal manure in equal proportions and left to grow in an insect proof screenhouse at MUARIK in Wakiso district. The numbers of cuttings for field planting were increased through repeated two node cuttings. To ensure sustained availability of virus free planting material, some of the materials were multiplied and conserved *in vitro* at MUARIK.

4.2.4 Generation and multiplication of planting materials infected with viruses

Virus indexed sweetpotato cuttings (from section 4.2.3 above) of cvs Dimbuka, Ejumula, Kabode, NASPOT 1 and Beauregard of 30 cm length were potted and left to grow for 2 weeks in a screenhouse at MUARIK. Each cultivar was planted in 15 pots making total of 75. The plants were divided into three groups, each having five cuttings per cultivar. One group was graft inoculated with SPFMV, another was graft inoculated with SPCSV and third was graft inoculated with SPFMV+ SPCSV. These were left to grow and monitored weekly for symptoms. After one month, infection was confirmed using DAS (for SPFMV) and TAS (for SPCSV) ELISA and the cuttings were multiplied using repeated 2 node cuttings in order to obtain enough planting material for the field experiment. The multiplied planting material were further tested by grafting on *I. setosa* to confirm their infection status before planting in the field.

4.2.5 Field experiment

Four field trials were conducted at MUARIK and at the National Semi Arid Resource Research Institute (NaSARRI) located in Serere district in eastern Uganda as showed in table 7. In the first trial, the yield of SPFMV infected sweetpotato was compared to that of virus indexed material at both MUARIK and NaSARRI.

Table 7: Sweetpotato vines used in each trial in different seasons at MUARIK and NaSARRI

Trials	Seasons	Vines used	Cultivar tested
1	2012A	SPFMV vs Healthy	Kabode, NASPOT 1, Ejumula, Dimbuka and Beuregard
2	2012B	SPFMV from screen house, SPFMV from the field in 2012A and healthy control	Kabode, NASPOT 1, Ejumula and Dimbuka
3	2013A	SPFMV, SPCSV, SPFMv+SPCSV and healthy control	Kabode, NASPOT 1, Ejumula and Dimbuka
4	2013B	SPFMV, SPCSV, SPFMv+SPCSV and healthy control	Kabode, NASPOT 1, Ejumula and Dimbuka

In this trial, a split plot randomised complete block design was used in which the SPFMV infected plants and healthy controls acted as the main plot (to minimise virus spread) while the cultivars acted as the sub-plots. Five cultivars namely Kabode, NASPOT 1, Ejumula, Dimbuka and Beuregard were evaluated. The experiment was replicated three times with each experimental block laid in an area of 5 m by 10 m. Each treatment was planted in five mounds per plot and each mound was made at a spacing of 1m by 1m and planted with three vine cuttings. In the second trial, a similar design was used except that an additional treatment of SPFMV infected cuttings from the 1st trial was included in order to evaluate the cumulative exposure to virus infection on yield. Also in the 2nd trial cultivar Beuregard was dropped and the remaining four cultivars were used in the study using the same design as above.

In the 3rd and 4th field trials, SPFMV, SPCSV, and combination of SPFMV+ SPCSV were evaluated. A randomized complete block design in split plot arrangement was used where the main blocks consisted of virus/ virus combination and the sub blocks were the cultivars. This was replicated three times. Four categories of planting materials (treatments) all obtained from screenhouse were used, that is healthy controls, SPFMV, SPCSV and SPCSV+ SPFMV and the same varieties except Beuregard. Systemic insecticide (imidacloprid) was applied monthly for trials at MUARIK to control aphids and white flies. Weeding was done 2 to 3 times depending on the weed intensity using a hand hoe.

4.2.6 Disease symptoms and yield measurement

Sweetpotato plants were monitored for virus symptom development and severity data were collected at monthly interval beginning one month after planting for period of four months. Severity scores of 1 to 5 were used, where 1 = plants showing no symptoms, 2 = virus symptoms just starting to appear and this can be as mild chlorotic spots on the older leaves or mild vein clearing or mild purpling at the leaf margin of the older leaves, 3 = the symptom above enlarge and become more visible, 4 = infected plants showing severe disease symptoms including leaf purpling, leaf chlorosis and leaf shape starts to get distorted and 5 = infected plants showing very severe virus disease symptom including total distortion in leaf shape, stunted growth, mosaic, leaf chlorosis and sometimes complete death of infected plant (Hahn *et al.*, 1981). Harvesting was done 5 months after planting. At harvest, total yield/mound, marketable yield, total root number, marketable root number, root diameter, root length and vine weights were determined. The root length and diameter were measured using a marked thread. For each sweetpotato root, the diameter was measured at 3 positions, at the neck end, the middle and the tail end and the average diameter recorded.

4.2.7 Evaluation of sweet potato virus disease recovery and virus infection

Detection of viruses that could have infected sweetpotato during the growing season in the 1st and 2nd field trials was carried out using NCM ELISA. Samples were collected from symptomatic leaves or a leaf from the basal, middle and upper parts of symptomless plants. At least 15 samples (5 samples per plot) per cultivar per treatment of each trial were collected and tested for 10 viruses i.e. SPFMV, SPMMV, SPLV, SPCFV, SPMSV, C-6 virus, SPCSV, SPCaLV, SPVG, and CMV for which antibodies were available. Positive control samples for each virus were provided in the NCM ELISA kit by CIP Lima, Peru. The plants were serologically evaluated at the end of each trial before harvesting. The presence of virus was confirmed visually based on the intensity of the colour change on the membrane. Also prior to harvesting, ten symptomless cuttings per cultivar from SPFMV infected plants were graft inoculated on *I. setosa* and monitored weekly for any virus symptom for period of five weeks. Any infected *I. setosa* plants were further tested with NCM ELISA to confirm the viruses present.

4.2.8 SPFMV and SPCSV accumulation in different sweetpotato cultivars

The virus quantification was carried out on the samples collected from the 3rd and 4th trial to determine the virus load of SPFMV and SPCSV in the field exposed materials in two agro-

ecologies. DAS and TAS ELISA were used as described previously (Gibson *et al.*, 1998) to estimate the virus titre.

4.2.9 Statistical Analysis

Statistical analyses were carried out using Genstat 13th Edition. Data on virus severity, storage root yield, storage root number, storage root diameter and length, and vine weight were subjected to Analysis of variance and the means separated using Fisher's protected least significant difference at 5% probability level.

4.3 Results

4.3.1 Sweetpotato symptom expression due to single and mixed virus infection under field conditions

Analysis of variance for disease severity indicated a significant ($P \leq 0.05$) cultivar, virus and cultivar by virus interaction effect on disease severity at both MUARIK and NaSARRI. Severity was observed to increase with time of field exposure in all the cultivars and in both locations (Figure 5). Dual infection of SPFMV and SPCSV caused the most severe symptoms in all the cultivars in both locations (Figure 5); the symptoms included vein chlorosis, purple spots on the leaves, mosaic, distorted leaf shape, and stunted growth. The second most severe symptoms were observed in single infection of SPCSV (Figure 5); the common symptoms were chlorotic spots, purple spots on mature leaves, and leaf chlorosis. Disease severity due to SPFMV alone was generally low in all cultivars in both locations (Figure 5). The symptom expression due to SPFMV was characterized by mild chlorotic spots and mild vein clearing in some cultivars. Virus indexed healthy control materials at MUARIK displayed greater disease severity than SPFMV infected plants (Figure 5). At NaSARRI, all the control plants had the least disease severity compared to virus infected ones for all the tested cultivars (Figure 5). Among the cultivars tested, irrespective of the virus status, Ejumula was the most severely affected, followed by Dimbuka and least in Kabode and NASPOT 1 (Figure 5). For cvs NASPOT 1 and Dimbuka, disease severity was high at 3 months after which, there was decline in severity in plants singly infected by SPFMV or SPCSV in both locations.

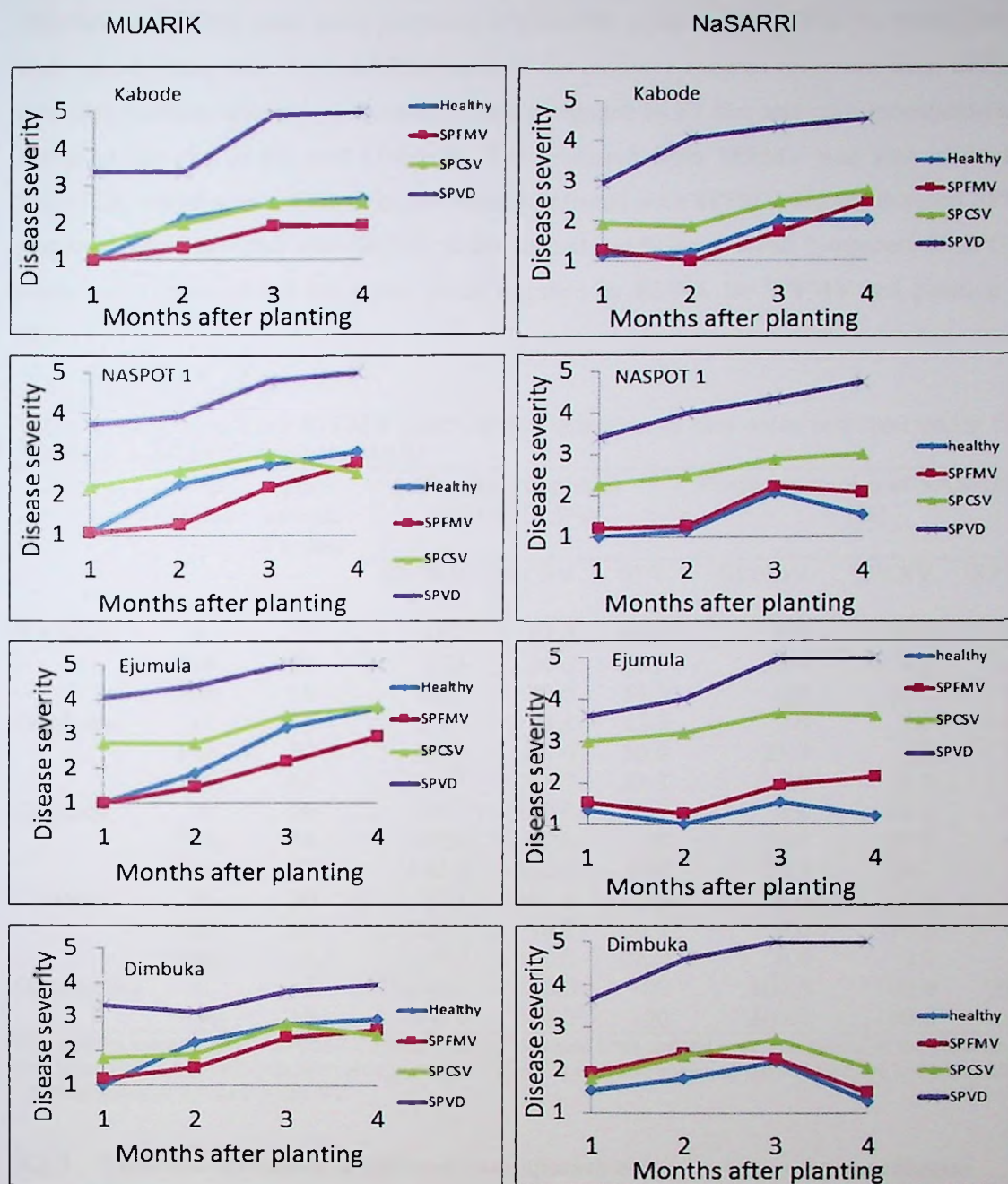


Figure 5: Disease severity curve showing response of sweetpotato cultivars to virus infection in two agro ecologies (MUARIK and NaSARRI). The disease severities plotted are the means of disease severity in the 3rd and 4th field trial.

