



Sweetpotato virus detection

Literature review March 2018

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Glossary, list of abbreviations and acronyms

ACIAR	Australian Centre for International Agricultural Research
DAF	Department of Agriculture and Fisheries
CIP	International Potato Centre
DNA	deoxyribonucleic acid
dsDNA	double stranded deoxyribonucleic acid
NCM-ELISA	nitrocellulose membrane enzyme-linked immunosorbent assay
EM	electron microscopy
LAMP	Loop mediated isothermal amplification
LFDs	Lateral Flow Devices
PCR	polymerase chain reaction
PT	pathogen tested
qPCR	real time - polymerase chain reaction or quantitative PCR
RC	russet crack
RT-PCR	reverse transcription-polymerase chain reaction
RT-qPCR	reverse transcription - real time - polymerase chain reaction
RNA	ribonucleic acid
SPC-3	Sweetpotato C-3 virus
SPC-6	Sweetpotato C-6 virus
CDD	Chlorotic dwarf disease
CMV	Cucumber mosaic virus
ICLCV	Ipomoea crinkle leaf curl virus
IYVV	Ipomoea yellow vein virus
SPCFV	Sweetpotato chlorotic fleck virus
SPCSV	Sweetpotato chlorotic stunt virus
SPCV	Sweetpotato collusive virus
SPFMV	Sweetpotato feathery mottle virus
SPLCV	Sweetpotato leaf curl virus
SPLL	Sweetpotato little leaf
SPLSV	Sweetpotato leaf speckling virus
SPLV	Sweetpotato latent virus
SPMaV	Sweetpotato mosaic associated virus
SPMMV	Sweetpotato mild mottle virus
SPMSV	Sweetpotato mild speckling virus
SPPV	Sweetpotato pakakuy virus
SPRSV	Sweetpotato ringspot virus
SPSMD	Sweetpotato severe mosaic disease
SPSMV-1	Sweetpotato symptomless virus-1
SPVC	Sweetpotato virus C
SPVCV	Sweetpotato vein clearing virus
SPVD	Sweetpotato virus disease
SPV G	Sweetpotato virus G
SPVMV	Sweetpotato vein mosaic virus
SPV 2	Sweetpotato virus 2
SPYDV	Sweetpotato yellow dwarf virus

Foreword

This literature review has been compiled as a requirement of M.Phil. undertaken through the University of Queensland in conjunction with DAF Queensland, conducted as part of the Australian sweetpotato growers Inc (ASPG), Hort Innovation funded project Vg13004 *“innovating new virus diagnostics and planting bed management in the Australian sweetpotato industry”*.

1.0 Introduction

Sweetpotato, *Ipomoea batatas* (L.Lam) is a perennial plant of the morning glory family (Convolvulaceae) that has been grown domestically as an annual for over 5000 years in its native Latin America (CIP n.d.). Sweetpotatoes produce higher food yields (energy and nutrition) per unit area, per time unit than most other crops (Tao et al, 2012). In recent decades, throughout the developed world, the popularity of the sweetpotato has been steadily increasing in line with the increasing consumer focus on health and nutrition. With skin and flesh colours ranging from white, cream, yellow, orange and pink through to red, brown and dark purple, sweetpotatoes have numerous health attributes. Gold fleshed types have high levels of the carotenoid form of Vitamin A (beta carotene), and are a source of vitamins B, C, E, iron and zinc. Pink and purple fleshed types have a high anthocyanin (antioxidant) content, while white fleshed types have a relatively low glycaemic index and the edible leaves contain a high percentage of beneficial polyphenols (antioxidants) (CIP n.d.).

Global productivity is also increasing in relation to increased investment in agronomic research specific to sweetpotatoes. The International Potato Centre now ranks sweetpotato as the sixth most important crop worldwide, with 105 million tonnes produced annually (CIP n.d.), mostly in China where sweetpotato is also grown for animal feed and processing. Sweetpotatoes continue to rank in the top five most important staples in many developing countries, with production in sub-Saharan Africa currently increasing faster, than that of more traditional staple crops. Sweetpotatoes can be grown in various soil types at a range of altitudes and have a long history of providing a fast growing, nutritious food source in countries affected by natural disasters. Sweetpotatoes played a critical role during the Chinese famine in the 1960s and in Uganda in the 1990s after virus decimated cassava crops (CIP n.d., Gibson et al.2009).

Sweetpotatoes are often misquoted as producing tubers, like potatoes (belonging to the nightshade family, Solanaceae) however, sweetpotatoes produce storage roots and planting these storage roots produces sprouts but not storage roots. Sprouts or vines that develop from storage roots must be planted to produce more storage roots. Due to the vegetative nature of sweetpotato propagation and the use of vine cuttings from field grown plants to provide planting material, crops are exposed to pest and disease pressure for multiple generations. This prolonged exposure invariably leads to a build-up of pathogens within plants. Sweetpotato vine cuttings have also historically been dispersed and traded amongst growers, increasing pest and pathogen distribution throughout entire growing regions.

1.1 Yield decline

During mid to late last century, many sweetpotato growing countries documented reductions in yields over consecutive growing seasons and subsequent research by world authorities nominated virus diseases as the major contributors to this decline as well as a subsequent decline in cultivars globally (Salazar and Fuentes 2000, Loebenstein *et. al.* 2009, Clarke and Valverde 2009). Work done by Villordon (2014) demonstrates the adverse effects of virus infection on early storage root development in the absence of nitrogen. Plants infected with Sweetpotato feathery mottle virus (SPFMV) produced a reduced number of lateral or feeder roots impairing storage root development and consequently, yield. This reduction in lateral roots is further impaired with the introduction of additional viruses. Tao (2012) states that virus free plants had more vegetative growth, exhibited earlier root development and quicker root expansion than virus infected plants, facilitating more efficient water and nutrient uptake and increased yield.

Russet crack disease of sweetpotato was attributed to the Potyvirus Sweetpotato feathery mottle virus (SPFMV) (Campbell et al. 1975) and prior to the 1990s, most research into viruses affecting sweetpotato crops, centred on this Potyvirus (Clark et al. 2012). Recently there has been a rapid increase in knowledge and advancement of molecular diagnostic technologies as a result many more sweetpotato virus species and strains have now been documented, particularly within sub Saharan Africa. To date 30 viruses from nine families have been reported to infect sweetpotato, some having many strains (Clark et al. 2012). One of the most economically significant findings is the discovery of the synergistic interaction as a result of simultaneous infection of SPFMV and SPCSV to result in the severely debilitating Sweetpotato virus disease complex (SPVD) (Clark et al. 2012).

One solution to combat this decline in yield and one that is often used in developing countries is to encourage propagation of locally preferred cultivars showing little deleterious effects of SPFMV infection and/or those to have some resistance or tolerance to local SPFMV infections (Gibson et al. 2012). The theory is that even if SPCSV was present, plants had tolerance to SPFMV and as a result SPVD would be less likely to develop therefore avoiding large losses in yield. Tolerant vine cuttings are then disseminated to local growers. Whilst these plants may have some resistance to a single virus or strain within a local area, moving plants to another region may expose them to different viruses or virus strains, to which they may have little or no resistance. Of concern is that viruses are still present and therefore they may effect yield. Due to the predisposition of viruses to mutate there is also the chance that any tolerance may be overcome.

The second solution is to breed virus “resistant” cultivars, currently the focus of large research projects in sub Saharan Africa. However this is hampered by the diversity of virus species, with sweetpotato varieties resistant to one virus for example SPFMV, are often susceptible to other viruses. There is also the possibility that if multiple virus infections happen to occur in resistant plants there is increased potential for the development of new viruses due to swapping of genetic material during replication. Whilst breeding for resistance to single viruses has been accomplished, (Gibson et al. 2012) resultant cultivars also need to be high yielding and possess tolerance to other agronomic restraints that contribute to yield decline, for example insect pests such as weevil, wireworm and nematodes. Any resultant cultivars must also have consumer appeal e.g. taste and consistency. The solution of breeding for resistance is a lengthy and expensive process with many years before growers will see any increase in income (Valverde et. al. 2007).

The third solution of pathogen testing (PT) not only alleviates the problem of progressive yield decline but also has the benefit of providing growers with increased yield opportunities and economic benefit in a more rapid timeframe. This solution involves removing viruses from infected locally preferred grower cultivars and returning them to growers. This strategy has proven to be a highly effective and rapid solution to combat yield decline in developed countries. Major sweetpotato producing countries such as China (Fuglie et al. 1999) and South Africa have had outstanding success with the use of PT planting material, with the United States implementing a PT seed scheme around the 1990s, (LaBonte et al. 2004, Gibson et al. 2009). Scientists used virus removal technologies such as those developed by Love (1987), such as meristem extraction with or without thermotherapy along with tissue culture technology to remove viruses from sweetpotato plants. The resulting PT plantlets were then grown out and tested for known viruses using currently available virus detection methods. Those testing negative were deemed to be free from known viruses and were then re-introduced into tissue culture for multiplication and subsequent production of PT planting material.

This solution not only provides sweetpotato growers with planting material free from viral pathogens but also free from insect pests, bacterial and fungal diseases. Readily available PT planting material empowers growers, giving them the capacity to produce higher yields from the first harvest. Although reinfection might occur at some stage once the PT planting material is introduced to the grower's fields, initial virus titres are much lower and virus complexes occur less commonly. Virus titres are not increasing across multiple generations, pest pressure is reduced and carry over fungal and bacterial diseases are markedly reduced.

1.2 Australian production

Starchy, white fleshed sweetpotatoes were originally grown in Australia, often in backyards as feed for dairy cattle and pigs. Australian growers now produce the world's highest yields per hectare of commercial grade gold fleshed sweetpotatoes with a current estimated annual farm gate value of \$90-100M (M Hughes 2017 pers. comm., 4th December) The DAF QLD investment in the development of a PT or "clean" seed system has been one of the most significant research and development outcomes since the late 1990s, enabling Australian commercial sweetpotato growers to access first generation, PT planting material through a now fully commercialised PT seed production operation. The provision of clean planting material and associated research on seed bed technology and agronomy has been instrumental in improving productivity leading to rapid industry expansion during the last decade. Recent research findings revealed that the use of PT planting vines or sprouts of optimal length produced via seedbed technology with the incorporation of best practice planting techniques and agronomy, have improved Australian yields by up to 80% (Coleman et al. 2006).

Once viruses have been removed and plants have tested negative on multiple occasions for known viruses, tissue culture plantlets are placed into insect proof igloos for vine multiplication. Vine cuttings are then field planted in an area well isolated from any commercial sweetpotato production. These cuttings produce first generation storage roots which are then sold to growers who use seedbed technology to propagate their own vine cuttings for use as planting material to produce their commercial crop. Conversely in the US, G1 storage roots and tissue culture multiplied vine cuttings are sold to nurseries who plant these roots into seedbeds and/or cuttings into igloos to produce clean planting material. The resultant clean material is then on sold to 2nd tier nurseries who also multiply planting material to sell to growers, but by now it is G2. This process is repeated with 3rd, 4th and 5th tier nurseries on selling planting material up G5. Yields generally decline over at least 2 seasons, (LaBonte et al. 2004) this is also the case in Australia where growers purchase new PT material every one to two years to avoid economic losses.

Although PT seed is produced in an area well isolated from any commercial sweetpotato production it is partly field produced, so there is a need to regularly test material to ensure freedom from virus infection. Any successful PT scheme is therefore highly dependent upon accurate virus detection to ensure planting material is free from known viruses and ensuring capacity to remove those viruses. Effective virus testing requires a comprehensive knowledge of both viruses present in country and any potential exotic virus threats and ability to rapidly and accurately diagnose them. Recent work funded as part of ACIAR projects SCMN/2004/071 and PC 2011/053 detected SPLCV and other virus species in Australian germplasm collections that had been assembled in the early 2000s (Hughes 2010).

There is limited knowledge on what sweetpotato viruses may be present throughout Oceania where sweetpotato was introduced around 1000-1900AD. Our neighbouring Melanesian countries

are considered to be home to the secondary centre of sweetpotato diversity to Latin America (Tairo *et. al.* 2005, Roullier *et al.* 2013). Sweetpotato is an important staple crop in neighbouring Indonesian Papua and Papua New Guinea, where hundreds of cultivars exist as well as in the Solomon Islands. It is largely unknown which virus species/strains are present throughout these regions and therefore the potential risks of an inadvertent introduction of unknown viruses to other areas such as Australia with its intensive commercial production system are also unknown and of concern to industry. The Increasing importation of fresh vegetables is also an emerging biosecurity threat to the sweetpotato industry.

Due to the nature of sweetpotato viruses, interactions with host plants, changing titres and absence of symptoms in host plants, the current Australian PT system relies on the following combination of complimentary diagnostic procedures.





- Phyto-diagnostics, to detect the presence of virus infection including new or “novel” viruses using biological indicator plants. As indicator plants are highly sensitive to most sweetpotato infecting viruses, leaf symptoms produced are more apparent than those observed on sweetpotato plants (when present). This sensitivity of indicator plants to virus infections also facilitates rapid increases in virus titres, enhancing downstream diagnostics such as NCM-ELISA and PCR. Due to the nature of this technology, phyto-diagnostic techniques do not always provide indications as to which virus species may be responsible for symptoms produced. Phyto-diagnostic indexing as part of the Australian PT protocol is conducted by experienced and highly skilled staff at Gatton research facility (GRF) as some virus symptoms can be transient (Potyviruses), difficult to discern at lower titres (SPLCV) and mixed infections influence symptom expression.
- Serology using a standard Nitrocellulose membrane enzyme linked immunosorbent assay (NCM- ELISA) test kit manufactured by the International Potato Center, Peru (CIP) to test for 10 known sweetpotato infecting viruses, (C-6, CMV, SPCaLV [now called SPCV], SPCFV, SPCSV, SPFMV, SPLV, SPMMV, SPMSV and SPVG).
- Molecular detection using new technology, quantitative or real time PCR (qPCR) to detect SPLCV.

Recently the US established a Sweetpotato National Clean Plant Diagnostic Network (NCPN) for sweetpotato with minimum virus testing protocols for sweetpotato (NCPN sweetpotato n.d.) for viruses found in country. CIP also has standardised protocols to detect sweetpotato viruses occurring in their part of the world and other countries of interest. Whilst some virus species are common to all countries, different viruses occur in Australia not covered within NCPN or CIP protocols. Another issue is that most NCPN and CIP assays are based on conventional PCR rather than the more sensitive qPCR. The NCPN only encompasses qPCR protocols for SPCSV-EA and SPCSV-WA with the CIP qPCR protocols limited to strains of SPFMV and SPCSV. Thus there are a lack up-to-date standardised protocols within the Australian PT regime to detect all viruses found in the country and it is also imperative that the Australian industry has the capacity to detect any potential exotic virus threats.

Project VG13004 has identified the viruses SPFMV and SPLCV to be endemic within Australian commercial sweetpotato production areas, while SPV2 and SPVC were detected in Western Australia and SPCFV and SPCV detected in North Queensland (Table 1). As farmers rely on field based seedbed technology to produce their PT planting material, those seedbeds are regularly

subjected to infestation from virus vectors. Thus it is essential to provide growers with rapid detection tools to identify these viruses empowering them to make informed decisions on when to renew their seedbeds to avoid a build-up of virus pathogens within planting material that would lead to an economic loss.

Table 1: Sweetpotato viruses and phytoplasmas reported from Australia.

Family	Genera	Anacronym	Species	Type	Structure
Potyviridae	Potyvirus	SPFMV (Gibb 1993)	Sweetpotato feathery Mottle virus Strains O, RC and EA	ssRNA +	 flexuous filaments
		SPV2 / IVMV / SPVY (Tairo 2006)	Sweetpotato virus 2. Syn: Ipomoea vein mosaic virus / Sweetpotato virus Y		
		SPVC (Tairo 2006)	Sweetpotato virus C		
Betaflexiviridae	Carlavirus	SPCFV (Jones 2007)	Sweetpotato chlorotic fleck virus	ssRNA +	 flexuous filaments
Geminiviridae	Begomovirus	SPLCV (Hughes 2010)	Sweetpotato leaf curl virus	ssDNA	 twinned icosahedra
Caulimoviridae	Cavemovirus	SPCV (Norkunas 2009)	Sweetpotato collusive virus	dsDNA-RT	 icosahedra
Phytoplasma	<i>Candidatus aurantifolia</i>	SPLL syn: SPLL4 (Davis 2003)	Sweetpotato little leaf	phytoplasma	

2. Detection of sweetpotato viruses

Virus particles consist of a core of nucleic acid, DNA or RNA, surrounded by a protective protein coat. They are extremely small, and only able to survive and multiply within their host plants. Viruses can be transmitted from plant to plant by direct contact, via tools at harvesting or planting, by propagation from infected cuttings or roots, by graft inoculation and by vectors such as sap-sucking insects, particularly aphids, whiteflies and jassids.

Moyer and Salazar (1989) suggest that viruses are present in most non PT material wherever sweetpotatoes are grown and that the “endemic nature” of these viruses has led to high levels of tolerance to local viruses within sweetpotato plants over time as farmers naturally select and propagate the most healthy looking, asymptomatic plants. This tolerance complicates virus detection which is often further confounded due to a lack of visual symptoms (most sweetpotato viruses are asymptomatic), many sweetpotato viruses are present in low titres, mixed infections are common, some viruses have diverse strains and virus particles are unevenly distributed within sweetpotato plants. The presence of inhibitors in sweetpotato sap also interferes with serological and PCR detection (Fuentes pers com. 2007). To further hinder accurate virus isolation and identification, the presence of SPFMV which is thought to be endemic in every sweetpotato growing country, often masks the presence of other viruses, especially other Potyviruses, (Valverde *et al.* 2007).

When present, virus symptoms are often difficult to distinguish, and expression often varies with virus strain, host cultivar, age of plants, abiotic factors, multiple infections and synergistic reactions. Nutritional deficiencies also cause symptoms sometimes mistaken for those caused by virus infection. Although infected sweetpotato plants are mostly asymptomatic, abiotic stresses can induce symptom expression or exacerbate existing symptoms. Visual symptoms include vein-clearing, chlorosis, leaf cupping both upward and downward, leaf curling and/or rolling, mottling, rugosity, stunting, leaf deformities, necrosis, flowering, poor root development, poor uptake of water and nutrients, reduced canopy growth, misshapen roots, low yields, and in some extremely severe infections, plant death.

Early detection and identification of sweetpotato viruses has previously relied on the use of herbaceous indexing, using indicator plants of the family Convolvulaceae, such as *Ipomoea setosa* and electron microscopy. Currently the three most successfully established sweetpotato virus detection methods in use are biological indexing, NCM-ELISA and PCR. These technologies offer high levels of repeatability and reproducibility. Boonham *et al.* (2013) suggests that qPCR will become the most widely used diagnostic technology in testing laboratories around the world.

There has been an increasing demand for rapid and accurate diagnosis of plant diseases during the last decade. Developing new detection techniques for rapid and accurate detection of multiple plant pathogens and strains is essential. Next generation sequencing technologies are rapidly becoming more affordable and are a promising platform for developing future portable devices for plant pathogen detection devices.

2.1. Biological indexing

The universal Indicator plant *Ipomoea setosa* (Brazilian morning glory), widely recognised as a highly sensitive indicator plant is used globally to detect the presence of sweetpotato infecting viruses. *I. setosa* plants express symptoms readily when infected with virus/es (Fuentes 2005). Moyer and Salazar (1989) suggest that almost all viruses that infect sweetpotato also infect *I. setosa*. As some sweetpotato viruses are often present in sweetpotato plants in low titres, the sensitivity of this indicator plant to virus infection, allows viruses to multiply rapidly increasing virus titres. This increase in titre also facilitates downstream serological and molecular testing such as NCM-ELISA and conventional PCR, with the additional advantage that *I. setosa* plants do not contain inhibitors that impede diagnosis.

Biological indexing using *I. setosa* is the first step in the Australian PT process. Graft inoculation commences in October and concludes in late April, a period when suitable weather conditions occur in south east Queensland. *I. setosa* growth is optimal at 25°C and favours increasing day length and warmer night time temperatures with symptom expression affected at higher or lower temperatures (Fuentes pers. comm.). Currently, there is limited available information on virus particle distribution and movement within sweetpotato plants. Also unknown is the influence that specific virus species and the number of species present within a plant have on virus particle distribution. To overcome this, vine sections are removed from both young vine (tip) and mature vine (distal end) of collected sweetpotato plants to be tested, as well as from known positive and negative control plants.

Sweetpotato vine sections (scion) containing at least one node are grafted onto *I. setosa* (stock) as per Beetham *et al.* (1992) (Figure 1). Moyer and Salazar (1989) suggest that SPFMV reaches peak concentrations in older leaves. Both an end cleft and a side veneer graft are performed on each plant and this is repeated five times (on five indicator plants). Grafted plants are observed twice

weekly over a 28 to 56 day period. As some symptoms can be transient, regular monitoring is important. This process is then repeated two to three times preferably some months apart. Sweetpotato plants should be severely pruned and allowed to grow to 10-15 nodes between each test (NCPN n.d.).



Figure 1. Graft inoculation, end cleft and side veneer grafts and *I.setosa* seedlings.

Biological indexing using *I.setosa* is a reliable and repeatable method to detect the presence of sweetpotato viruses, including novel or unknown viruses but is non-specific and individual virus species are always identifiable. Symptom expression is very reliable, however, poor nutrition, insecticide applications, chimeras and abiotic stresses may cause confusion in symptom recognition.