4.3.2 Reversion from virus infection and detection of other viruses

Among the ten viruses tested using NCM-ELISA, only SPCSV and SPFMV were detected in both locations. The remaining eight viruses were negative in all the samples tested. SPCSV

followed by SPFMV were more prevalent at MUARIK while in NASARRI, the incidences of both viruses were low. At NaSARRI, most of the cultivars showed reversion from SPFMV infection, initially infected plants mostly testing negative by ELISA and graft inoculation to *I. setosa* at the end of the trial (Table 8). The reversion from SPFMV was also evident at MUARIK where some of the cultivars initially infected with SPFMV did not develop SPVD symptom when infected with SPCSV under natural condition. Instead symptoms of SPCSV alone was expressed and the plants tested negative by ELISA for SPFMV and positive for SPCSV (Table 8).

Table 8: Reversion from SPFMV infection and detection of new virus infection under field condition at MUARIK and NaSARRI

Cultivar	Virus status	#plant samples tested	Positive samples at MUARIK (%)			Positive samples at NASARRI (%)		
			SPFMV	SPCSV	SPV D	SPFMV	SPCSV	SPVD
NASPOT 1	H	30	46.7	93.3	46.7	0.0	20.0	0.0
	FM ₁	30	33.3	60.0	23.3	20.0	6.6	0.0
	FM ₂	15	60.0	80.0	33.3	0.0	26.7	0.0
Dimbuka	H	30	13.3	93.3	13.3	0.0	3.3	0.0
	FM ₁	30	20.0	40.0	20.0	26.7	0.0	0.0
	FM ₂	15	66.7	86.7	33.3	6.0	6.7	0.0
Ejumula	H	30	86.7	100.0	86.7	0.0	13.3	0.0
	FM ₁	30	100.0	80.0	80	53.3	20.0	6.7
	FM ₂	15	100.0	100.0	100	13.3	26.7	3.3
Kabode	H	30	13.3	100.0	0.0	0.0	16.7	0.0
	FM ₁	30	26.7	86.7	26.7	6.0	10.0	0.0
	FM ₂	15	26.7	100.0	26.7	0.0	0.0	0.0
Beauregard	H	15	100.0	100.0	100	100.0	100.0	100.0
	FM ₁	15	100.0	100.0	100	100.0	100.0	100.0

H = healthy control, FM₁ = SPFMV-infected cuttings obtained from screenhouse and grown in the field for the first time; FM₂ = SPFMV-infected cuttings obtained from 1st trial and grown in the field for the 2nd time. SPVD = combination of SPFMV + SPCSV

4.3.3 Virus accumulation in different sweetpotato cultivars in two agro-ecologies

Results from DAS and TAS ELISA indicated high virus accumulation in plants grown at MUARIK than that at NASARRI irrespective of the type of virus (Figure 6). Higher absorbance values were observed in plants with mixed infections than those with single infection in both locations and in all the cultivars tested (Figure 6).

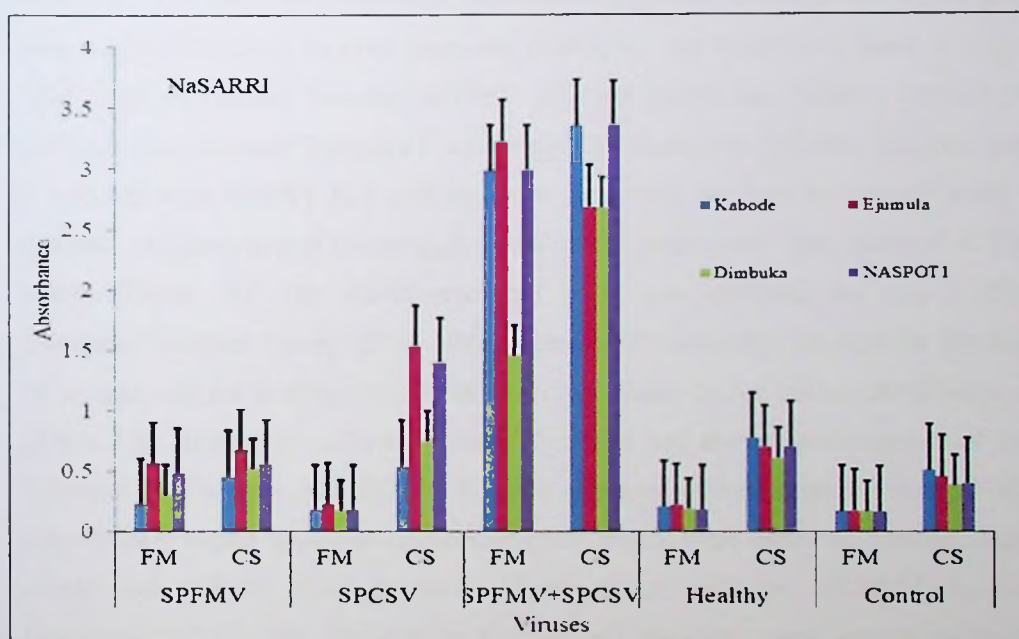
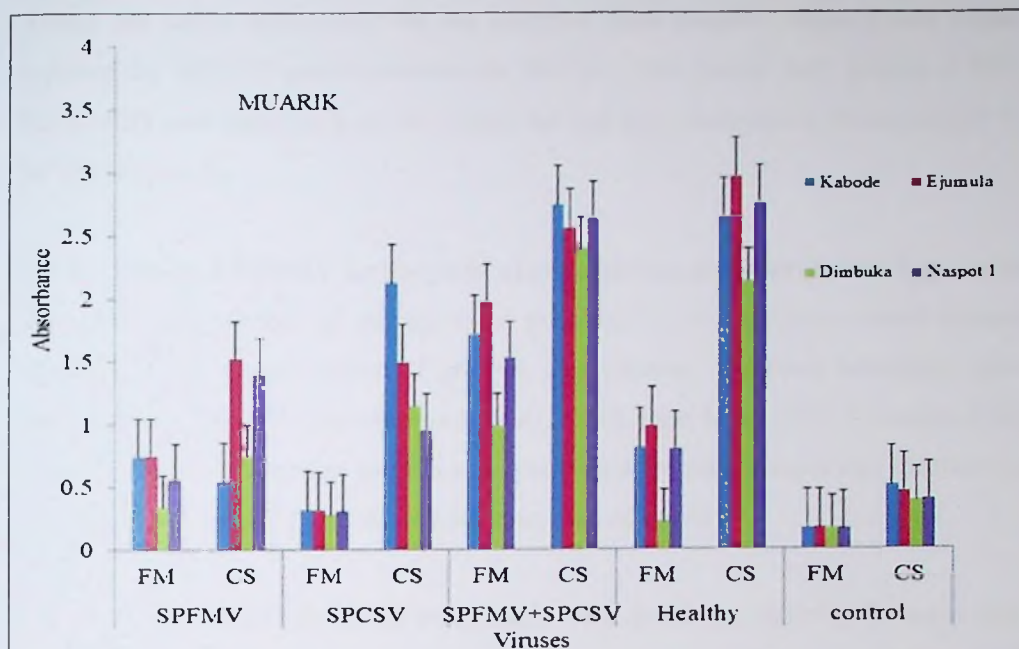


Figure 6: Absorbance values from detection of SPFMV antigens by DAS- ELISA and SPCSV antigen by TAS ELISA in leaves of four sweetpotato cultivars five months after planting in MUARIK and NaSARRI. FM= sweet potato feathery mottle virus absorbance values, CS = sweetpotato chlorotic stunt virus absorbance values

Also plants singly infected by SPCSV accumulated more viral antigen and never showed recovery as most of them tested positive as compared to those plants infected by SPFMV alone in which most of them tested negative. The results also showed high spread of SPCSV

within the fields since most of the samples from healthy controls and those previously infected by SPFMV tested positive for SPCSV. The spread was greater at MUARIK than NASARRI with samples from MUARIK having high absorbance values except in the case of SPVD (Figure 6).

4.3.4 Effect of SPFMV on the yield of sweetpotato cultivars in two agro-ecologies

The yield and number of storage roots produced by sweetpotato varied depending on the cultivars, virus status, season of growth, and location. Between locations, more yield and storage root numbers were observed at MUARIK than NaSARRI. Because of high variation observed between location and seasons, yield data for each season and location was analysed separately individually and the results presented as below:

For the 1st field trial significant differences were detected ($<0.05\%$) for total and marketable root yield, and total and marketable root numbers among cultivars, vine status and cultivar by vine status interaction in both locations (Table 9). At MUARIK, there was no significant total yield difference between SPFMV infected plants and healthy control plants of all cultivars tested except Dimbuka (Table 9 and 10). However, cultivars, Kabode, and NASPOT 1 infected with SPFMV had slightly more yield than the healthy control while the healthy controls of Ejumula and Beauregard had slightly better yield than those of SPFMV infected plants (Table 10). The marketable root yield was different for cvs NASPOT 1 and Dimbuka. The total (except for cv. Beauregard) and marketable (except for Dimbuka) number of storage root for healthy and SPFMV infected plants within each cultivar were not different (Table 10). Among the cultivars tested, Dimbuka had the greatest number of storage roots, followed by Ejumula, NASPOT 1, Kabode and least in Beauregard. For the NaSARRI trial, significantly higher total and marketable yield effects were observed between healthy control plants and SPFMV infected plants (Table 9) of cultivars NASPOT 1, Dimbuka and Beauregard (Table 10). For cultivar Kabode and Ejumula, there was no difference in yield between the healthy control and SPFMV infected plants (Table 10). The yield loss due to SPFMV in this location ranged from 14% to 67% depending on the cultivar. The number of storage roots was greatest in Dimbuka followed by NASPOT 1, then Kabode, Ejumula and least in Beauregard. The storage root numbers within cultivar were not different (Table 10).

Table 9: ANOVA to test for the difference on yield and storage root number of five sweetpotato varieties grown from virus tested plants compared with virus tested plants graft inoculated with SPFMV at MUARIK and NasARRI during the 1st field trial

Source of variation	D.f	Mean square							
		MUARIK				NASARRI			
		Total yield	Marketable yield	Total root number.	Marketable root number	Total yield	Marketable Yield	Total root number	Marketable root number
Block	2	12.11	16.98	16.32	112.48	0.86	1.25	32.09	9.73
Vine status	1	8.40*	11.48*	42.87	9.79	3.62**	2.62*	138.24**	17.34*
Cultivar	4	34.68**	39.65**	332.03**	147.04**	8.94**	12.45**	31.95*	82.08**
VXC	4	5.49*	6.44*	75.31*	39.79*	1.33*	1.61*	36.65**	15.52*
Residual	138	1.93	2.03	30.43	11.07	0.34	0.41	7.54	3.68
total	149								

VXC= virus status by cultivar interaction, d.f = degree of freedom, * and ** = significant at 0.05 and 0.001

Table 10: Mean yield and storage root number of five sweetpotato varieties grown from virus tested plants compared with virus tested plants graft inoculated with SPFMV under two agro-ecologies during the 1st field trial

Cultivar	Virus status	MURARIK				NASARRI					
		Yield (Kg/m ²)		Root numbers (per mounds)		Yield loss (%)	Yield (Kg/m ²)		Root numbers (per mounds)		Yield loss (%)
		Total	Marketable	Total	Marketable		Total	Marketable	Total	Marketable	
Kabode	H	2.0a	1.8a	6.4a	5.2a	0.0	1.1a	0.8a	3.7a	2.2a	0.0
	FM	2.6a	2.3a	7.5a	5.7a	-30.0	1.3a	1.0a	4.3a	3.1a	-18.2
NASPOT 1	H	3.0a	2.6b	9.9a	6.1a	0.0	2.3a	2.1a	5.3a	4.9a	0.0
	FM	3.8a	3.7a	8.6a	6.5a	-26.6	1.4b	1.1b	3.7a	2.8b	39.1
Ejumula	H	2.1a	1.4a	12.4a	4.9a	0.0	0.7a	0.2a	3.1a	0.4a	0.0
	FM	1.9a	1.3a	13.3a	4.4a	10	0.6a	0.1a	2.8a	0.5a	14.3
Dimbuka	H	2.7b	2.1b	15.1a	6.0b	0.0	1.4a	1.1a	7.9a	4.0a	0.0
	FM	4.4a	4.0a	15.5a	10.2a	-63	0.9a	0.6b	3.3b	1.7b	35.7
Beauregard	H	1.2a	0.7a	12.3a	3.1a	0.0	0.6a	0.0a	5.3a	0.0a	0.0
	FM	0.7a	0.2a	5.8b	1.1a	41.7	0.2b	0.0a	1.5b	0.0a	66.7