Herbaceous indexing is relatively inexpensive requiring minimal specialised equipment and, as long as seeds of *I. setosa* are available as well as access to an insect proof shade house, it can be conducted almost anywhere. It is however, time consuming and requires considerable labour, glasshouse space, an optimal temperature of 25 °C and practitioners skilled at grafting and symptom observation to detect viruses present in low titres.

A related species, *I. nil*, also produces symptoms to most sweetpotato viruses (Valverde *et al.* 2007). Other indicator plants used are *Chenopodium quinoa*, *C. quinos*, *I. hederifolia*, *I. incarnnata*, *I. nil*, *I. purpurea*, *Nicotiana benthamiana*, *N. clevelandii* and *N. glutinosa* (Valverde *et al.* 2007) as well as *I.coccinea* (Coleman 2017).

2.2 Serological detection

There are a variety of serological tests available to detect viruses. These are based on a reaction between the proteins in the pathogen and an antibody produced against them. Animals produce an immune response to foreign bodies, particularly large molecules such as proteins. A purified suspension of plant virus proteins can be injected into an animal host, such as a rabbit, to produce antibodies, which can then be used in various diagnostic methods, to detect the presence of plant viruses (Wilson 2014).

Nitrocellulose membrane, enzyme linked immunosorbent assay (NCM-ELISA) is the main serological method currently used to detect viruses in sweetpotato (Figure 3). It is recommended that infected material is first grafted on *I. setosa*, as the virus concentration will be higher in the

indicator species and the ELISA will not be affected by inhibitors present in sweetpotato sap (Fuentes pers com.).

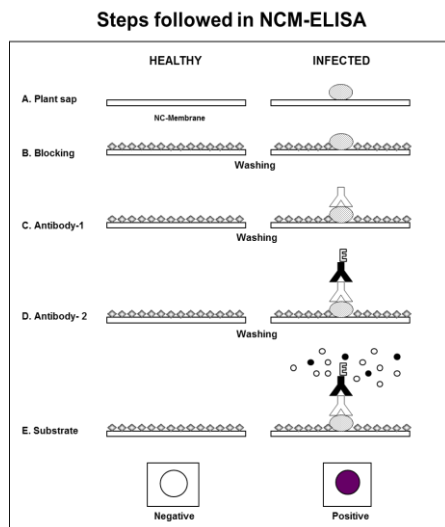


Figure 2. Steps in NCM-ELISA, (Fuentes CIP n.d.).

This test detects proteins on the virus particle coating, using a detection antibody conjugated to an enzyme, which produces a reaction, resulting in a colour change (Figure 2). There are many different forms of ELISA.

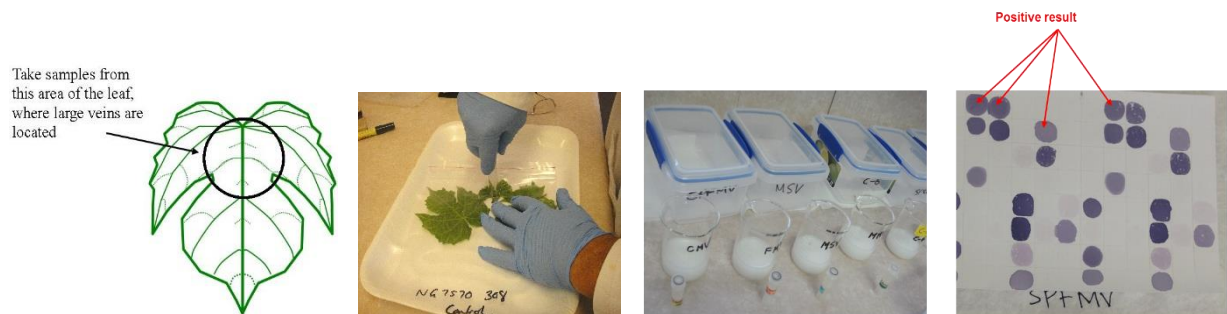


Figure 3. Sample area for leaf discs, adding antibodies, positive results indicated by colour change.

Another common test used to detect sweetpotato viruses is the double antibody sandwich ELISA (DAS-ELISA). Research at the International Potato Center (CIP) in Peru led to the development of a serological test using NCM-ELISA (Salazar 2000). An NCM-ELISA kit containing all required reagents and antisera for ten sweetpotato viruses (C-6, CMV, SPCaLV [now called SPCV], SPCFV, SPCSV, SPFMV, SPLV, SPMMV, SPMSV and SPVG) is available for purchase from CIP (Fuentes 2005; Fuentes 2010; Boonham *et al.* 2013).

NCM-ELISA does not necessarily require a laboratory and can be completed in a shorter period of time by placing antibodies on membranes for three hours instead of overnight. It also has another very important advantage: the samples can be spotted on the nitrocellulose membrane and stored for several months before continuing with the test, or it can be sent to another laboratory for

testing. This test can identify up to 10 known viruses, requires minimal laboratory equipment, and comes in a ready to use kit form and 96 samples can be processed at a time.

NCM-ELISA is not as sensitive as PCR, may not detect very low virus titres, takes 24 hours to complete and only contains antibodies for 10 viruses. There are no antisera currently available for Begomoviruses and as sweetpotato sap interferes with the test's efficiency, sap samples from *I. setosa* grown under optimal conditions are required.

2.3. Electron microscopy

Developed in the 1930's, this microscope provides much higher resolution than typical microscopes, using electromagnetic or electrostatic lenses to control a beam of electrons providing up to two million times magnification. Leaf tissue or sap samples are negatively stained, this stain then surrounds minute particles present in the sample such as viruses, and enables the user to view their physical structure. The morphology of the virus particle can be observed, providing information on its identity and/or the family to which it belongs (Wilson 2001). Electron microscopy is able to visualise virus particles but requires very expensive equipment and operators with specialised skills. Samples need special preparation to enable them to withstand the process which is often difficult and time consuming. Electron microscopes are only able to process a limited number of samples per day compared to other detection methods (Microscopy n.d.) and is only available at larger diagnostic laboratories and Universities.

2.4. Nucleic acid spot hybridization (NASH).

Through nucleic acid hybridization, the degree of sequence identity between nucleic acids can be determined and specific sequences detected in them. The hybridization process uses heat denaturation and can be carried out in solution, or on a gel, or most commonly, on nitrocellulose paper (Wilson 2014).

2.5. Polymerase chain reaction (PCR).

Polymerase chain reaction (PCR) and Reverse transcriptase polymerase chain reaction (RT PCR) is a technology that increases the amount of genetic material in a sample that initially contains very small amounts, to a level that it can be detected. The products of qPCR are then separated by placing them on an agarose gel and subjecting this to an electric current. Pieces of the genetic material settle at different positions, depending on their size.

DNA or RNA is firstly extracted from plant samples and in the case of RNA, converted to complementary DNA (cDNA). Specific primers to detect parts of the virus genome to be screened for and DNA polymerase are added and placed into a thermocycler. During a process of heating and cooling in the thermocycler, DNA strands are split and primers (small pieces of matching genetic sequence) bind to matching areas on the split DNA strands, forming a duplication or amplification of the original strands (Figure 4). This process is repeated many thousands of times resulting in millions of duplicate strands of DNA being produced to a level that they can be detected. The build-up of the large amounts of the target virus sequence in the final PCR cycle can then be visualised on stained agarose gels. (Figure 5).

The PCR products generated can then be used for further analysis or sequencing. Although PCR and RT-PCR are very sensitive methods for the detection of viruses, it is recommended that infected material is first grafted on *I. setosa*, as the virus concentration will be significantly higher in the indicator species (Fuentes pers.com.).

Also, if tests are to be done on RNA/DNA, it is best to test extracts from *I. setosa*, as those from *I. batatas* may include PCR inhibitors (Fuentes pers com.). Agarose gels are obtained at end point of the PCR or RT-PCR process, with results based on the size of the DNA, gels have low resolution that require post PCR processing (i.e. specially trained staff, expensive equipment and laboratory facilities (Fuentes 2005; Boonham *et al.* 2013; Wilson 2014).

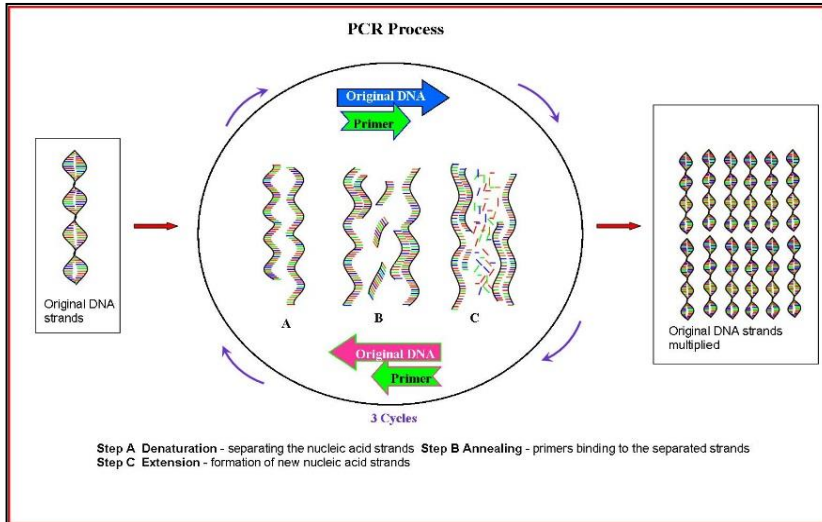


Figure 2. The PCR process (Dennien 2013).

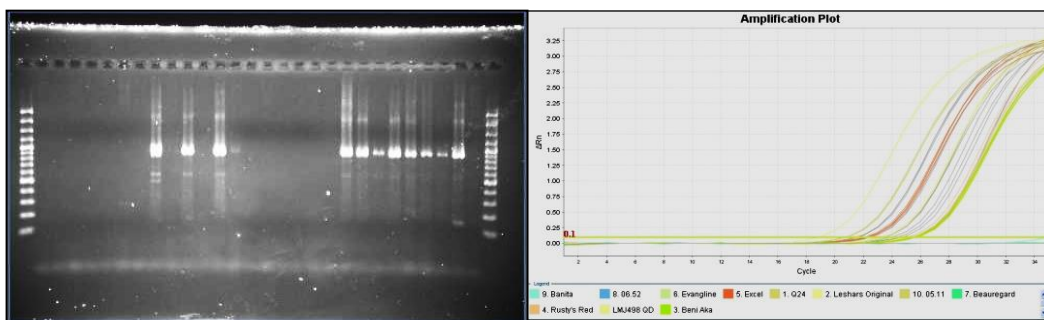


Figure 3. Agarose gel (McMichael's, DAF, SPLCV qPCR amplification plot Dennien, and Sukal (2014).

PCR is more sensitive than ELISA; able to detect very small amounts of virus at low levels in a plant; can be used to rapidly test a large number of plants required for clean planting material schemes, or for screening in the event of a virus outbreak in a production region.

2.6. Real time PCR (qPCR)

Real time PCR or quantitative polymerase chain reaction qPCR and RT-qPCR works in the same way as PCR, but measures DNA/cDNA amplification as it occurs by capturing and measuring fluorescence released by fluorescently-tagged probes (Figure 5). By comparing the cycle threshold (Ct) values of samples of unknown DNA concentration with a series of sample dilutions from a known DNA concentration, the amount of DNA in an unknown reaction can be accurately determined. qPCR results can be obtained faster and with less variability than standard PCR due to sensitive fluorescent chemistry and elimination of post-PCR detection procedures. qPCR is a useful for the investigation of gene expression, viral load, pathogen detection, and numerous other applications.

For qPCR run time is around 2 hours, it is able to be multiplexed (multiple targets run at once) and is high throughput, excluding sample preparation and extraction. The whole reaction takes place inside the instrument therefore PCR products are contained, resulting in less chance of contamination of work areas. qPCR is up to 100 times more sensitive than conventional PCR (Wilson 2014). The test is also quantitative as it measures the amount of DNA/cDNA produced.

qPCR requires expensive equipment and currently there are limited published primer/probe sets available for sweetpotato viruses. Sequencing of other viruses is needed along with primer probe design to detect a wider range of sweetpotato viruses using this method (Fuentes 2012; Boonham et al. 2013; Fuentes 2014; Wilson 2014).

2.7. Lateral flow devices (LFDs)

LFDs, also known as lateral flow immunoassays, have been commercially available for some time, such as home pregnancy test kits. Many lateral flow devices have been developed for plant pathogen testing. They are commonly used by farm advisors and agronomists to detect the presence of pathogens in-field. This method of virus detection has not yet been developed for sweetpotato viruses. LFD's provide rapid diagnosis, are easy to use, portable and robust.

The widespread uptake of this technology remains limited, maybe due to a lack of available tests for many crop pathogens. Research for this review has not revealed any test that has been developed for sweetpotato viruses. Loop-mediated isothermal amplification (LAMP) end products are suitable for lateral flow devices (S. Fuentes pers. comm. November 2014). Costs involved in the development of specific tests may also be a limiting factor.

2.8. Loop-mediated isothermal amplification (LAMP)

Loop-mediated isothermal amplification or LAMP enables the detection of specific nucleic acid sequences within a single tube within 30 minutes by amplifying DNA or cDNA to a detectable amount. LAMP uses 6 primers (forward, reverse, internal and external primers) and a DNA polymerase. Isothermal amplification is carried out at a constant temperature and therefore does not require a thermal cycler and can result in the accumulation of 10^9 copies of the target sequence within 30 minutes (Figure 6).

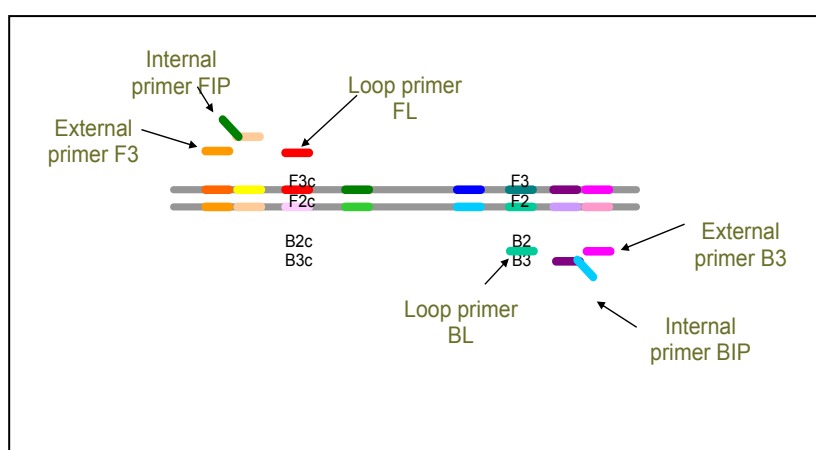


Figure 4. LAMP mode of action, adapted from Fuentes (2014).

Like PCR, and qPCR, LAMP can be used to detect RNA viruses, by incorporating a reverse transcription step, to generate cDNA prior to amplification. Reverse transcription and LAMP can be carried out in one tube, at a single temperature. Commercial kits are available for some plant viruses for example the tomato yellow leaf curl virus (TYLCV) kit from Nippon Gene Co, Japan.



Figure 7. LAMP workflow

The Genie II® instrument manufactured by OptiGene, Horsham, UK is a portable, low-power platform for real-time fluorescence monitoring of isothermal amplification reactions such as LAMP which is suitable for on-site use. It can be run with or without connection to a computer and is powered by a rechargeable battery.

Like qPCR, results are displayed on amplification screen that plots fluorescence over time taken for amplification to occur. When connected to a computer, results can be displayed on 2 additional screens: Isothermal Ratio (showing first derivative of fluorescence vs time during amplification) and annealing (fluorescence vs temperature during melt analysis).

LAMP is a relatively low cost, fast, robust and sensitive detection technology with minimal processing of samples required. It is suitable for onsite or in-field detection of plant viruses (Figure 7). LAMP can be operated on 240 volt power or battery power making it fully portable and ideal for in field detection. LAMP has also been observed to be less sensitive than PCR to inhibitors in the sweetpotato plant, likely due to use of a different DNA polymerase, (typically *Bst*DNA polymerase rather than the *Taq* polymerase used in PCR).

The disadvantages of LAMP include that it requires at least three sets of primers with very few primer sets for specific sweetpotato viruses currently available. Primers for SPFMV and SPCSV, two of the most economically important sweetpotato infecting viruses may be released commercially in the future. Sequencing and primer design is needed to detect other sweetpotato viruses using this method (Tomlinson and Boonham 2008; Fuentes 2014; Wilson 2014; Boonham *et al.* 2013).

2.9. Microarrays

Microarrays enable large number of samples to be tested for a range of pathogens at the one time. Hundreds or thousands of individual hybridization reactions are contained on a glass slide or chip. Plant samples are extracted and labelled with a fluorescent dye. The labelled samples are applied to the slide and many probe sequences for a range of pathogens can be placed onto the

slide or chip. This is incubated for up to 24 hours, then washed, and a scanner is used to measure the fluorescence at each probe placement on the slide or chip. The sensitivity is comparable to ELISA (Boonham *et al.* 2013; Fuentes 2014; Wilson 2014). Microarrays require costly equipment and specially trained staff as well as known sequence information of viruses to be detected.

2.10. Next Generation Sequencing

Next-generation sequencing technology is a promising universal sequence-based diagnostic tool, to sequence and assemble genetic information. The biggest limitation to virus detection is the requirement for knowledge of sequence information, to enable design of primers and probes. This is vital to developing new technologies for detection of known sweetpotato viruses, and for the detection of new viruses. Next generation sequencing provides rapid identification and sequencing of both known and new viruses and virus strains existing within a plant.

This procedure simultaneously detects all genetic information within a plant, both DNA and RNA, (Figure 8). Sophisticated bioinformatics software is then used to analyse and assemble the short sequences, to provide full length viral genomic data. This requires skilled, experienced staff and high powered computers with specialised software to analyse the large data sets produced. Several commercial laboratories offer next generation sequencing throughout Australia (Boonham *et al.* 2013; Fuentes 2014; Wilson 2014).

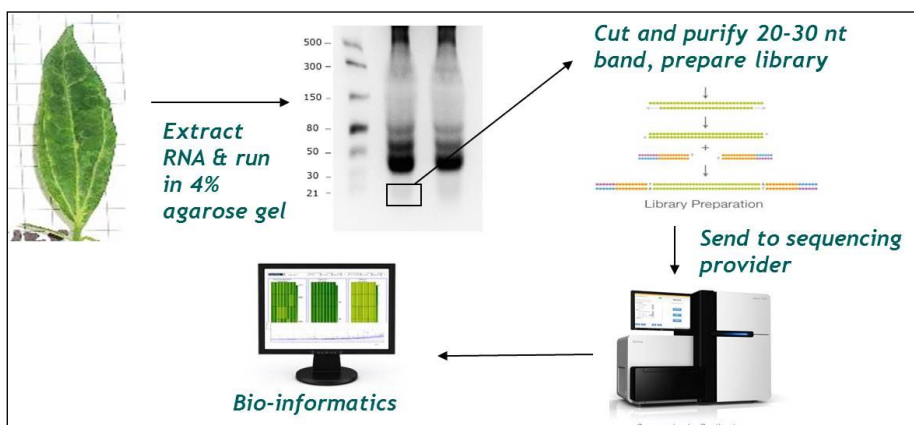


Figure 5. Next Generation sequencing workflow, (Fuentes 2014).

2.11. Mini-microarrays

Mini-microarray in a tube is a tube-based microarray system developed by ClonDiag® (www.clondiag.com/) and currently being validated at the CIP virology lab in Peru (Figure 9).

This technology is very simple to use and less time consuming than other virus detection methods. Up to 80 samples per chip can be tested. Mini arrays (Figure 9) are suitable for 'diagnostic' testing, screening & identification.



Figure 6. ClonDiag® Microarray in a tube,

This technology is promising to be a cost effective detection tool in the future, as it uses a relatively cheap scanner to detect fluorescence, and a smart phone app is being developed to interpret results (via the CIP web portal). Mini-microarrays can detect known viruses, but is not able to detect novel viruses, and is limited to small sample numbers compared to large commercial laboratories using traditional detection technologies (Fuentes 2014).

2.12. Microfluidics

Currently virus diagnostic testing largely relies on PCR, which amplifies a small sample of nucleic acid, such as DNA, and makes it easier to detect. Optics-based methods such as microfluidics are able to determine the exact viral load of a sample.

Targeted virus particles are labelled with specific dyes, which enable individual virus particles to be counted as they pass through the fluorescence detector on the chip, in less than 30 minutes, with minimal sample preparation or extraction.

The instrument can be compact and lightweight, and can be connected to a smart phone making it suitable for in-field testing, even in remote areas (Figure 10). This new technology is cheaper and faster than current technologies and is currently under development (Fuentes 2014).

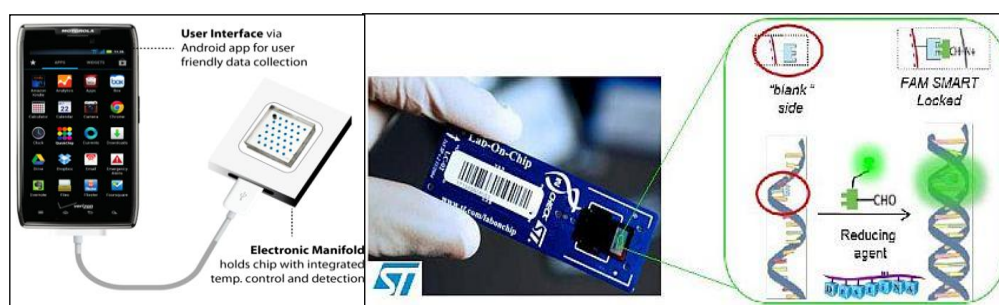


Figure 7 Left to Right; Phone detector, biochip form "lab on a chip" (Fuentes 2014).