H= virus indexed material (healthy), FM= virus indexed material graft inoculated with SPFMV. Cultivar treatment in a column with the same letters are not significantly different. Column with the same letter for individual cultivar is not significantly different. A negative figure implies a percentage yield gain in a column of a yield loss

4.3.5 Cumulative effects of SPFMV on the yield of four sweetpotato varieties in two agro-ecologies

For the second field trial, significant differences were detected among cultivars, vine status and interaction between cultivars by vine status in both locations for total yield, marketable yield, total number of storage root and marketable number of storage roots (Table 11). In both locations, the yield of the healthy controls was greater than the SPFMV infected ones. For the MUARIK trial, the newly SPFMV-infected materials from the screenhouse had more total and marketable yield and storage root number than the SPFMV infected materials obtained from 1st field trial. The total yield loss due to SPFMV infected material from screenhouse ranges between 0% and 23.8% while the total yield loss due to SPFMV infected materials obtained from the 1st field trial ranges between 14% and 26.1% (Table 12). The exception was in cultivar Kabode in which the field exposed material had greater yield than screenhouse infected materials though statistically not different from the healthy control. For the NaSARRI trial, SPFMV infected materials from the 1st field trial yielded more than the screenhouse infected materials planted in the field for the first time in all the cultivars except Dimbuka. The total yield loss due to screenhouse infected materials ranged between 33% and 42% while that due to field exposed material ranged between 26%-48% (Table 12). The total root numbers within cultivars were not different but the marketable root numbers were different for NaSARRI trial. Dimbuka had more storage roots, followed by NASPOT 1, Ejumula and least in Kabode (Table 12).

Table 11: ANOVA to test for the difference on yield and storage root number of four sweetpotato varieties grown from virus tested plants compared with SPFMV inoculated plants from screenhouse and SPFMV infected cuttings from 1st field trials for both MUARIK and NaSARRI during 2nd field trials

Source of variation	d.f	Mean square							
		MUARIK				NaSARRI			
		Total yield	Marketable yield	Total root number.	Marketable root number.	Total yield	Marketable yield	Total root number	Marketable root number.
Rep	2	8.67	7.039	281.11	32.87	1.9376	1.6676	29.27	35.317
Cultivar	3	1.44	5.25	161.35**	3.62	3.2211**	3.7855**	196.73**	35.919**
Vine status	2	0.37	0.74	16.71	10.09	10.358**	13.498**	18.22	96.867**
V x C	6	3.32*	2.96	35.56*	15.48*	0.2392	0.2586	7.21	12.719
Error terms	166	1.07	1.42	15.86	6.85	0.2485	0.2369	11.73	5.394
Total	179								

Rep = Replicate, Vx C= Vine status by Cultivar interaction, d.f = degree of freedom, * and ** = significant at 0.05 and 0.001

Table 12: Mean yield and storage root number of four sweetpotato varieties grown from virus tested plants compared with SPFMV inoculated plants and previously field exposed SPFMV infected materials under two agro-ecologies during the 2nd field trials

Cultivar	Virus status	MUARIK						NaSARRI					
		Yield (Kg/m ²)		Root number (per mound)		Yield loss (%)		Yield (Kg/m ²)		Root numbers (per mound)		Yield loss (%)	
		total	marketable	total	marketable			total	marketable	total	marketable		
Kabode	H	2.1 ^a	1.6 ^a	6.7 ^{ab}	3.6 ^a	0.0		1.5 ^a	1.5 ^a	5.6 ^a	4.7 ^a	0.0	
	FM ₁	1.6 ^b	1.2 ^a	5.0 ^b	2.7 ^a	23.8		1.0 ^b	0.9 ^b	6.7 ^a	4.2 ^a	33.3	
	FM ₂	2.7 ^a	2.1 ^a	8.1 ^a	4.5 ^a	-28.6		1.1 ^{ab}	1.0 ^b	5.9 ^a	4.3 ^a	26.7	
NASPOT 1	H	2.8 ^a	1.7 ^a	4.9 ^a	3.3 ^a	0.0		2.4 ^a	2.3 ^a	9.7 ^a	7.9 ^a	0.0	
	FM ₁	2.5 ^a	1.8 ^a	7.7 ^a	3.2 ^a	10.7		1.4 ^b	1.2 ^b	8.4 ^b	5.8 ^b	41.6	
	FM ₂	2.4 ^a	2.1 ^a	6.1 ^a	4.1 ^a	14.3		1.7 ^b	1.5 ^b	8.1 ^b	5.7 ^b	29.2	
Ejumula	H	2.3 ^a	1.5 ^a	7.5 ^a	4.2 ^a	0.0		2.1 ^a	2.1 ^a	8.9 ^a	7.3 ^a	0.0	
	FM ₁	1.8 ^{ab}	1.4 ^a	9.7 ^a	3.2 ^a	22.7		1.3 ^b	1.1 ^b	7.7 ^a	4.5 ^b	38.1	
	FM ₂	1.5 ^b	0.8 ^b	7.5 ^a	2.4 ^a	34.8		1.5 ^b	1.3 ^b	8.1 ^a	5.7 ^{ab}	28.6	
Dimbuka	H	2.3 ^a	1.6 ^a	10.9 ^a	4.2 ^a	0.0		1.9 ^a	1.7 ^a	12.3 ^a	8.7 ^a	0.0	
	FM ₁	2.3 ^a	1.3 ^{ab}	11.5 ^a	3.3 ^{ab}	0.0		1.1 ^b	0.8 ^b	10.9 ^a	5.2 ^b	42.1	
	FM ₂	1.7 ^a	0.6 ^b	8.7 ^a	1.5 ^b	26.1		1.0 ^b	0.8 ^b	10.1 ^a	4.3 ^b	47.4	
LSD (5%)		0.75	0.86	2.87	1.88			0.36	0.35	2.47	1.67		

H= virus indexed material (healthy), FM₁= SPFMV-infected cutting obtained from screenhouse and grown in the field for the first time. FM₂= SPFMV-infected cuttings obtained from 1st trial and grown in the field for the second time

Cultivar treatment in a column with the same superscript letters are not significantly different. LSD = least significant difference at 5% probability level

A negative figure implies a percentage yield gain in column of a yield loss

4.3.6 Effects of single or mixed infection by SPFMV and SPCSV on the yield under field conditions

In general, the total yield, marketable yield, total storage root number and marketable root number were significantly influenced by seasons within location, cultivars tested, the viruses used and their interactions (Table 13). Storage root yield was greater in field trial 4 than in field trial 3 in both locations. Combination of SPFMV and SPCSV severely reduced the yield followed by the single infection of SPCSV and least in single infection by SPFMV and healthy controls.

The total and marketable yields of sweetpotato cultivars singly infected by SPFMV were less than the yields from healthy controls in field trial 3 and 4 in both locations except for NASPOT 1 and Ejumula trial 4 at MUARIK although these differences were not statistically significant. The total yield loss due to SPFMV ranged between 7% and 39% for all the cultivars in both locations except for NASPOT 1 and Ejumula during the 4th field trial at MUARIK where there was yield gain of 111.1 and 114% respectively. SPFMV alone did not affect the total number of storage root but reduced the marketable root number in all the cultivars in both seasons and locations except for cultivars NASPOT 1 and Ejumula in the 4th field trial where the total number of storage roots was less than in the healthy control (Table 14).

Single infection by SPCSV significantly reduced the total and marketable yield and total and marketable number of storage roots as compared to healthy plant in both locations and in all the field trials (Table 13). The yield loss due to SPCSV ranged between 14% - 60% for all the cultivars tested in both locations for 3rd and 4th field trial (Table 14). The marketable yield, total storage root number and marketable storage root number were less in SPCSV infected plants compared to the healthy control. Cultivars, Ejumula and Dimbuka had the greatest total yield loss due to SPCSV during 3rd field trials in both locations. In the 4th field trial, Dimbuka had greatest yield loss (45%) at MUARIK while at NaSARRI, yield loss was greater in cvs. NASPOT 1 and Ejumula (50%) (Table 14). Co-infection of SPFMV and SPCSV had an even larger effect on total yield, marketable yield and total and marketable storage root number. The yield loss ranged between 60% and 95% with Ejumula and Dimbuka having the greatest total yield loss in both locations in all seasons (Table 14).

Table 13: ANOVA to test for the difference on yield and storage root number of four sweetpotato varieties grown from virus tested plants compared with virus tested plants graft inoculated with SPFMV, SPCSV and combination of the two viruses under two agro-ecologies during the 3rd and 4th field trial

Source of d.f	Mean square					
	MUARIK			NASARRI		
	Total yield	Marketable yield	Total root number	Marketable root number	Total yield	Marketable yield
3rd field trial						
Block	2	3.7200	3.1876	42.954	44.254	0.4720
Cultivar	3	0.3968	0.3929	80.106**	2.200	1.2968**
Vine status	3	18.4368**	9.3010	298.539**	82.444**	25.149**
VX C	9	0.6927*	0.3400**	36.465**	1.837	0.9532**
Error	222	0.2267	0.2707	7.582	2.788	0.1455
Total	239					
4th field trial						
Block	2	1.8858	1.7176	25.317	9.404	1.0073
Cultivar	3	1.9196*	2.2783*	331.949**	69.015**	0.2174
Vine status	3	59.4037**	61.7262**	204.971**	411.626**	29.7908**
VX C	9	1.2569*	1.1414	17.463	15.663*	1.3570**
Error	222	0.6496	0.6598	9.519	5.085	0.1938
Total	239					

VXC= interaction of vine status by cultivar, * and ** = significant at 0.05 and 0.001 respectively

NASPOT 1	SPCSV	1.9 ^b	1.8 ^c	5.3 ^a	3.7 ^b	38.7	1.3 ^a	1.2 ^a	5.2 ^{ab}	4.1 ^a	18.8
	SPVD	0.7 ^c	0.6 ^d	3.7 ^b	1.9 ^c	77.4	0.7 ^b	0.6 ^b	4.5 ^b	2.5 ^b	56.3
	Healthy	2.7 ^a	2.6 ^a	7.9 ^a	6.9 ^a	0.0	2.2 ^a	2.0 ^a	10.5 ^a	8.2 ^a	0.0
	SPFMV	3.0 ^a	2.9 ^a	7.3 ^a	6.5 ^a	-11.1	1.8 ^b	1.6 ^b	8.0 ^c	5.4 ^b	18.2
Ejumula	SPCSV	2.0 ^b	1.9 ^b	7.1 ^a	5.7 ^a	25.9	1.1 ^c	1.0 ^c	5.7 ^c	4.7 ^b	50.0
	SPVD	0.4 ^c	0.3 ^c	3.0 ^b	1.5 ^b	85.2	0.1 ^d	0.0 ^d	1.3 ^d	0.0 ^c	95.4
	Healthy	2.1 ^{ab}	2.1 ^{ab}	7.9 ^a	7.1 ^a	0.0	2.1 ^a	1.9 ^a	10.9 ^a	8.7 ^a	0.0
	SPFMV	2.4 ^a	2.3 ^a	9.5 ^a	8.7 ^a	-14.3	1.4 ^b	1.1 ^b	8.0 ^b	4.9 ^b	33.3
Dimbuka	SPCSV	1.8 ^b	1.6 ^b	7.9 ^a	5.1 ^b	14.3	1.0 ^c	0.8 ^b	6.3 ^b	4.0 ^b	52.4
	SPVD	0.4 ^c	0.2 ^c	3.7 ^b	1.0 ^c	80.9	0.3 ^d	0.2 ^c	2.8 ^c	1.2 ^c	85.7
	Healthy	3.1 ^a	3.0 ^a	13.4 ^a	10.3 ^a	0.0	2.3 ^a	2.0 ^a	13.0 ^a	9.8 ^a	0.0
	SPFMV	2.5 ^b	2.3 ^b	11.5 ^a	8.3 ^b	19.3	1.4 ^b	1.2 ^b	11.6 ^a	7.7 ^b	39.1
S.E	SPCSV	1.7 ^c	1.5 ^c	9.1 ^b	5.7 ^c	45.2	1.6 ^b	1.3 ^b	11.5 ^a	7.5 ^b	30.4
	SPVD	0.8 ^d	0.6 ^d	8.8 ^b	3.1 ^d	74.2	0.1 ^c	0.0 ^c	1.7 ^b	0.0 ^c	95.7
		0.208	0.2097	0.797	0.582		0.1114	0.1137	0.672	0.475	
	LSD	0.580	0.5845	2.220	1.623		0.3105	0.3168	1.872	1.323	

Cultivar treatment in a column with the same superscript letters are not significantly different. S.E = standard error of means, LSD = least significant difference at 5% probability level
A negative figure implies a percentage yield gain in a column of yield loss, SPFMV=sweet potato feathery mottle, SPCSV=Sweet potato chlorotic stunt virus, SPVD= combination of SFMV and SPCSV

4.3.7 Effects of SPFMV and SPCSV on sweetpotato root length and diameters

Significant differences were detected ($P \leq 0.05$) in the length and diameter of storage roots due to cultivar, virus infection and their interaction (Table 15). Both single infection and co-infection of SPFMV and SPCSV reduced the diameter of storage roots. Storage roots of plants infected by both SPFMV and SPCSV had the smallest diameter followed by that infected by SPCSV alone and then SPFMV (Table 16). Healthy plants overall, storage roots with the greatest diameter and therefore less length diameter ratio were produced by the healthy control plants.