A summary of the main virus detection methods used globally in sweetpotato and their ability to identify known or unknown viruses is listed below (Table 2).

Table 2: Detection methods used for sweetpotato viruses

Diagnostic method	Identify virus presence	Identify specific known viruses	Identify new or novel viruses
Biological indexing	✓		✓
NCM-ELISA		✓	
PCR		✓	
qPCR		✓	
LAMP		✓	
Next gen sequencing		✓	✓


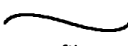


3.0. Viruses reported in sweetpotato









To date 24 viruses (excluding various SPLCV related viruses), assigned to 9 families and at least 4 virus complexes (mixed infections with synergistic reactions) have been reported infecting sweetpotato (Moyer and Salazar 1989, Valverde *et al.* 2007, Salazar and Fuentes 2000, Clark *et al.* 2012) (Table 3). Multiple strains of SPFMV and SPCSV have been identified as well as numerous recently described DNA viruses belonging to the families Geminiviridae and Caulimoviridae (Clark *et al.* 2012). Most of these new viruses or virus strains are associated with symptomless infections in sweetpotato.

Some sweetpotato infecting viruses are present worldwide, with others having a limited geographical distribution (Komolong & Coleman 2005). However, despite rapid progress in virus diagnostics during the last decade, there is still a great lack of knowledge and understanding of virus diseases occurring within sweetpotato plants, their aetiology, epidemiology and the interactions between mixed infections leading to disease complexes (Souto *et al.* 2003, Clark *et al.* 2012).

To date 5 viruses have been reported in Australia: The Potyviruses Sweetpotato feathery mottle virus, Sweetpotato virus 2 and Sweetpotato virus C, the Begomovirus, Sweetpotato leaf curl virus, the Carlavirus, Sweetpotato chlorotic fleck virus and the Cavemovirus, Sweetpotato collusive virus (previously Sweetpotato Caulimo-like virus, SPCaLV) along with the Phytoplasma *Candidatus aurantifolia* (Table 3). A further virus has not been confirmed in Australia, Sweetpotato mild mottle virus (detected in NCM-ELISA 2004, D. Persley), has not yet been confirmed and it is if *Ipomoea setosa* or *Ipomoea batatas* leaves was used in the assay.

Table 3. Viruses reported from sweetpotato (ICTV n.d.).

Family	Genus	Acronym	Species	Type	Structure
Potyviridae	Potyvirus	SPFMV	Sweetpotato feathery mottle virus Strains O, RC and E	ssRNA +	 flexuous filaments
		SPV2 / IVMV / SPVY	Sweetpotato virus 2. Syn: Ipomoea vein mosaic virus /Sweetpotato virus Y		
		SPVC	Sweetpotato virus C		
		SPVG	Sweetpotato virus G		
		SPLV	Sweetpotato latent virus		
		SPMSV	Sweetpotato mild speckling virus		
		SPVMV	Sweetpotato vein mosaic virus		
	Ipomovirus	SPMMV	Sweetpotato mild mottle virus		
		SPYDV	Sweetpotato yellow dwarf virus		
Betaflexiviridae	Carlavirus	SPCFV	Sweetpotato chlorotic fleck virus	ssRNA +	 flexuous filaments
		SP C-6	Sweetpotato C6 virus		
Bromoviridae	Cucomovirus	CMV	Cucumber mosaic virus	ssRNA +	 icosahedra
Bunyaviridae	Phlebovirus	SP C-3	Sweetpotato C-3 virus	ssRNA - and +/-	 enveloped spheres

Closteroviridae	Crinivirus	SPCSV	Sweetpotato chlorotic stunt virus Strains, EA and WA	ssRNA +	 flexuous filaments
Secoroviridae / Comoviridae	Nepovirus	SPRSV	Sweetpotato ring spot virus	ssRNA +	 Icosahedra
Leuteoviridae	Leuteovirus	SPLSV-Peru	Sweetpotato leaf speckling virus	ssRNA+	 Icosahedra
Geminiviridae	Begomovirus	SPLCV	Sweetpotato leaf curl virus multiple strains	ssDNA	 twinned icosahedra or icosahedra
		IYVV	Ipomoea yellow vein virus		
		ICLCV	Ipomoea crinkle leaf virus		
	Mastrevirus	SPSMV-1	Sweetpotato symptomless virus -1		 twinned icosahedra
Caulimoviridae	Badnavirus	SPPV	Sweetpotato pakakuy virus Badna virus A, Badna virus B	dsDNA-RT	 Bacilliform
	Cavemovirus	SPCV	Sweetpotato collusive virus		 Icosahedra
	Solendovirus	SPVCV	Sweetpotato vein clearing virus		 Icosahedra
Phytoplasma	Candidatus aurantifolia	SPLL	Sweetpotato little leaf	phytoplasma	

Green indicates viruses reported occurring in Australia

3.1. Sweetpotato feathery mottle virus (SPFMV)

Sweetpotato feathery mottle virus Family Potyviridae, Genus Potyvirus is the most common and widespread of all viruses known to infect sweetpotato. SPFMV is present in all areas of the world where sweetpotato is grown (Moyer *et al.* 1980) and is the most studied virus infecting sweetpotato.

This virus has been reported in Australia in:

- From the Darwin region of the Northern Territory (Gibb 1993).
- In field multiplication plots in Queensland (Heisswolf 1994).
- Strains RC (Russet crack) and C (common) were documented from Western Australia (Tairo *et al.* 2005).
- In roots purchased at a Perth supermarket in 2006, cultivars *Beauregard* and *Northern Star*.

SPFMV was also detected from plants grown in Broome, Kununurra, and Katherine in cultivars *Jewel*, *Beerwah Gold* and *Beauregard*, strains RC (Russet crack) and C (Common) or RC alone in *Beauregard*, and strain C only in cultivars *Jewel* and *Northern Star* (Tairo *et al.* 2005; Tairo *et al.* 2006b).

SPFMV has also been reported in Papua New Guinea and the Solomon Islands unpublished (Fuentes 2007; Hughes *et al.* 2009) Africa, China, Europe, Japan, Korea, Peru, Taiwan and USA (Kreuze 2008; Clark *et al.* 2012) and Fiji (Amit Sukal pers. com unpublished data 2013).

SPFMV can be detected using Electron Microscopy (EM), herbaceous indexing using indicator plant such as *Ipomoea setosa*, NCM-ELISA (included in the International Potato Center [CIP] kit), RT-PCR (Beetham *et al.* 1992; Souto *et al.* 2003) and RT-qPCR (Kokkinos 2006).

Symptoms seen on sweetpotato plants infected with SPFMV range from vein clearing, vein feathering (feathered chlorosis along the veins), mottling and chlorotic spots with or without purple margins (Hughes *et al.* 2009) and internal cork or cracking of storage roots. Plants infected with SPFMV may be asymptomatic, or symptoms may be transient or mild, however severe infections have been reported with reduced plant vigour and considerable yield reduction. Experiments have shown that virus-free sweetpotato plants yield from 20 to over 100% more than infected plants (Hughes n.d.).

Virus-free plants recently infected with SPFMV in the field typically displaying the symptoms associated with an acute SPFMV infection, a flush of vein clearing and feathering over a two to three week period, in the mid-area of the vine are shown (Figure 11). This sharp peak in infection then levels off, during subsequent weeks, leaving the older leaves with chlorotic spots sometimes surrounded by purple margins (figure 12) that appear quickly and is referred to as a chronic infection (Chris Clark pers. com.).



Figure 9. Acute SPFMV infection DAF 2014.

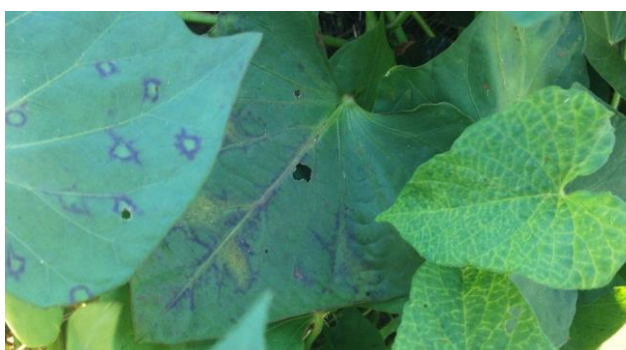


Figure 8. Chronic SPFMV symptoms DAF 2014.

SPFMV is spread by aphids, including *Aphis gossypii*, *Aphis craccivora*, *Myzus persicae* and *Lipaphis erysimi*, in a non-persistent manner (Loebenstein 2009). This virus has a narrow host range being limited to plants in the Convolvulaceae family, mainly the Ipomoeas, *I. batatas*, *I. setosa*, *I. nil*, *I. incarnata* and *I. purpurea*. However it is also reported that some strains have been transmitted mechanically to infect *Chenopodium* spp., including *C. quinoa* as well as *Nicotiana benthamiana* and *N. clevelandii* (Loebenstein 2009) as well as the some plants in the Solanaceae family. Wild Ipomoea species could also act as a reservoir for this virus.

Four different strains of the virus, based on molecular sequencing, have been recognized and recombinations of different strain groups of SPFMV have also been recently reported (Untiveros et al. 2007).

1. Russet crack (RC), as shown in Figure 13, causing characteristic symptoms on storage roots, reported from Australia, Africa, Asia and North and South America.
2. Common (C) reported from Australia, Africa, Asia and North and South America. The C-strain is genetically distant to the remaining strains (Tairo et al. 2005), and this is now classified as a separate virus.
3. Ordinary (O) reported from Africa, Asia and South America.
4. East African (EA) the least widely distributed so far, being reported from East Africa, Spain, Peru and Easter Island. Untiveros et al. 2007, reports some recombination between SPFMV strains.



Figure 13. Russet crack symptoms Fuentes 2005.

3.2. Sweetpotato virus 2 (SPV2)

Sweetpotato virus 2, family Potyviridae, Genus Potyvirus, synonym Sweetpotato virus Y (SPVY) and Ipomoea Vein Mosaic Virus (IVMV) is part of the SPFMV 'group' of viruses (Kreuze 2008). SPV2 was first detected in Taiwan and has since been detected in Barbados, China, Peru Portugal, Spain, South Africa, Australia and USA.

SPV2 was reported by Tairo *et al.* (2006b) for the first time in Australia in 2006. Plants from far north Western Australia and plants grown from roots purchased in a Perth supermarket were found to be infected with SPV2, at the time a tentative member of the potyvirus genus. These samples were also co-infected with strains RC and/or C of SPFMV. The Australian isolates were most similar to isolates of SPV2 from North America.

SPV2 is transmitted in a non-persistent manner by the aphid, *Myzus persicae*, and is able to be mechanically transmitted to several species in the genera *Chenopodium*, *Datura*, *Nicotiana*, and *Ipomoea* (Loebenstein 2009).