Table 15: ANOVA table for length and diameter of sweetpotato cultivars under different virus infection at MUARIK and NaSARRI during 3rd and 4th field trial

Source of variation	d.f	Mean square					
		MUARIK			NaSARRI		
		Length	Diameter	L/D	Length	Diameter	L/D
Field trial 3							
Block	2	2.711	34.353	0.069	7.146	6.038	0.017
Cultivar	3	9.757	51.210*	0.267**	1.156	14.607	0.021
Virus	3	48.971**	199.301**	0.273**	29.345**	241.310**	0.558**
CXV	9	6.464	15.382	0.019	4.320	23.291*	0.034
Error	30	3.737	9.213	0.017	3.544	6.601	0.017
Total	47						
Field trial 4							
Block	2	6.891	9.589	0.033	8.206	1.317	0.011
Cultivar	3	45.030**	22.467*	0.142**	51.316**	4.312	0.123**
Virus	3	113.706**	195.039**	0.058*	139.273**	142.821*	0.037
CXV	9	4.131	2.783	0.008	9.768**	6.542	0.006
Error	30	1.942	3.996	0.009	2.059	2.932	0.016
Total	47						

*, ** significantly different at 0.05 and 0.001. L/D= Length Diameter ratio

Table 16: Summary of mean length and diameter of storage roots of sweetpotato cultivars under different virus infection during 3rd and 4th field trial in two locations of MUARIK and NaSARRI

NaSARRI		MUARIK			NaSARRI		
Cultivar	Virus infection	Length	Diameter	L/D	Length	Diameter	L/D
Field trial3							
Kabode	Healthy	22.0 ^a	20.1 ^a	1.1 ^b	16.9 ^a	19.4 ^a	0.9 ^b
	SPFMV	18.4 ^b	17.3 ^a	1.1 ^b	15.4 ^a	17.8 ^a	0.9 ^b
	SPCSV	14.3 ^c	11.8 ^b	1.3 ^{ab}	14.5 ^a	19.6 ^a	0.8 ^b
	SPVD	15.5 ^{bc}	10.3 ^b	1.5 ^a	14.6 ^a	11.6 ^b	1.3 ^a
NASPOT 1	Healthy	18.3 ^a	22.9 ^a	0.8 ^b	19.3 ^a	24.4 ^a	0.8 ^b
	SPFMV	14.4 ^{bc}	17.1 ^b	0.9 ^{ab}	16.8 ^{ab}	20.1 ^b	0.8 ^b
	SPCSV	16.7 ^{ab}	18.2 ^{ab}	0.9 ^{ab}	14.7 ^{bc}	15.9 ^{bc}	0.9 ^{ab}
	SPVD	12.6 ^c	11.9 ^c	1.1 ^a	12.6 ^c	11.8 ^c	1.1 ^a
Ejumula	Healthy	17.8 ^a	15.9 ^a	1.2 ^a	16.8 ^a	18.9 ^{ab}	0.9 ^b
	SPFMV	16.1 ^a	13.7 ^a	1.2 ^a	15.2 ^a	15.8 ^{bc}	1.0 ^b
	SPCSV	16.1 ^a	14.9 ^a	1.1 ^a	15.6 ^a	20.5 ^a	0.8 ^b
	SPVD	14.7 ^a	11.3 ^a	1.3 ^a	15.2 ^a	12.3 ^c	1.3 ^a
Dimbuka	Healthy	18.8 ^a	26.9 ^a	0.8 ^b	16.5 ^a	22.1 ^a	0.7 ^b
	SPFMV	15.1 ^b	17.1 ^b	0.9 ^b	16.6 ^a	24.4 ^a	0.7 ^b
	SPCSV	15.6 ^b	16.4 ^b	1.0 ^{ab}	15.3 ^{ab}	21.5 ^a	0.7 ^b
	SPVD	15.7 ^{ab}	12.8 ^b	1.2 ^a	12.3 ^b	9.2 ^b	1.4 ^a
S.E		1.116	1.752	0.0763	1.537	1.483	0.0763
LSD		3.223	5.061	0.2203	3.139	4.284	0.2205
Field trial4							
Kabode	Healthy	25.0 ^a	25.6 ^a	1.0 ^b	25.1 ^a	19.3 ^a	1.3 ^a
	SPFMV	25.4 ^a	22.3 ^b	1.1 ^{ab}	20.9 ^b	16.1 ^b	1.3 ^a
	SPCSV	24.1 ^a	21.4 ^b	1.1 ^{ab}	20.1 ^b	15.6 ^{bc}	1.3 ^a
	SPVD	18.4 ^b	15.9 ^c	1.2 ^a	19.8 ^b	13.9 ^c	1.4 ^a
NASPOT 1	Healthy	21.7 ^a	25.8 ^a	0.8 ^c	25.1 ^a	22.0 ^a	1.1 ^c
	SPFMV	22.7 ^a	23.8 ^{ab}	0.9 ^{ab}	21.8 ^b	18.2 ^b	1.2 ^b
	SPCSV	21.2 ^a	21.8 ^b	1.0 ^a	20.6 ^b	17.2 ^b	1.2 ^b
	SPVD	16.0 ^b	15.8 ^c	1.0 ^a	13.8 ^c	10.0 ^c	1.4 ^a
Ejumula	Healthy	24.1 ^a	21.6 ^a	1.1 ^a	24.3 ^a	19.8 ^a	1.2 ^a
	SPFMV	24.1 ^a	19.9 ^a	1.2 ^a	22.1 ^{ab}	18.8 ^a	1.2 ^a
	SPCSV	23.3 ^a	19.8 ^a	1.2 ^a	20.5 ^b	17.1 ^a	1.2 ^a
	SPVD	15.7 ^b	13.6 ^b	1.2 ^a	14.7 ^c	11.5 ^b	1.3 ^a
Dimbuka	Healthy	20.5 ^a	24.6 ^a	0.8 ^b	17.9 ^a	17.7 ^a	1.0 ^a
	SPFMV	20.9 ^a	22.9 ^a	0.9 ^b	19.1 ^a	17.4 ^a	1.1 ^a
	SPCSV	17.2 ^b	18.4 ^b	0.9 ^b	18.1 ^a	16.3 ^a	1.1 ^a
	SPVD	16.2 ^b	14.5 ^c	1.1 ^a	11.9 ^b	10.8 ^b	1.1 ^a
S.E		0.804	1.154	0.056	0.829	0.989	0.0742
LSD		2.323	2.333	0.1625	2.393	2.855	0.2144

Column with the same superscript letters for each cultivar is not significantly different. S.E = standard error of means, LSD = Least significant difference at 5% probability level, L/D= length diameter ratio

4.4 Discussion

Symptom expression differed with the infecting viruses and cultivars. SPFMV infected plants produced mild symptoms in all the cultivars, which later disappeared. This suggests that these cultivars have different levels of resistance to SPFMV infection. Such resistance may have been developed through unintentional selection by farmers (for landraces) or breeders growing crops under strong disease pressure and high vector population. This finding supports reports that most Ugandan cultivars are resistant to SPFMV and when infected can revert to healthy status (Gibson *et al* 1997; Mukasa *et al.*, 2006; Wasswa., 2012; Gibson and Kreuze, 2015). This reversion was further confirmed when some of the previously infected plants from the field at NaSARRI tested negative for SPFMV using NCM-ELISA and when grafted on *I. setosa*. Also reversion from SPFMV was evident at MUARIK where initially SPFMV infected plants did not develop SPVD when infected naturally with SPCSV, instead testing negative for SPFMV at the end of the trial. Beauregard which is a cultivar from America is very susceptible to SPFMV (Bryan *et al.*, 2003; Clark and Hoy, 2006), it was discarded for later trials. This cultivar cannot be used by farmers in Uganda because it cannot be maintained, reaching 100% infection by both viruses within one season (Table 8).

Infection by SPCSV alone induced clear symptoms under field conditions in all the cultivars. Typical symptoms of SPCSV observed were chlorotic spots, purpling and yellowing of middle and mature leaves similar to symptoms reported by Gibson *et al.* (1998). Co-infection of SPFMV and SPCSV resulted in severe disease symptoms due to a synergistic interaction between them (Gibson *et al.*, 1998; Untiveros *et al.*, 2007).

The titre of both SPFMV and SPCSV was high in co-infected plants as compared to single infected plants for all the cultivars evaluated, suggesting that the symptoms observed were the result of higher accumulation of both viruses in infected plants. This is contradicting the previous finding that the titres of SPCSV in co-infected plants either declines or remain constant (Karyeija *et al.*, 2000; Kokkinos and Clark, 2006b). However, their finding was based on experiments under screenhouse conditions. In this experiment, field conditions might have influenced virus replication. In addition, other viruses such as begomoviruses which were not evaluated in this study and are known to be common in some cultivars in Uganda (Wasswa *et al.*, 2011) could have infected the plants leading to synergistic interaction among them hence higher titre (Cuellar *et al.*, 2015). Also SPCSV seems to be the major determinant in symptom development as most of the plants infected by SPCSV alone displayed very clear symptoms and produced high titre value indicating their accumulation in

plants. On the other hand SPFMV when infecting alone did not easily accumulate in the plant, and therefore, little or no symptoms were produced.

Effects of single infection of SPFMV on yield of sweetpotato were variable ranging from better apparent yield performance to yield loss as high as 40% depending on the agro-ecology, season or cultivar. Yield of cultivars NASPOT 1, Dimbuka and Kabode infected with SPFMV were greater or did not differ from healthy controls at MUARIK. This could be because the healthy control became largely infected by SPFMV but also SPCSV whilst some of the plants initially infected with SPFMV reverted to healthy so was able to compensate for the observed yield. It could also be that prior infection with SPFMV conferred a specific protection against itself thus limiting its multiplication and accumulation in the infected plants. Alternatively it could have conferred nonspecific protection against other viruses probably due to more activated RNA silencing pathway (Kreuze *et al.*, 2005). Similar results have also been reported in Peru (Gutierrez *et al.*, 2003) and Israel (Milgram *et al.*, 1996). This finding justifies why these varieties are widely grown by farmers in central Uganda where high virus disease pressure occurs and therefore supports the use of field derived planting material of resistant or tolerant varieties to reduce the impact of the virus. However, previously field exposed material when re-used in the subsequent season yielded less than freshly SPFMV infected material raised in a screenhouse especially in the susceptible cultivar Ejumula. When tested using ELISA, most of the sweetpotato plants were positive for SPCSV and accumulation of this virus may have resulted in less yield. This therefore discourages farmers from obtaining cuttings from their field for such a susceptible variety in areas with high virus incidence like MUARIK.

At NaSARRI however, SPFMV reduced yield of all cultivars tested despite less visible virus symptoms and the initially healthy control largely remaining healthy in contrast to what was observed at MUARIK (Figure 5). The lower infection rate in the healthy control could be a result of few aphid vectors of SPFMV or few whitefly vectors of SPCSV so that there were few SPVD- affected plants to act as excellent sources of SPFMV.

Single infection of SPCSV significantly reduced the yield of all sweetpotato cultivars in both locations and proved clearly to be the most economically important virus in the central region of Uganda. The yield loss ranged from 14% to 60% in all the cultivars tested in two locations. In other studies, yield losses of between 15% and 88% have been reported (Milgram *et al.*, 1996; Gibson *et al.*, 1998; Gutierrez *et al.*, 2003; Njeru *et al.*, 2004; Untiveros *et al.*, 2007).

However, the situation was worse when SPCSV co-infected with SPFMV, resulting in yield losses ranging from 60%-95% similar to that reported earlier (Sheffield, 1957; Schaefer and Terry, 1976; Milgram *et al.*, 1996; Gibson *et al.*, 1998; Gutierrez *et al.*, 2003). The reduced root yield in sweetpotato has been attributed to decrease in size of photosynthetic organs resulting from severe stunting and other symptoms of SPVD due to synergistic interaction (Hahn *et al.*, 1981; Njeru *et al.*, 2004).

In addition to yield loss, planting virus infected cuttings also reduced the root quality in terms of size and number of roots produced. Virus infected plants produced roots with small diameter and therefore giving higher ratios of length/diameter as compared to the healthy control plants. The length/diameter ratio was greatest in co-infection followed by SPCSV infection and least with SPFMV infection. This result is consistent with other studies, for example, Kano and Nagata (1999) found that SPFMV infected plants produce roots with a small diameter than storage roots produced from healthy plants. Similarly Bryan *et al.* (2003) reported that SPFMV infected plants produce roots with greater length diameter ratio compared to healthy control. On the other hand, the total number of storage roots was significantly less in SPCSV infected plants and plants infected by combinations of SPFMV and SPCSV compared to healthy controls.

The variation in yield and number of storage roots of the same cultivar in different locations or seasons could be due to differences in environmental factors. Sweetpotato yield is greatly influenced by the environment (Collins *et al.*, 1987; Kanua *et al.* 1988; Bryan *et al.*, 2003). The widely differing environmental conditions between MUARIK and NaSARRI undoubtedly impacted the yield of controls and virus infected plants. At MUARIK rainfall is uniformly distributed throughout the year favouring production of sweetpotato and thus better yield as compared to NaSARRI which is hot and dry in most part of the year thus reducing the yield of sweetpotatoes.

The continuous production of sweetpotato at MUARIK allows survival of pathogens and their vectors; thus, continuous disease spread leading to virus accumulation in the plant as was evidence by the high disease severity. This was further supported by the fact that healthy controls became infected and some of the SPFMV infected plants developed SPVD symptoms by the end of the growing season despite the use of pesticides to control whiteflies in the 3rd and 4th trials at MUARIK. This implies that pesticides may not be of use in managing virus vectors. More emphasis should instead be put on breeding and growing

resistant cultivars for such areas with high vector and disease pressure. In NaSARRI, however, the hot dry spell in some months doesn't allow sweetpotato production thus breaking the pathogen and pest cycle and may be the cause of reduced virus spread. Its worth noting that no pesticides were applied in this area but most of the healthy controls did not develop virus symptoms and tested negative with ELISA and *I. setosa*. This, therefore, implies that both susceptible and resistant varieties can successfully be grown in this area. Further, field multiplication of virus indexed sweetpotato materials can be carried out in this area provided drought is addressed.

CHAPTER FIVE

VIRUS MOVEMENT FROM INFECTED SWEETPOTATO VINES TO ROOTS AND SYMPTOM EXPRESSION ON ROOT SPROUTS

5.1 Introduction

Sweetpotato propagation in Uganda usually involves the use of vine cuttings from mature fields obtained from a farmer's own garden or from the neighbours' (Bashaasha *et al.*, 1995; Gibson *et al.*, 2009). This however, may not be the case in areas with a long dry season in which the existing sweetpotato fields dry out, resulting in a lack of planting material at the onset of the next rains (Gibson *et al.*, 2011). Most farmers in such areas tend to wait for the surviving roots in the soil to sprout in order to obtain planting materials leading to late planting (Yanggen and Nagguja, 2006; Gibson *et al.*, 2009; Namanda *et al.*, 2011; Namanda, 2012). The health status of such sprouts is not known and, if infected by virus, they may act as a local source of inoculum from which insect vectors can quickly spread it. This may result in virus accumulation and perpetuation over generations thus reducing sweetpotato quality and yield (Karyeija *et al.*, 1998a; Gibson *et al.*, 2009).

To overcome the challenge of late planting by ensuring timely access to planting material in areas prone to drought, a method of sprouting storage roots has been developed (Namanda, 2012) and is being promoted in Uganda, Tanzania, and Malawi by the International Potato Centre (CIP). The source of seed roots used by farmers for this technology is from their fields and in most cases farmers do the selection after harvesting the entire field, implying that, the health status of the mother plants of selected roots is not known. In addition, some farmers have the tendency of getting roots that have desired attributes from neighbours or market places and sprouting them to generate planting materials. A major limitation with using storage root as source of planting material is that little or no information is available on the virus status of roots and therefore the decision on which root to save as seed is difficult.

Sweet potato feathery mottle virus (SPFMV) and *Sweet potato chlorotic stunt virus* (SPCSV) are the major economically important sweetpotato viruses in Uganda although other viruses like *Sweet potato mild mottle virus* (SPMMV), *Sweet potato caulimo like virus* (SPCaIV), *Sweet potato chlorotic fleck virus* (SPCFV) and *Sweet potato leaf curl Uganda virus* (SPLCUV) have also been reported (Aritua *et al.*, 2000; Mukasa *et al.* 2003; Aritua *et al.*, 2007; Ndunguru *et al.*, 2009; Wasswa *et al.*, 2011). Single infection by these viruses causes mild or no foliar symptoms and this has made selection against infected material difficult

leading to their continued use. Vines infected by a single virus especially SPFMV have the ability to revert to healthy status but whether roots or root sprouts can equally revert when infected by either SPFMV or SPCSV is not clear and requires investigation. Co-infection of SPFMV and SPCSV results in severe disease symptoms characterized by a combination of vein clearing, chlorosis, leaf purpling, mosaic, leaf distortion, mottling and complete plant stunting described as sweetpotato virus disease (SPVD) (Milgram *et al.*, 1996; Gibson *et al.*, 1998; Gutierrez *et al.*, 2003). These clear symptoms make it easy for a farmer to select against such vines when sourcing for planting materials. It is however not known if the storage roots derived from infected vines among cultivars grown by Ugandan farmers can produce symptomatic sprouts to enable farmers rogue them out during the time of sprouting in screenhouse before field planting. In some USA sweetpotato cultivars like Beauregard, roots show external cracking and internal corkiness when singly infected with SPFMV and when planted, the virus symptoms on sprouts are very clear making it easier to select against such roots (Clark and Moyer 1988; Gutierrez *et al.*, 2003). This study was designed to determine whether viruses (single or mixed infection) can move from infected vines to roots and whether the infected sprouts from roots can revert to healthy status.

5.2 Materials and methods

5.2.1 Sources of roots

Three sources of roots were used in this experiment: Roots randomly selected from already harvested sweetpotato; roots obtained from symptomless plants selected prior to harvesting; and roots generated by planting sweetpotato vines and graft inoculating them with SPFMV and SPCSV under screenhouse conditions.

5.2.2 Sweetpotato roots selected from already harvested sweetpotato as source of planting material in areas with high and low disease pressure

In this trial, sweetpotato roots sourced from two locations, MUARIK and NaSARRI were evaluated. The roots were randomly selected from amongst already harvested ones that were physically good with no defect. Thirty (30) roots of each of the four cultivars NASPOT 1, Ejumula, Dimbuka and Beauregard were selected. The roots were planted in plastic pots (one root per pot) filled with a mixture of soil, sand and animal manure in equal proportions and arranged following a randomized complete block design and left to sprout in an insect proof under screenhouse. One month after sprouting, a visual assessment of the sprouts for virus symptoms was done and disease incidence recorded. The root sprouts were tested using

NCM-ELISA to detect and identify some of the viruses present in the roots. The symptomless root sprouts were grafted on *I. setosa* and then tested with NCM ELISA to detect any latent infection.

5.2.3 Roots sourced from symptomless plants selected prior to harvesting

The second source of roots was obtained by selecting mounds with symptomless plants prior to harvesting in two locations (MUARIK and NaSARRI). The selected plants were harvested and 40 roots per cultivar with no physical damage were obtained from each location and planted as already described in 5.2.2 above. In this trial three sweetpotato cultivars, NASPOT 1, Dimbuka and Ejumula were sampled. One month after sprouting, the root sprouts were assessed for virus symptoms through visual observation and the virus present confirmed using NCM ELISA. Data were recorded on the number of root sprouts and number of plants with disease symptoms.

5.2.4 Reaction of sweetpotato cultivars to virus infection and generation of sweetpotato roots under screenhouse condition

Virus indexed sweetpotato cultivars Dimbuka, Ejumula, Kabode and NASPOT 1 were planted in a screenhouse. 20 cuttings of each cultivar (80 cuttings in total) were planted in pots containing a mixture of soil, sand and animal manure in equal proportions. Plants were left to grow for a period of one month and thereafter graft inoculated with an appropriate virus. Before inoculation, each cultivar was divided into four groups each having five plants. One group was graft inoculated with SPFMV, a 2nd group was graft inoculated with SPCSV, a 3rd group was graft inoculated with combination of SPFMV and SPCSV and a 4th group was mock inoculated as controls. These plants were left to grow for four months during which their response to virus infection was monitored visually. The grafted plants were also tested by grafting their shoots to the indicator plants *I. setosa* and NCM-ELISA was done to confirm the presence of the viruses. After four months, the roots were harvested and their weight recorded. The harvested roots were used to evaluate whether viruses moved from infected shoots to roots. This experiment was done twice.

5.2.5 Evaluation of virus movement from roots to sprouts

The harvested roots above were evaluated to determine whether roots from infected plants can produce infected sprouts. In this trial, 10 roots from each treatment per cultivar (160 roots in total) were planted in pots containing equal proportion of sand, soil and manure and left to sprout under screenhouse condition. One month after sprouting, the root sprouts were

assessed for the number of shoots produced and virus symptoms expressed. The symptomatic plants were tested by DAS and TAS ELISA to confirm whether symptoms expressed were actually due to virus infection. Also asymptomatic shoots were grafted onto *I.setosa* to rule out latent infection. The sprouted shoots were left to grow for a further four months in a screenhouse during which progress on symptom expression and disease severity were monitored. At five months before the termination of the experiment, the shoots were grafted onto *I.setosa* and also tested by ELISA to determine if there was any reversion.

5.2.6 Virus detection and quantification by ELISA

Two types of virus tests were used. One involved grafting *I. setosa* seedlings and observing whether symptoms developed. This was considered to be a very sensitive test for virus infection but relatively poor at identifying which virus was involved. The second type involved ELISA tests, which identified the specific virus involved. Three ELISA methods were used: NCM, DAS and TAS ELISA. NCM ELISA test was conducted to detect and identify the viruses that were present in the sprouts from field derived roots. The standard protocol for the NCM ELISA kit obtained from CIP Lima was followed and the results were based on visual assessment of the colour change. DAS and TAS ELISA were used to detect and estimate the virus load due to SPFMV and SPCSV in the samples following the protocol by Clark and Adams (1977). All the antibodies used for both DAS (IgG, and IgG-AP-Conjugate) and TAS ELISA (IgG, monoclonal antibodies-MAb and RAM-AP) were from Leibniz-Institut DSMZ-Deutsche sammlung von Mikroorganismen und Zellkulturen GmbH, Germany. The p-nitrophenyl phosphate and micro plates were from Sigma Chemical Co. The absorbances were measured at 405nm after one and a half hours using a micro plate reader (model 680).