SPV2 causes vein clearing and leaf distortion (Figure 14) on *N. benthamiana*, chlorotic local lesion on *Chenopodium spp.*, vein mosaic on *I. nil*, *I. setosa* and *I. tricolour*. Obvious ring symptoms with red margins were observed on infected sweetpotato plants in Australia. Symptoms on *I. setosa* included chlorotic spots, blotches, rings and vein banding (Tairo *et al.* 2006b).



Figure 14. SPV2 symptoms on *I. setosa* (Fuentes 2007).

Tairo *et al.* (2006b) reports that SPV2 was found in one plant of cultivar *Jewel*, grown in Broome in the far north of Western Australia and in two roots of cultivar *Northern Star*, grown from roots sourced at a Perth supermarket. Both of these samples were also found to be co-infected with SPFMV. Tairo *et al.* (2006b) also suggested that due to the widespread occurrence of SPFMV and possibly SPV2 in Australian sweetpotato crops that to provide a reliable virus free planting material program, specific tests for SPV2 should be done in addition to indexing.

3.3. Sweetpotato virus C (SPVC)

Sweetpotato virus C or SPVC, family Potyviridae, genus Potyvirus was previously known as Sweetpotato feathery mottle virus strain C (common) but now renamed Sweet potato virus C (ICTVn.d.).

Like SPFMV, SPVC is transmitted by aphids. Detection methods include RT-PCR, RT-PCR/ restriction fragment length polymorphism (RFLP) and RT- qPCR. Very little information on this virus was found at the stage of compiling this review. The economic impact of this virus is unknown.

3.4. Potyvirus Sweetpotato virus G (SPVG)

Sweetpotato virus G, SPVG, family Potyviridae, genus Potyvirus, was first reported from China (Kreuze 2008), and was previously known as C8 virus. To date, it is known to occur in China, Africa, Barbados, Egypt, Ethiopia, Japan, Nigeria, Peru, Spain, Java, New Zealand, Hawaii, French Polynesia, Easter Island and USA (Rannali 2008). SPVG positive reactions have been observed recently for SPVG in NCM-ELISA from samples in Papua New Guinea, and the Solomon Islands (Hughes undated). Different trains of SPVG have been reported from China.

SPVG is transmitted in a non-persistent manner by the aphids *M. persicae* and *Aphis gossypii*, and is also able to be transmitted mechanically by grafting to other *Ipomoea* species. SPVG causes mottling in *I. nil* and chlorotic spotting in *I. setosa* (Figure 15) and *I. tricolor* (Loebenstein 2009). Infected sweetpotato plants rarely display symptoms and titres are too low to be effectively detected using serological tests (Rannali 2008).

SPVG is able to react synergistically with SPCSV to produce sweetpotato virus disease. Co-infection with SPFMV reduced yields by 14% (Clark 2006). This virus is able to be identified using the NCM-ELISA kit from CIP. There is very little data on the economic impact of SPVG.



Figure 10. SPVG symptoms, (Fuentes 2007).

3.5. Sweetpotato latent virus (SPLV)

Sweetpotato latent virus, family Potyviridae, Genus Potyvirus was first reported in China, but is now known to occur in all major sweetpotato growing areas in Asia. It has been recorded from China, Egypt, India, Indonesia, Japan, Kenya, Korea, New Zealand, Philippines, South Africa, Taiwan and Uganda, (Kreuze 2008). SPLV is a component of the complex viral disease Camote Kulot in the Philippines.

Isolates of SPLV in China and Japan were transmitted by the aphid *Myzus persicae*. This virus can also be transmitted by mechanical inoculation and by grafting, but it is not transmissible by seed.

SPLV is able to be detected using the herbaceous indicators *N. benthamiana*, causing systemic mosaic and stunting; *N. clevelandii*, causing systemic pin-prick chlorotic lesions; *C. quinoa*, *C. amaranticolor*, causing brown necrotic lesions; and *I. setosa*, causing systemic mottle.

SPLV infection is mostly symptomless in *I. batatas*, but occasionally causes mild chlorosis that often disappears soon after infection of sweetpotato plants. SPLV is able to be detected using NCM-ELISA, RT-PCR and reverse transcription - real time - polymerase chain reaction (RT-qPCR). The economic impact of this virus is unknown.

3.6. Sweetpotato vein mosaic virus (SPVMV)

SPVMV family Potyviridae genus *Potyvirus* was first reported from Argentina. SPVMV virions were found in the cytoplasm in all parts of the host plant and this virus has not been reported elsewhere and the original culture and antiserum are no longer available (Loebenstein 2009).

SPVMV causes a virus disease referred to as 'batata crespa' in Argentina. Infected plants show symptoms of vein clearing, mosaic, twisting of leaves, stunting, and reduction in number and size of roots and yield reductions of 84% in cultivar *Criolla Amarilla* in 1970 (Di Feo 1999) (Loebenstein 2009).

SPVMV can be transmitted in a non-persistent manner by *M. persicae*, by grafting and by mechanical inoculation. SPVMV was successfully transmitted to *I. alba*, *I. angulata*, *I. fistulosa*, *I. hederacea*, *I. kurtziana*, *I. lacunose*, *I. nil*, *I. trichocarpa*, *I. tricolor* and *I. setosa*, causing systemic vein clearing and mosaic. In *I. batatas*, *I. hederacea*, *I. nil*, and *I. setosa*, SPVMV also causes twisting, chlorosis and small leaves.

3.7. Sweetpotato mild speckling virus (SPMSV)

Sweetpotato mild speckling virus, family Potyviridae, genus Potyviridae, was first described from Argentina (Kreuze 2008). SPMSV has also been detected in China, Egypt, Indonesia, Peru, Philippines, New Zealand, Nigeria, and South Africa. Recently colleagues in Papua New Guinea, using NCM-ELISA found samples positive for SPMSV, but this has not been confirmed.

SPMSV is transmitted in a non-persistent manner by *M. persicae*. The host range of SPMSV includes plants from the Chenopodiaceae, Convolvulaceae, and Solanaceae families (Loebenstein 2009). Symptoms observed on *I. setosa* and *I. nil* are vein clearing, blistering, leaf deformation and mosaic patterns, while on *N. benthamiana*, vein clearing and reduction, deformation and down rolling of leaves. SPMSV is able to be detected using NCM-ELISA kit from CIP.

SPMSV is a component in Sweetpotato chlorotic dwarf disease (CD) along with SPFMV and SPCSV, synergism exists between SPMSV and SPCSV but not SPMSV and SPFMV. Plants infected with SPFMV and SPCSV show milder symptoms than those additionally infected with the three viruses. The severity of symptom expression is increased when SPMSV is present. The economic impact of this virus is unknown.

3.8. Sweetpotato mild mottle virus (SPMMV)

Sweetpotato mild mottle virus, SPMMV (Figure 16), family Potyviridae, genus *Ipomovirus*, has been reported throughout Africa and in China, Egypt, India, Indonesia, New Zealand, Papua New Guinea and the Philippines. A study of the variability of SPMMV in Uganda using nucleotide sequences showed the virus consisted of a population of distinct sequence variants in the coat protein.

SPMMV differs from most other sweetpotato viruses in that it has the ability to infect a very broad range of host plant species in 14 families including Convolvulaceae, Chenopodiaceae and Solanaceae. SPMMV is transmitted in a non-persistent manner by whiteflies; however it has not been possible to confirm this report (Kreuze 2008). This virus is able to be identified using the NCM-ELISA kit from CIP.



Figure 11. SPMMV infected sweetpotato plant (Fuentes 2007)

3.9. Sweetpotato yellow dwarf virus (SPYDV)

Sweetpotato yellow dwarf virus SPYDV, also from the family Potyviridae, genus Ipomovirus, was first reported from Taiwan in 1979 and Brazil (Loebenstein 2009). SPYDV is transmitted in a persistent manner by *B. tabaci*, and by mechanical inoculation or grafting.

Susceptible hosts include *Chenopodium spp.*, *Gomphrena globosa*, *Datura stramonium*, *Cassia occidentalis* and *Sesamum indicum*. Symptoms on sweetpotato are systemic leaf chlorosis and stunting. Symptoms induced on *I. setosa* include stunting, general leaf chlorosis, small distinct chlorotic spots and vein chlorosis. SPYDV is able to be removed for infected plants by meristem tip culture and thermotherapy (Loebenstein 2009).

Very little other information on this virus was found during this review. The economic impact of this virus is unknown.

3.10 Sweetpotato chlorotic fleck virus (SPCFV)

Sweetpotato chlorotic fleck virus SPCFV, family Flexiviridae, genus *Carlavirus* (formerly known as the Carnation virus group, is an RNA virus with reportedly larger RNA than other Carlaviruses. There is some evidence that different strains may occur. SPCFV has a narrow host range in the families' Convolvulaceae and Chenopodiaceae, but some strains/isolates infect *N. occidentalis* (Kreuze 2008).

SPCFV was detected in a mixed infection with SPFMV in a plant grown from a root purchased at a supermarket in the Australian Capital Territory in 2006 and in sweetpotato from Australia (Jones and Dwyer 2007, Maina 2016). SPCFV has also been repeatedly detected in NCM-ELISA and *I. setosa* in Queensland germplasm (Dennien 2016 in publication), (Figure 17).

Colleagues in Papua New Guinea have also detected SPCFV in *I. setosa* and NCM-ELISA, (unpublished data D. Homare, 2010; A. Kawi, 2013; W. Maso & M. Deros, 2014, NARI). SPCFV has also been reported from Asia, Bolivia, Brazil, China, Colombia, Cuba, India, Indonesia, Japan, Korea, New Zealand, Panama, Philippines, Taiwan and Uganda (Loebenstein 2009).



Figure 12. Symptoms on *I. setosa* graft inoculated with SPCFV and SPFMV DAF 2009.

To date there are no known insect vectors capable of transmitting this virus, although it is able to be mechanically transmitted via grafting to *I. setosa*. It is not transmissible by seed. However, a close relative of SPCFV, Melon yellowing associated virus (MYaV), a proposed Carlavirus from Brazil, has been found to be transmissible by whiteflies (Loebenstein 2009). Other Carlaviruses SPCFV is mostly symptomless in its natural host; hence, it was also referred to as Sweet potato symptomless virus in Japan (Kreuze 2008).

No symptoms have ever been observed on sweetpotato plants infected with SPCFV in Queensland. Symptoms on *I. nil* include fine chlorotic spots and vein clearing on the first and second true leaves (Loebenstein 2009). Symptoms on *I. setosa* in Queensland include vein clearing, short chlorotic areas along minor veins and mottling.

This virus is able to be identified using the NCM-ELISA kit from CIP. Synergism was observed in plants infected by SPCFV and SPCSV (Untiveros *et al.* 2007). The economic impact of this virus is unknown. Only one plant in the Queensland germplasm collection has been found to be infected with this virus so far.

3.11. Sweetpotato C-6 virus (SPC-6)

Sweetpotato C-6 virus SPC-6, family Flexiviridae, genus *Carlavirus* was isolated from sweetpotato in the Dominican Republic and has since been reported in Cuba, Egypt, Indonesia, Kenya, New Zealand, Peru, Philippines, Puerto Rico, South Africa and Louisiana, USA (Clark *et al.* 2009; Loebenstein *et al.* 2009).

Attempts to transmit the virus with *M. persicae* were unsuccessful, C-6 is also poorly transmissible by mechanical inoculation using sap from *I. nil* roots but not leaves (Loebenstein 2009). The host range of C-6 is low, being restricted to the Convolvulaceae family.

Chlorotic spots were observed in black ornamental sweetpotatoes Blackie, Ace of Spades and Black Beauty in Louisiana (Clark and Valverde, 2000). Fine chlorotic spots and vein clearing symptoms were induced on *I. nil* and *I. setosa*, however on *I. batatas* only chlorotic spots were seen (Figure 18). This virus is able to be identified using the NCM-ELISA kit from CIP.



Figure 13. SPC-6 infected sweetpotato plant (Fuentes 2007).

3.12. Sweetpotato C-3 virus (SPC-3)

Sweetpotato C-3 virus SPC-3, is suspected to belong to the family Bunyaviridae genus *Phlebovirus*. SPC-3 was isolated from a sweetpotato plant also infected with SPFMV in Brazil. SPC-3 is transmitted by grafting, but not by mechanical methods or aphids.

Symptoms observed on sweetpotato were vein clearing, leaf deformation, mottling and interveinal mottling. C-3 was found to induce mosaic, leaf deformation and vein clearing on *I. setosa*, and on *N. benthamiana* vein yellowing, mosaic and leaf deformation. Graft inoculation to *I. nil* did not produce symptoms (Loebenstein 2009). The economic impact of this virus is unknown.

3.13. Sweetpotato chlorotic stunt virus (SPCSV)

Sweetpotato chlorotic stunt virus (SPCSV), family *Closteroviridae*, genus *Crinivirus* was previously known as sweetpotato sunken vein virus, SPCSV. The particles of SPCSV are similar to other Criniviruses. To date SPCSV has not been detected in Australian sweetpotato producing areas, and has not been recorded in publications in the South Pacific. SPCSV has possibly recently been detected by NCM-ELISA by colleagues in Papua New Guinea (currently awaiting confirmation). Using NCM-ELISA a positive reaction to SPCSV from a sample collected in Solomon Islands was found in 2009.

Due to its ability to mediate severe synergistic viral diseases with several other sweetpotato infecting viruses including Potyviruses, Caulimoviruses, Cucumoviruses, Ipomoviruses, Cavemoviruses and Carlaviruses, SPCSV is worldwide, probably the most damaging virus of sweetpotato, due to its involvement in SPVD, causing permanent symptoms even when in a single infection (Clark *et al.* 2012). However, most single infections cause mild stunting, combined with slight yellowing or purpling of older leaves (Kreuze 2008).

Two strains of SPCSV have been reported:

- SPCSV EA (East Africa) was first identified in East Africa, and later in Peru and Southern Europe.
- SPCSV WA (West Africa) was first identified in West Africa and has been found in the Americas, and the Mediterranean, but not East Africa (Kreuze 2008).

SPCSV is transmitted in a semi persistent manner by whiteflies (e.g. *Bemisia tabaci*, *B. afer* and *Trialeurodes abutilonea*) and is not mechanically transmissible (Kreuze 2008). The host range of

SPCSV is limited mainly to the family Convolvulaceae and the genus *Ipomoea*, although *Nicotiana* spp. and *Amaranthus palmeri* have been reportedly susceptible. SPCSV has also been detected in the wild species *Lisianthus* (*Eustoma grandiflorum*) (Kreuze 2008). Single infections of SPCSV may be relatively mild, including mild stunting with chlorotic and purple areas (Figure 19), some sunken secondary veins on adaxial leaf surfaces, and swollen veins on abaxial surfaces and mild vein yellowing (Cohen *et al.* 1992). The virus may also be asymptomatic.



Figure 14. SPCSV infected plant (Fuentes 2007).

Due to the structure of the SPCSV genome having two unique proteins, p22 and RNase3, (not known to occur in any other RNA viruses), SPCSV is able to suppress RNA-silencing in plants. This depresses the plant's antiviral defence mechanisms, enabling synergistic reactions to occur when plants are co-infected with SPCSV and other unrelated viruses; mainly RNA viruses, but also Pararetroviruses such as those from the Caulimoviridae family (Kreuze 2008).

Plants co-infected with SPCSV produce more severe symptoms and higher titres of the co infecting RNA or Pararetroviruses – up to 600-fold greater (Kreuze 2002) than in single infections (Cuellar 2011). A singular SPCSV infection may cause only small yield losses in first-generation planting material, but up to 30% in second generation plants. Co-infection with SPFMV can cause sweet potato virus disease (SPVD), a severe disease attributed to almost complete yield loss in Africa, Asia and Central America.

Detection methods available are NCM-ELISA, Nucleic acid spot hybridization (NASH), RT-PCR, RT-qPCR and herbaceous indexing. Yield losses over 80% and death of plants have been attributed to mixed sweetpotato virus infections leading to Sweetpotato virus disease, comprising SPCSV (Carey 1998).

3.14. Sweetpotato ring spot virus (SPRSV)

Sweetpotato ring spot virus SPRSV, family Comoviridae, genus *Nepovirus*, was first observed in sweetpotato from Papua New Guinea imported into the UK (Brown *et al.* 1988). It has since been recorded in Kenya. Virions can be found in leaves and roots (Loebenstein 2009). SPRSV has chemical properties resembling nepoviruses, however there is no serological relationship to any of the 13 recognised nepoviruses.

Sweetpotato plants infected with the virus are often symptomless, or show various degrees of chlorotic spotting on the leaves, depending on the susceptibility of the cultivar. O'Sullivan *et al.* (2005) observed SPRSV to cause occasional chlorotic ring spots that disappear soon after infection. This virus is transmissible by grafting to *I. setosa*, causing faint systemic

chlorotic leaf mottling; to *N. megalosiphon*, causing necrotic rings and systemic mottling; to *N. benthamiana*, causing systemic leaf deformation; and to *C. quinoa* and *C. murale*, leading to transient systemic chlorosis. It is also transmissible via seed, but not by plant to plant contact. No vector is known.

I. batatas is the only known natural host, although experimentally SPRSV infects species in several families (e.g. *Capsicum annum*, *Chenopodium sp.*, *Glycine max*, *Hibiscus esculentum*, *I. setosa*, *Nicotiana spp.*, *Phaseolus vulgaris* and *Vigna unguiculata*).

SPRSV can be detected using EM, *I. setosa* as indicator plant, ELISA and immunosorbent electron microscopy. It is not included in the NCM-ELISA kit from CIP and no primers are available for detection by PCR. The economic impact of this virus is currently unknown.

3.15. Sweetpotato leaf speckling virus (SPLSV)

Sweetpotato leaf speckling virus SPLSV, family Leuteoviridae, genus *Poleovirus* has been reported in sweetpotato from Cuba and Peru. SPLSV has isometric particles 30 nm in diameter. SPLSV is most similar to Potato leaf roll virus (PLRV), (Fuentes 1996).

SPLSV is transmitted in a persistent manner by the potato aphid, *Macrosiphon euphorbiae*. NASH and RT-PCR have been used to detect this virus. Very little other information on this virus was found during this review. The economic impact of this virus is currently unknown.

3.16. Sweetpotato leaf curl virus (SPLCV)

Sweetpotato leaf curl virus (SPLCV), family Geminiviridae, genus Begomoviruses, virus was first reported from Japan and Taiwan in 1998 (Kreuze 2008). Begomoviruses have become an increasing problem in cropping systems globally and are currently a major concern for the Australian Sweetpotato industry. Several new Geminivirus strains have been reported since the 1990s infecting sweetpotato (Kreuze 2008; Rodríguez 2012). These are now sometimes referred to as Sweepoviruses. (Loebenstein 2009), (Table 3).

There has been a rapid emergence and spread of Begomoviruses globally since the 1990's. This is linked to the spread of their whitefly vector, increased human activity and international trading (Caranta 2011). SPLCV was detected in Queensland in 2007, by indexing to *I. setosa* at Gatton and use of PCR (DAF virology group) as part of an Australian Centre for International Agricultural Research (ACIAR) funded project on pathogen testing of sweetpotato, Queensland with international collaborator Segundo Fuentes. This virus was previously not known to exist in Australia; this discovery was simply fortuitous, as an unanticipated outcome of international research. Begomovirus was also detected in Papua New Guinea and the Solomon Islands as part of this project.

SPLCV is transmitted by the whitefly *Bemisia tabaci* in a persistent manner. The host range of *Ipomoea* infecting Begomoviruses is restricted to members of the Convolvulaceae, especially to the genus *Ipomoea*; however *Nicotiana benthamiana* can also be infected experimentally. Many *Ipomoea* species are susceptible to SPLCV, such as *I. purpurea* causing leaf curl and stunt; *I. aquatica* – yellow vein symptoms; *I. nil*; *I. setosa* and *N. benthamiana* – leaf curl symptoms.

Begomoviruses are often asymptomatic and virus symptoms can be transient and mild and often present in low titres (Tao 2012). Visual detection in the field can be difficult. SPLCV causes upward curling and rolling of young leaves, stunting, (Figure 20), vein swelling and chlorosis or yellowing of

leaves (O'Sullivan *et al.* 2005). O'Sullivan (2005), also reports that storage roots can develop longitudinal grooves or ribs. These become more pronounced when SPLCV occurs in a complex with SPFMV. Symptoms on *I. setosa* may be mild, only visible for a short time and generally only appear around 21 days after grafting (Fig. 6). Co-infections of SPFMV and SPLCV in *I. setosa* and *I. nil* induce severe leaf distortion, general chlorosis and stunting (Kreuze 2008).



Figure 15. SPLCV symptoms on sweetpotato left and centre and on *I.setosa* right.

SPLCV has been detected using electron microscopy, after herbaceous indexing to *C. quinoa*, *C. quinos*, *I. incarnnata*, *I. hederifolia*, *I. nil*, *I. purpurea*, *I. setosa*, *N. benthamiana*, *N. clevelandii* and *N. glutinosa*. Plants co-infected with SPFMV and SPLCV may be grafted to *I. aquatica* which is not susceptible to SPFMV, to reveal vein yellowing induced by SPLCV (Valverde *et al.* 2007). SPLCV can also be detected by ELISA (not the NCM-ELISA kit from CIP as this does not contain an antibody to SPLCV), by PCR (Beetham *et al.* 1992; Souto *et al.* 2003) and qPCR (Barkley *et al.* 2011; Clark *et al.* 2012).