5.3 Results

5.3.1 Effects of selecting roots as a source of planting material from high and low virus pressure areas.

Sweetpotato roots that were randomly selected after field harvesting from MUARIK all developed virus symptoms (Plate 1 A, B and C). The symptoms observed were combinations of vein clearing, mosaic, distorted leaf shape and stunted growth. These symptomatic root sprouts when tested by NCM ELISA mostly reacted positively for both SPFMV and SPCSV (Table 17). In addition, the sprouting capacity of some roots was poor especially for cv Dimbuka at MUARIK (Table 17) where only 19 roots sprouted while the rest of the roots rotted. In Serere, however the sprouting capacity of roots was over 90% in all the cultivars

tested (Table 17). For sweetpotato roots from NaSARRI that were obtained after field harvest, no virus symptom was visible on the sprouts of NASPOT 1, Dimbuka and Ejumula (Plate 1 D, E, F), respectively. The exception was in cultivar Beauregard where all the root sprouts developed typical virus symptoms which when tested by NCM ELISA, indicated the presence of both SPFMV and SPCSV. Some of the symptomless sprouts when grafted onto *I. setosa* developed characteristic feathery mottle symptoms (Plate 1 G, H and I) and the virus was confirmed with ELISA.



Plate 1: symptom expression on root sprouts from roots randomly selected after harvesting for different sweetpotato cultivars from two locations A) Symptomatic root sprout of NASPOT 1 from MUARIK B) symptomatic root sprout of Dimbuka from MUARIK, C) symptomatic root sprout of Ejumula from MUARIK, D) asymptomatic root sprout of NASPOT 1 from NaSARRI, E) asymptomatic root sprout of Dimbuka from NaSARRI and F) asymptomatic root sprout of Ejumula from NaSARRI. G) Vein clearing H) vein banding and I) vein chlorosis

Table 17: Proportion of sweetpotato sprouts tested positive for virus infection from root samples randomly selected after field harvest from MUARIK and NaSARRI

Cultivar	Number of roots planted	Roots from MUARIK				Roots from NaSARRI			
		Number of roots sprouted	Visual virus symptoms on root sprout	% root sprouts positive by <i>I. setosa</i> and NCM ELISA		Number of root sprouted	Visual virus symptoms on root sprout	% root sprout positive by <i>I. setosa</i> and ELISA	
				(%)	SPFMV	SPCSV	(%)	SPFMV	SPCSV
NASPOT 1	30	24	100	100	100	100	0	46.4	0
Beauregard	30	26	100	100	100	100	100	100	100
Ejumula	30	28	100	100	100	100	0	86	17
Dimbuka	30	19	53	79		28	0	46	0

5.3.2 Effects of selecting roots from symptomless plants as source of planting material

In this trial where sweetpotato roots were got from symptomless plants, most of the sprouted shoots of cvs NASPOT 1 and Dimbuka obtained from MUARIK were symptomless while that of Ejumula, all the sprout developed virus symptoms (Table 18). When symptomless sprouts were grafted on *I. setosa* and tested by ELISA, 43% of Dimbuka, 50% of NASPOT 1, and 100% Ejumula were positive for a single or combination of viruses mainly SPFMV and SPCSV. For NaSARRI samples, none of the root sprouts were symptomatic and on grafting to *I. setosa* and testing with ELISA only 25% of Ejumula, 20% of Dimbuka and 17% of NASPOT 1 tested positive for SPFMV with no other virus detected (Table 18). It was also observed that some root sprout of NASPOT 1 (Plate 2 A) developed leaves that did not resemble the parent plants (Plate 2 B) but when grafted on *I. setosa*, no viral symptom was observed and when tested with ELISA, none of the samples reacted positive.

Table 18: Proportion of roots from symptomless plants that reacted positive for different viruses

Cultivar	Number of roots sprouted	Number of Samples positive for virus infection using NCM			%age of infected roots
		SPFMV	SPCSV	SPFMV+SPCSV	
Kabanyolo trial					
Ejumula	40	2	0	38	100
Dimbuka	40	6	2	9	42.5
NASPOT1	40	5	12	3	50
Serere trial					
Ejumula	40	10	0	0	25
Dimbuka	40	8	0	0	20
NASPOT1	40	7	0	0	17.5



Plate 2: Sweetpotato root sprout of NASPOT 1 showing deviation of leaf morphology from normal. A) NASPOT 1 with interveinal chlorosis and distorted leaf shape. B) normal NASPOT 1 leaves

In the screenhouse environment, sweetpotato cultivars reacted differently to different viruses or combinations of viruses by expressing varied symptoms. For plants infected by SPFMV alone, the symptoms were generally mild in most of the cultivars tested and sometimes no symptoms were observed especially in cultivar Kabode. These symptoms later disappeared in all the cultivars with the subsequent leaves growing normally as in healthy plants. For plants infected by SPCSV alone, symptoms started as chlorotic spots which later turned to purple or red spots on mature leaves depending on the cultivar. For dually infected plants, there was a mixture of symptoms characterized by vein chlorosis, purple spots on the leaf, mosaic, the leaf shape was distorted and remained small and plants were stunted.

In addition to foliar symptoms, these viruses also significantly reduced the root yield of all the cultivars irrespective of whether it was single or dual infection (Table 19). The greatest yield reduction was observed in plants infected by both SPFMV and SPCSV, followed by the plants singly infected by SPCSV and least in plants infected by SPFMV alone while healthy control had overall better yield performance except for Kabode in trial 1 and Ejumula in trial 2. Among the cultivars tested, NASPOT 1 had the greatest yield followed by Kabode, Ejumula, and least in Dimbuka (Table 19)

Table 19: Yield of four sweetpotato cultivars graft inoculated with single or combination of SPFMV and SPCSV under screenhouse environment

Virus	Total yield per plant (g)			
	Ejumula	Kabode	Dimbuka	NASPOT 1
Trial 1				
Healthy	48.3a	70.0b	112.4a	80.4a
SPFMV	29.7b	86.6a	25.8b	60.0b
SPCSV	26.7b	27.1c	19.2b	48.5c
SPFMV+SPCSV	21.7b	9.7d	18.6b	52.6bc
Trial 2				
Healthy	95.3ab	76.5a	60.2a	112.4a
SPFMV	104.8a	64.9a	68.6a	87.0b
SPCSV	80.5b	66.6a	62.0a	84.4b
SPFMV+SPCSV	46.2c	32b	39.9b	59.1c

Column with the same letter for each trial is not significantly different

5.3.5 Evidence of movement of SPFMV and SPCSV into roots and root sprouts

Sprouted roots were assessed visually for the presence of virus symptoms. For roots derived from plant infected with SPFMV, cv Ejumula had the highest number of root sprouts that expressed symptoms followed by Dimbuka, NASPOT 1 and no symptom was observed in all the root sprouts of cv Kabode (Figure 7). The symptom severity was very mild with no

symptom observed within the first month after planting. However, as time progressed, from 2nd month, mild symptoms characterized by mild purpling of the margin or mild chlorotic spots on the lower leaves were observed in Ejumula, Dimbuka and NASPOT 1 and none in Kabode. These symptoms disappeared with time and by 5th month after planting most sprouts did not have symptom (Figure 8A) and the shoots grew normally as did the healthy control plants.

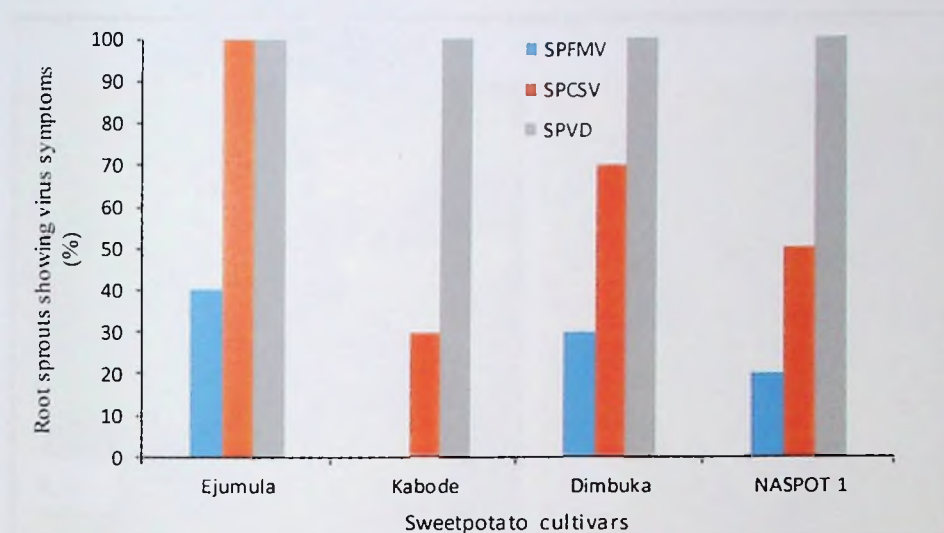


Figure 7: Percentage of sweetpotato root sprouts expressing virus symptoms from roots derived from plants graft inoculated with SPFMV, SPCSV and their combination for the four different sweetpotato cultivars

For roots derived from SPCSV infected plants, the virus incidence was greater in Ejumula, followed by Dimbuka, NASPOT 1 and least in Kabode (Figure 7). Disease severity however was low at the beginning of sprouting (one month after planting) but with time, the disease severity increased in all the cultivars with Ejumula having the greatest severity score at three months after planting. The rest of the cultivars had mild symptoms with mean severity scores of 2.0 or less (Figure 8). The symptoms were mainly leaf purpling and chlorotic spots on the mature leaves.

For roots obtained from plants infected with a combination of SPFMV and SPCSV, all the sprouted shoots were symptomatic in all the cultivars (Figure 7). The symptoms were very clear from the time of sprouting with disease severities of more than 3.0 and remained visible throughout for all the cultivars tested (Figure 8). In some cases the symptoms became more severe leading to death of some sprouts. The symptoms were characterized by vein chlorosis, mosaic, leaf purpling, distorted leaf shape and stunted growth (plate 3).

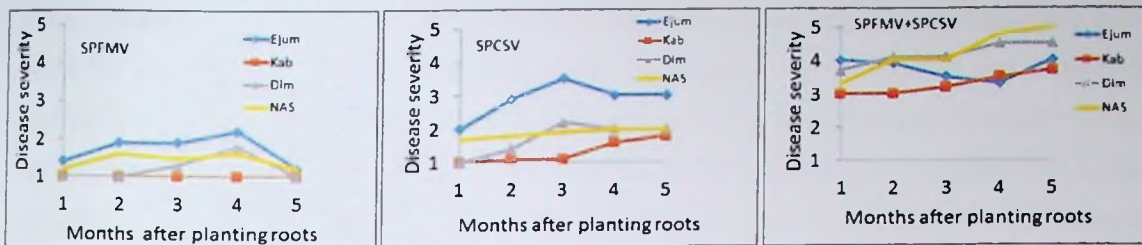


Figure 8: Progress in disease severity due to virus infection (SPFMV and SPCSV) on root sprouts of four sweetpotato cultivars over time under screenhouse condition



Plate 3: Symptom expression on root sprout due to dual infection by SPFMV and SPCSV. A) = symptoms on Cvs Dimbuka (mottling vein clearing, distorted leaf shape), B) = symptom on Ejumula (mottling and vein clearing), C) = Symptom on NASPOT 1 (vein clearing mottling and distorted leaf shape and D) = symptom on Kabode (vein clearing)

5.3.6 Detection of SPFMV or SPCSV on the root sprouts of four sweetpotato cultivars

After the visual assessment of the sprouts, three leaf samples were collected from each root and subjected to ELISA test to confirm the presence of the viruses. Of the 40 samples of root sprout tested for SPFMV, 20 were positive for the virus. Most of the samples that tested positive for the virus were from cv Ejumula, followed by Dimbuka, NASPOT 1 and least in Kabode (Table 20). For root sprouts singly infected with SPCSV, of the 40 samples tested, only 16 were positive. Again cv. Ejumula had the most samples testing positive followed by Dimbuka, and least in Kabode and NASPOT 1. For dual infected root sprouts, all the 40 samples tested were positive for SPFMV and SPCSV in all the cultivars (Table 20).

Table 20: Proportion of sweetpotato root sprouts that tested positive for SPFMV and SPCSV using ELISA

Virus	Root sprouts positive for the virus				
	Ejumula	Kabode	Dimbuka	NASPOT 1	Total
SPFMV	9/10	2/10	6/10	3/10	20/40
SPCSV	6/10	2/10	5/10	3/10	16/40
SPFMV+SPCSV	10/10	10/10	10/10	10/10	40/40

5.3.7 Virus titre and reversion of root sprouts from single infection by SPFMV or SPCSV

Virus titre was measured at two month after planting and at five months after planting using DAS ELISA for the case of SPFMV and TAS ELISA for the case of SPCSV. The virus titre for SPFMV just after the root sprout was greater than the titre at five months after planting. At 2 MAP, SPFMV titre was greater in cultivar Ejumula followed by NASPOT 1 and least in Kabode and Dimbuka (Table 21). At five months, cvsNASPOT 1 and Kabode had reverted from SPFMV infection since they tested negative for the virus by DAS ELISA. Cultivars Ejumula and Dimbuka did not revert from SPFMV by the 5th month however their titre significantly reduced (Table 21). For SPCSV infected root sprouts, the virus titre was greater at 2 MAP while at 5 MAP, there was reduction in virus titre in all the cultivars. Reversion however, did not occur on root sprouts infected with SPCSV since all the sprouts tested positive for the virus by TAS ELISA at 5 MAP (Table 21). Among the cultivars tested, Ejumula had the greatest SPCSV titre, followed by NASPOT 1, Dimbuka and Kabode (Table 21).

Table 21: Absorbance values from detection of SPFMV antigens by DAS-ELISA and SPCSV antigens by TAS-ELISA, in leaves of sprouted roots of cv. Ejumula, Kabode, Dimbuka and NASPOT 1 2 months and 5 months after planting

Cultivar	Virus titre					
	SPFMV			SPCSV		
	2 MAP	5 MAP	-ve control	2 MAP	5 MAP	-ve control
Ejumula	1.458	0.556	0.138	1.618	1.237	0.221
Kabode	0.74	0.263	0.163	0.606	0.422	0.212
Dimbuka	0.682	0.256	0.116	0.708	0.474	0.208
NASPOT1	0.93	0.213	0.116	0.830	0.525	0.268

MAP= months after planting, -ve = negative control plants. Absorbance value of the test plant which is less than double that of the control plant, implies the plant has tested negative of the virus.

Effect of SPFMV and SPCSV on the number of root sprouts produced.

Comparing the number of sprouted shoots produced by each virus treatment per cultivar indicated no significant virus effect on the number of sprouts produced by SPFMV, SPCSV and healthy control except for cultivar Dimbuka where SPFMV infected roots produced greater sprouts. Difference was observed on number of root sprouts produced by NASPOT 1 and Dimbuka infected with combination of SPFMV and SPCSV in which very few sprouts were produced (Table 22).

Table 22: Mean root sprouts produced by different sweetpotato cultivars infected with SPFMV and SPCSV under screenhouse conditions

Virus	Mean number of sprouts			
	Ejumula	Kabode	Dimbuka	Naspot 1
Healthy	4.5a	4.6a	3.3b	3.5a
SPFMV	4.8a	4.2a	5.9a	3.9a
SPCSV	5.1a	4.2a	4.4ab	4.4a
SPFMV+SPCSV	4.5a	3.7a	2.2b	2.8b

5.4 Discussion

Use of sweetpotato roots as source of planting material has not been a formal practice in Uganda until a recent study by Namanda (2012) who developed the sand storage and root sprouting method for rapid multiplication of sweetpotato planting material in dry areas. Although this technology is under promotion in Uganda, Tanzania, and Malawi, little has been done to understand virus epidemiology in storage roots. In this study, roots obtained from farmers' naturally infected fields and from those obtained from plants graft inoculated with viruses in the screenhouse both developed symptoms on their sprouts although, the extent of symptom expression was dependent on the cultivar and the infecting virus. The symptoms expressed were confirmed to be caused by either SPFMV, SPCSV or both viruses

using serology and this provided clear evidence that these viruses actually move from infected vines to roots and then to sprouts. For singly infected sprouts by either SPFMV or SPCSV, the symptoms were very mild and in some cases there was no symptom at all implying that farmers can easily use such sprouts as planting material thus facilitating continued spread of the virus. However, roots that are dually infected by SPFMV and SPCSV, produced sprouts with very clear symptoms and can easily be discarded or rogued.

The fact that storage roots can carry viruses, has implication on the long distance spread of these viruses since fresh roots are the most marketable product of sweetpotato in Uganda and as such are moved from the production sites to market and then to consumers. At each of the stages where storage roots are moved, farmers may intentionally save roots for further multiplication especially if the variety has desired qualities. Alternatively unconsumed roots or root wastes like peelings may sprout and farmers may use such as planting material when there is scarcity. This may act as source of inoculum aiding further spread by the vectors. It has been observed that some farmers plant a variety whose planting material is available and not necessarily a preferred variety. Spread of viruses by roots can be worse if the roots are from a region with high disease pressure and the status of mother plant where the root is obtained is not known. This was supported in this study where all the roots randomly sampled after harvest from MUARIK developed symptomatic sprouts which tested positive for both SPFMV and SPCSV. However, for areas like NaSARRI with low disease pressure, there was greater chance of obtaining roots with either single virus infection or no infection at all. This finding discourages use of roots or root sprouts whose status is not clear. Since there are no proper sweetpotato seed channels in Uganda where clean seed roots are produced and distributed, the use of seed roots can be improved by pre-selecting the sweetpotato hills/mounds that look apparently healthy in order to increase the chances of obtaining virus free seed roots. This is supported by findings from this study when selection was made prior to harvesting but there was still risk of obtaining roots with single infection by either SPFMV or SPCSV. However as a long term strategy, there is a need to establish proper seed channels for sweetpotato where the seed stock are continuously cleaned and made available to farmers.

In addition to virus symptoms, some root sprouts of cultivar NASPOT 1 developed leaves that were morphologically different from the mother plant (plate 2) and did not test positive for any virus when grafted on *I. setosa* and when tested by NCM ELISA. This morphological variation could be due to mutation as the adventitious sprouts obtained from storage roots are

much more variable than those produced from meristematic tissues (Villordon and LaBonte, 1996). Such variation may alter genetic combination resulting in either better or deleterious mutations that may affect sweetpotato yield either positively or negatively. In Uganda, the use of root sprouts is not common but with the current effort by CIP to promote use of root sprouts especially in dry areas, there is need to determine the consequent rate of mutation in Ugandan sweetpotato cultivars. Nevertheless, leaf morphological variation could also be due to unknown viruses for which the tests were not available.

Also, some roots derived from plants infected by single SPCSV or SPFMV did not show any virus symptoms on the sprouts and also tested negative when grafted on *I. setosa* and with ELISA. Similar findings were reported in potato in which some infected plants produced virus free tubers which also produced virus free shoots (Wilson, 2001). The mechanism by which infected sweetpotato plant can produce healthy roots is not fully understood and requires further investigation. Alternatively, failure to detect virus from root sprout derived from infected plant could be because of very low virus titre that could not be detected in ELISA. Use of more sensitive methods like real time quantitative PCR would provide conclusive results on infection status of these roots.

This study further showed that root sprouts singly infected with SPFMV revert to healthy in both resistant cultivars like NASPOT 1 and moderately susceptible cultivars like Kabode since both tested negative for the virus. Cultivars Dimbuka and Ejumula showed recovery from virus infection as was evidence by the drastic reduction in virus titre. Similar results have been reported in most Ugandan landraces and station bred cultivars that have varying levels of resistance (Gibson *et al.*, 1997; Aritua *et al.*, 1998b; Wasswa 2012). This confirms reports that some symptomless vines in farmers' fields are actually virus free (Gibson *et al.*, 1997). The reversion or recovery also seems to influence the selection of a given cultivar for a given region. For example NASPOT 1 that is resistant and has the ability of quick reversion is widely grown in the central region that has high disease pressure. This is in support of findings by Gasura *et al.* (2009) who noted that cultivars with ability to recover were common in the high SPVD pressure zones in central and western regions of Uganda. Also the reversion of most Ugandan cultivars from SPFMV infection makes it easier to clean infected plants through meristem tip culture and thermotherapy (Rukarwa *et al.*, 2010). On the other hand, no cultivar reverted from SPCSV although there was reduction in virus titre by the fifth month after sprouting. Lack of reversion by SPCSV infected plants was also

observed under field conditions (Adikini *et al.*, 2015) and this makes SPCSV a limiting factor in cleaning sweet potato planting material. This is supported by Rukarwa *et al.* (2010) in which they did not obtain any virus cleaned material from plants infected with SPCSV when subjected to meristem tip culture and thermotherapy.

Among the cultivars tested under screenhouse, Ejumula was the most susceptible to all the viruses tested both in single and mixed infections since most of them were able to develop symptoms but also tested positive with ELISA. This finding is similar to earlier work under field conditions (Adikini *et al.*, 2015). Cultivars NASPOT 1, Dimbuka, and Kabode only appear tolerant to single virus infection and breaks down to mixed infection of SPFMV + SPCSV as earlier reported by other researchers (Adikini *et al.* 2015; Miano, 2008). In terms of yield effects, mixed infections severely reduced the yield of all the cultivars tested followed by the infection by SPCSV and least in SPFMV which is a similar trend to that observed under field conditions (Adikini *et al.*, 2015) although the root performance under screenhouse condition was poor.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General discussion

Sweetpotato viruses coupled with the current seed system where farmers obtain vines or seed roots from their own fields or neighbours has remained a great challenge to sweetpotato production in Uganda. From this study, it was proved that virus indexed material once grown under exposed field conditions with high disease pressure, as for the case of MUARIK will get infected with viruses within one month of planting and by the end of the growing season susceptible varieties like Beauregard and Ejumula become so severely infected that a farmer can hardly obtain any symptomless vines for the subsequent season. Such poor quality vines due to virus infection could be one of the major reasons why farmers abandon some cultivars irrespective of their yield and nutritional value. High rates of reinfection of virus indexed material have been reported in other countries (Pozzer *et al.*, 1994; Milgram *et al.*, 1996; Bryan *et al.*, 2003). Currently, multiplication of sweetpotato planting material is field based, and this implies farmers have been obtaining vines from crops which have already been exposed to virus infection, even for the newly released varieties and therefore give low yield at farmer level compared to on station. Although emphasis needs to be put on breeding for resistant varieties and encouraging farmers especially in areas with high disease pressure to grow them, the breeder material and foundation seed stocks need to be virus indexed before distributing them to farmers. To ensure that farmers get quality planting material, there is need for an effective certification program to regulate production and distribution of high quality planting stock to farmers at all times. In this study, degeneration of vine quality at NaSARRI was less than at MUARIK, as evidenced by low rates of re-infection of virus indexed material and most of the vines remained apparently healthy when grafted on *I. setaosa* and tested by ELISA. This may be good news for vine multipliers as open field multiplication can be achieved in such areas with low virus pressure provided other factors like drought are addressed. Low virus incidence at NaSARRI also implies that commercial production of both moderately susceptible cultivars like Ejumula and tolerant varieties like Dimbuka and NASPOT 1 can be achieved in such areas.

This study also revealed that virus indexed material (G1) overall had a greater yield than the subsequent field derived materials (G2, G3, G4 and G5) in all cultivars in both locations. In USA and China, use of virus indexed material has been found to greatly improve sweetpotato

yield and root quality and has made farmers to continue to grow high yielding, nutritious but susceptible varieties like Bearegard (Clark and Hoy, 2006; Fuglie *et al.*, 1999). In Uganda, however, commercial use of virus indexed planting material has not been implemented and farmers have continued to recycle their own planting material which is of low quality hence giving poor yield. This study has shown that storage root yield degeneration was greater in G2 irrespective of the cultivar and location and after which the yield remains relatively stable as for the case of MUARIK or increases with time of field exposure as for the case of NaSARRI (Figure 3). This contradicts the earlier degeneration studies conducted in which degeneration in root yield was reported to be gradual with time of field exposure (Bryan *et al.*, 2003; Lewthwaite *et al.*, 2011). This could be because some of the symptomless planting material selected did not have the virus as earlier reported by Gibson *et al.* (1997) that most symptomless planting material in Uganda are actually virus free as a result of the reversion phenomenon. This may explain why some varieties have been grown for long under field conditions without declining and it supports the use of symptomless planting materials of resistant varieties by farmers. However, further studies are needed to elucidate the possible confounding effects of environment, mutation and virus accumulation leading to cultivar decline. It's also worthy studying decline in other cultivars as this study suggests that the rate of decline is genotype dependent.

The most common viruses detected to infect sweetpotato grown under field conditions were SPCSV and SPFMV although, some samples obtained from G2, G3, G4 and G5 plants also tested positive for SPLV, C6, SPMSV, SPCV and CMV (Tables 3 and 4). This implies that the more times sweetpotato is grown in the field, the higher the chances of getting infected with more viruses and the contribution of such viruses to yield decline cannot be ruled out. Similar results were reported in USA for potyviruses like SPVG and SPV2 which were found to increase gradually in seed stocks with time of field exposure (Clark *et al.*, 2010). Although some of these viruses have been reported to occur in Uganda, little has been done to determine their interaction with other viruses and their effects on yield of sweetpotato is not known; this requires further research. The ELISA method used to detect these viruses in this study is limited to only 10 viruses yet over 30 viruses have been reported to infect sweetpotato worldwide (Clark *et al.*, 2012). This implies that more viruses other than those detected could be infecting sweetpotato in Uganda and therefore it is important to carry out a country-wide survey using appropriate diagnostic techniques to identify and characterize unknown viruses that could be infecting sweetpotato in Uganda and determine their effects on yield.

SPFMV and SPCSV being the most prevalent in Uganda, their effects on the yield of sweetpotato grown under field conditions were determined in two agro-ecologies. It was found that, both single infection and dual infection by SPFMV and SPCSV significantly reduced the yield of sweetpotato although the extent varied with cultivar and agro-ecology. Infection by SPFMV alone caused mild or no symptoms with yield loss ranging from no loss up to 40% depending on the cultivar. In the high disease pressure area like MUARIK, the yield of healthy control plants were not different from plants infected with SPFMV. This could be because the healthy control quickly became infected with the virus as was evidenced by the high incidence and severity (Figure 4) in this study. When SPFMV infected vines were recycled, the resultant yield became less than that of the healthy control and this could be attributed to infection by other viruses like SPCSV that was found to spread very fast in the field due to presence of high vector population at MUARIK. In the low disease pressure area like NaSARRI, the healthy control plants had greater yield than the SPFMV infected plants. This implies that, depending on agro-ecologies and cultivar, SPFMV alone can have significant impact on the yield and yet farmers may not be aware of this due to lack of symptoms. When SPFMV infected vines were recycled, there was slight increase in yield. This increase in yield could be largely due to reversion from infection as was evident from this study when most of the previously infected plants tested negative for the virus using both the indicator plant and ELISA. Reversion from infection due to SPFMV has been reported in most sweetpotato cultivars grown in Uganda (Aritua *et al.*, 1998b; Gasura *et al.*, 2009; Wasswa, 2012). In this study reversion from SPFMV infection was observed to be greater at NaSARRI compared to MUARIK (Table 8). This could be because of the differences in environmental conditions such as temperature, relative humidity and rain fall but no studies has so far been done to prove this. Also the high reversion rate at NaSARRI could be favoured by the low vector population that would continuously inoculate the plants as in the case of MUARIK. Therefore, further research is needed to establish the effect of environmental condition (i.e. temperature, rainfall and relative humidity) on the rate of reversion on sweetpotato due to virus infection. Also there is a need to determine the relationship between vector population and rate of reversion on sweetpotato due to virus infection

Infection by SPCSV alone was found to produce clear symptoms and caused significant yield loss ranging from 14% up to 60% in all the cultivars in both locations implying that this virus plays very key role in cultivar decline. Also, the plants infected with SPCSV did not show

any form of reversion and its titre remained high. This lack of reversion may make cleaning/ indexing virus free planting material difficult and supports the finding by Rukarwa *et al.* (2010) in which they did not obtain any virus cleaned material from plants infected with SPCSV when subjected to meristem tip culture and thermotherapy. The situation was worse with dual infection of SPFMV and SPCSV resulting in yield loss of up to 95% similar to earlier reports (Mukiibi, 1977 Karyeija *et al.* 1998a; Aritua *et al.*, 2000;). The titre of both SPFMV and SPCSV was found to be high in co-infected plants contradicting the early report that titre of SPCSV either remain constant or decline in co-infected plant. The high titre of SPCSV could be due to interaction with unknown viruses or due to environmental factors but this needs further research. Also the presence of vectors that continuously inoculate the plants grown under field conditions could have contributed to the higher virus titre observed. In this study, an attempt to control vectors using systemic insecticides at MUARIK which has high disease pressure was unsuccessful as healthy plant became severely infected with the virus. This implies there is a need to investigate the alternative methods to reduce the spread of virus disease. In the mean time the available integrated pest and disease management options should be exploited. For example farmers should be encouraged to grow tolerant/ resistant varieties while following the right cultural practices like planting virus indexed material away from existing sweetpotato, selecting vines/ seed roots from symptomless plants and following appropriate crop rotation. In addition to reduced yield, both single and dual infection by SPFMV and SPCSV also affected the root quality by reducing the storage root diameter and increasing the length hence high length diameter ratio.

This study further found that planting virus infected vines results in production of infected roots which if used as seed produces infected sprouts. Roots co-infected by SPFMV and SPCSV produce sprouts with very clear virus symptoms and therefore, farmers can easily identify and rogue just like in the case when vines are used. Unfortunately those with single infection had either mild or no symptoms at all on the sprouts making it very complicated for the farmer to rogue. This therefore, calls for the use of virus indexing method like indicator plants to quickly eliminate the infected roots during sprouting. Using roots from unknown sources must be avoided as this can increase the risk of virus spread and since currently there is no system through which seed roots are produced, farmers should be encouraged to preselect vigorous, apparently healthy plants from which to obtain roots prior to harvesting. As a long term strategy, it is important to design a simple technology incorporating virus indexing methods to ensure production of virus free seed roots that can be accessible to the

farmers timely and in right quantities.

In this study it was observed that sweetpotato plants previously singly infected with either SPFMV or SPCSV produced some roots that did not test positive for the virus. Uneven distribution of virus in the vines have been reported (Dje and Diallo, 2005; Miano, 2008) but no studies on the distribution of virus in sweetpotato roots have been done. It's therefore important to repeat this study and try to understand the mechanism under which infected plant can produce non infected roots. Also the morphological variation observed in some root sprouts particularly NASPOT 1 requires further investigation and understanding of mutation in Ugandan sweetpotato cultivars. Mutation has been reported as one of the factors contributing to cultivar decline in sweetpotato but no work has yet been done among cultivars grown in Uganda.

6.2 Conclusions

Viruses rapidly accumulated in sweetpotato through vine propagation leading to detectable levels of degeneration in all the cultivars, and was greater at MUARIK than NaSARRI. Cultivars Beauregard and Ejumula degenerated within one generation of field cultivation under high disease pressure area. Cultivars Dimbuka and NASPOT 1 showed gradual decline in vine quality and storage root yield after generation 2 in both location.

This study has also proved that both single and dual infection by SPFMV and SPCSV caused yield loss in terms of quality and quantity of vines and storage roots. SPFMV alone caused upto 40%, SPCSV caused up to 60% and combination of the two viruses caused up to 95% yield loss in both locations. Although SPFMV reduced yield, its effect alone in some cultivars was not very great and some reverted from virus infection. Plants infected with SPCSV alone and combination of SPFMV and SPCSV did not revert to healthy status.

The study further proved that, sweetpotato vines once infected with any virus whether single or combination, these viruses are capable of translocating into storage roots which if used as a seed will produce infected sprouts leading to continuous virus spread. Roots infected by single virus produce mild or no symptoms on the rootsprouts and the plant has the ability to revert to healthy in the case of SPFMV and only show recovery in case of SPCSV. Roots infected by both SPFMV and SPCSV, the virus symptoms on root sprouts are very clear and farmers can easily rogue out such seed roots at an early stage of sprouting.

6.3 Recommendations and areas for further research

This study has clearly showed that the rate of virus infection, and vine quality degeneration is greater at MUARIK than NaSARRI. Therefore the virus management package must be location specific depending on the virus pressure and farming system of a given agroecology. In high SPVD zones like MUARIK, for farmers to produce high value but susceptible cultivars like Ejumula, virus indexed material must be used every season. However use of vines of tolerant or resistant varieties like NASPOT 1 is recommended for these areas, and the vines should be renewed every after three generations with virus indexed clean planting material. This therefore calls for prioritizing sweetpotato virus indexing at the national program to ensure that there is constant supply of clean breeder and foundation seed that can be accessible to vine multipliers and farmers. Also open field multiplication of planting material in high disease pressure areas should be avoided. Instead, low cost alternative methods that can reduce virus spread whiling increasing vine production in a timely manner needs to be developed.

In areas with low disease pressure like NaSARRI, high yielding, nutrient rich but susceptible cultivars like Ejumula can be grown together with tolerant or resistant varieties up to between four to six generations. However, very susceptible cultivars like Beauregard should be avoided. Open field multiplication of planting material can be done in these areas provided the seed field is well isolated from the existing sweetpotato fields and the challenge of drought is well addressed.

It is also recommended that in both low and high SPVD zones, when a farmer is given a clean virus free planting material to begin with, it should be grown away from already existing sweetpotato in the field as this may jeopardise the yield of new materials by acting as source of virus inoculum. This should be implement along with other existing disease management package like roguing of infected plants within one month after planting and crop rotation.

From this study it was further proved that sweetpotato roots and their sprouts are good resvior for viruses. Therefore, seed roots should be selected from hills or mounds with vigorously growing and symptomless sweetpotato mother plants. But as a long term solution, technology

for rapid multiplication of clean seed roots like use of hydroponics needs to be tested for efficient supply of clean planting material.

Viruses like SPLV, C6V, and CMV that has not been reported in Uganda were detected at low frequency using ELISA in this study. This implies that more viruses other than those detected could be infecting sweetpotato in Uganda and therefore it is important to carry out a country-wide survey using appropriate diagnostic techniques to identify and characterize unknown viruses that could be infecting sweetpotato in Uganda and determine their effects on yield.

This study suggested that cultivar decline is genotype dependent. Therefore, it's worthy studying decline in other cultivars and try to understand the possible confounding effects of environment, mutation and virus accumulation leading to cultivar decline. In particular, it is important to monitor changes in virus load with time under different environmental conditions

In this study, it was observed that sweetpotato plants singly infected with either SPFMV or SPCSV produce some roots that did not test positive for the virus. It's therefore important to investigate why these roots did not test positive for the viruses, and also try to understand the mechanism under which infected plant can produce none infected roots.

The morphological variation observed in some root sprouts particularly NASPOT 1 requires further investigation and understanding of mutation in sweetpotato cultivars grown in Ugandan. Mutation has been reported as one of the factors contributing to cultivar decline in sweetpotato but no work has yet been done on this aspect among the Ugandan cultivars

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