Worldwide, there are many different strains of Begomovirus reported to infect sweetpotato (Table 4). Begomoviruses are able to recombine with other viruses from the same genera, if they have a common host plant (Zhang and Ling 2011). The result is a new virus that is then able to infect different hosts. Alternatively, the suites of viruses present in a plant interact, to cause a synergistic reaction, potentially producing severe effects on crop yields in a relatively short period of time. Worldwide, Begomoviruses now rank as the most destructive viruses across all tropical and subtropical agricultural areas, impacting severely on economic and socially important crops. Yield losses of 25-30% have been reported from a single SPLCV infection, as well as reductions in sweetpotato marketable root quality (Barkley *et al.* 2011; Clark *et al.* 2012).

3.17. Sweetpotato symptomless virus -1 (SPSMV-1)

Sweetpotato symptomless virus -1 SPSMV-1, family Geminiviridae, genus *Mastrevirus*, was reported from Peru and later in Tanzania, there was very little information on this virus found during this review. The economic impact of this virus is unknown.

Table 4. Geminiviruses reported from Sweetpotato (ICTV n.d.)

Geminiviruses	Acronym	Reported from	Vector
Sweetpotato golden vein associated virus	(SPGVaV)	Brazil	unknown, possibly whiteflies
Sweet potato leaf curl virus	(SPLCV-Br)	Brazil	unknown, possibly whiteflies
Sweet potato mosaic associated virus	(SPMaV)	Brazil	unknown, possibly whiteflies
Sweet potato leaf curl virus	(SPLCV- Pr)	Puerto Rico	unknown, possibly whiteflies
Sweet potato leaf curl virus	(SPLCV-Pr-Me)	Puerto Rico	unknown, possibly whiteflies
Sweetpotato leaf curl Spain virus	(SPLCESV)	Spain	whiteflies
Sweet potato leaf curl virus Spain	(SPLCV-Sp)	Spain	whiteflies
Sweetpotato leaf curl Canary virus	(SPLCCaV)	Canary Islands	whiteflies
Sweetpotato leaf curl virus	(SPLCV-CN)	China	unknown, possibly whiteflies
Sweet potato leaf curl China virus	(SPLCCNV)	China	unknown, possibly whiteflies
Sweet potato leaf curl virus	(SPLCV-F-p3)	China	unknown, possibly whiteflies
Sweet potato leaf curl virus Korea	(SPLCV-K)	Korea	unknown, possibly whiteflies
Sweet potato leaf curl virus Japan	(SPLCV-Jp)	Japan	unknown, possibly whiteflies
Sweet potato leaf curl virus USA	(SPLCV-USA)	USA	unknown, possibly whiteflies
Sweet potato leaf curl virus USA MS	(SPLCV-MS)	USA	unknown, possibly whiteflies
Sweetpotato leaf curl Georgia virus	(SPLCGoV)	Georgia, USA and Puerto Rico	<i>B. tabaci</i> biotype B
Sweetpotato leaf curl South Carolina virus	(SPLCSCV)	South Carolina US	whiteflies
Sweetpotato leaf curl Uganda virus	(SPLCUV)	Uganda	whiteflies
<i>Ipomoea</i> crinkle leaf curl virus	(ICLCV) (IVYY)	Israel	whiteflies
<i>Ipomoea</i> yellow vein virus	(IVYY- mal)	Malaga, Spain	whiteflies

3.18. Sweetpotato pakakuy virus (SPPV)

Sweetpotato pakakuy virus family Caulimoviridae, genus Badnavirus, synonym Sweetpotato Badnavirus A, SPVa and Sweetpotato Badnavirus B, SPVb, referring to two distinct sequences reported from Peru (Kreuze 2011).

Badnaviruses are one of the most important plant virus groups and have emerged as serious pathogens affecting the cultivation of several horticultural crops in the tropics, especially banana, black pepper, cocoa, citrus, sugarcane, taro, and yam.

Many Badnaviruses are asymptomatic and are also known as endogenous viruses because they are integrated into their host genomes but some can be “awakened” through abiotic stresses causing symptoms to appear. Some Badnaviruses are transmitted by mealybugs and a few species by aphids in a semi-persistent manner, however the vector for sweetpotato infecting Badnaviruses has not been determined.

As PCR methods can result in false positives due to the presence of endogenous badnaviruses, this poses an additional challenge to the complex issue of sweetpotato virus detection and management of clean planting material.

Economic impact, symptoms and host range is unknown. The economic impact of this virus is currently unknown.

3.19. Sweetpotato collusive virus (SPCV)

Sweetpotato collusive virus SPCV, family Caulimoviridae, genus Cavemovirus was previously known as Sweetpotato Caulimo-like virus (SPCaLV) and originally thought to belong to the *Badnavirus* genus, this virus has now been placed into a new genus designated *Cavemovirus*. As part of an international ACIAR funded project on pathogen tested planting material production in Papua New Guinea (PNG), Queensland researchers detected SPCV (Figure 21), using the NCM-ELISA kit purchased from CIP Peru, after indexing sweetpotato plants to *I. setosa* in 2007 in Queensland germplasm. This was confirmed by PCR in 2009. This virus was previously not known to exist in Australia (Hughes undated; Norkunas *et al.* 2009).

SPCV was first reported in Puerto Rico (Moyer and Salazar 1989). It has since been reported throughout several countries in the South Pacific; New Zealand, Papua New Guinea, Tonga, and the Solomon Islands; and the Caribbean Islands, Central America, China, East Africa, Egypt, Kenya, Madeira, and Uganda, (Cuellar *et al.* 2011).

SPCV has a circular, double-stranded DNA (dsDNA) genome (Atkey and Brunt 1987; Moyer and Salazar 1989). Caulimoviruses are pararetroviruses i.e. they contain a reverse transcription stage in their replication cycle, meaning they replicate through an RNA intermediary.



Figure 16. Sweetpotato and ipomoea setosa plants infected with SPCV.

Sweetpotato plants usually do not display symptoms when infected with SPCV (formerly SPCaLV) (Moyer and Salazar 1989). This has been the experience in Queensland, however small chlorotic spots on leaves of plants co-infected with SPCV and SPFMV have been observed on three occasions in a 10 year period on Queensland germplasm (Figure 21). These symptoms have only been observed when plants have been under stress i.e. during cold weather. In contrast, sweetpotato plants co infected with SPCV and Begomovirus kept under greenhouse conditions have been asymptomatic for more than a decade.

When grafted onto *I. setosa* SPCV and SPFMV, mixed infections regularly produce tiny chlorotic spots and flecks, leading to necrosis. Mixed infections of SPCV and Begomovirus on *I. setosa* reliably produce tiny chlorotic flecks along minor veins, and small chlorotic spots leading to necrosis along with downward leaf cupping (Figure 8). Death of the older affected leaves generally occurs 3-5 weeks after grafting.

SPCV can be detected using EM, ELISA and NCM-ELISA (included in the CIP kit) (Beetham *et al.* 1992, Souto *et al.* 2003, Hughes undated, Norkunas 2009) and by PCR (Cuellar *et al.* 2011). Little is known on the biology and epidemiology of SPCaLV. The virus is located in the cytoplasm of the epidermal and vascular parenchyma causing wilting and early

abscission of leaves. There is no insect vector yet identified able to transmit any of the Cavemoviruses (Norkunas *et al.* 2009; Cuellar *et al.* 2011) however SPCV is able to be transmitted by grafting. Sweetpotato is the only known host, though the virus can be made to infect *I. setosa* and *N. megalosiphon*. The impact on yield is not known and it often occurs in a complex with SPFMV.

3.20. Sweetpotato vein clearing virus (SPVCV)

Sweetpotato vein clearing virus SPVCV, family Caulimoviridae, genus Solendovirus, has been reported from Central America, Dominican Republic, East Africa, Guatemala, Jamaica, Kenya, Panama and Uganda. The vector is unknown and detection is by PCR. SPVCV can be synergized with SPCSV in an unnamed complex. Very little information on this virus was found during this review. The economic impact of this virus is unknown.

4.0. Virus complexes

At least four virus complexes incorporating a range of viruses, mainly Potyviruses, have been reported globally to date (Figure 5). These multiple virus infections are associated with synergistic interactions within plants, enabling rapid increases in virus titre.

Table 5. Virus complexes reported in sweetpotato globally

Name	Anacronym		Species	Genus	Yield loss %
Sweetpotato virus disease	SPVD	SPFMV	Sweetpotato feathery Mottle virus	Potyvirus	Up to 80%
		SPCSV	Sweetpotato chlorotic stunt virus	Crinivirus	
Chlorotic dwarf disease	CD	SPFMV	Sweetpotato feathery Mottle virus	Potyvirus	Up to 80%
		SPMSV	Sweetpotato mild speckling virus	Potyvirus	
		SPCSV	Sweetpotato chlorotic stunt virus	Crinivirus	
Sweetpotato severe mosaic disease	SSMD	SPMMV	Sweetpotato mild mottle virus	Ipomovirus	Up to 80%
		SPCSV	Sweetpotato chlorotic stunt virus	Crinivirus	
Camote kulot	Camote kulot	SPFMV	Sweetpotato feathery Mottle virus	Potyvirus	50 %

4.1. Sweetpotato virus disease (SPVD)

Sweetpotato virus disease or SPVD is caused by a synergistic interaction of the aphid transmitted potyvirus SPFMV and the whitefly transmitted Crinivirus, SPCSV. SPVD is the most harmful diseases of sweetpotato globally, severely impacting affected plants, with reports of yield reductions of 80% (Carey 1998). This debilitating virus complex may have been reported from the Belgian Congo in 1939 and was possibly described by Sheffield in East Africa in 1953. SPVD emerged in Peru following an increase in whitefly populations during 1997 and 1998 and led to significant yield reduction. SPVD is the most serious disease of sweetpotato in Africa and is common in both eastern and western Africa. It has also been found in Asia, Israel, Taiwan, China, USA, Argentina, Brazil and Peru (Guitierrez 2003).

Sweetpotato plants infected with SPFMV alone can be asymptomatic, or may produce mild vein-clearing, or chlorotic spots with purple borders. Sweetpotato plants infected with SPFMV and SPCSV however, produce severe symptoms, including leaf reduction and deformation, vein clearing or mosaic, purpling, stunting and significant yield reductions, but not a reduction in root quality (Guitierrez 2003).

Experimentally, SPCSV can induce synergism with all tested Potyviruses (including SPMMV), CMV as well as Carlaviruses and is always associated with an increase in the titres of the co-infecting virus and reduced yield of storage roots (Kreuze 2008). The severity of yield reductions depends on which isolate of each virus is involved (Figure 22).



Figure 17. Plants of Jonathan variety infected with SPFMV and SPCSV isolates (Fuentes 2007).

Incidence and severity of this disease in Uganda is closely associated with the prevalence of whiteflies (Aritua 1998). Varieties differ in their susceptibility to SPVD, some local African germplasm exhibiting resistance, but this is mostly associated with low yielding, later maturing varieties (Carey 1998). The use of virus tested planting materials was recommended to control SPVD in Peru.

4.2. Chlorotic dwarf disease (CD)

Chlorotic Dwarf disease, CD is caused by a co-infection of the Potyviruses SPFMV, SPMSV, and the Crinivirus SPCSV, and is the most economically important sweetpotato disease in sweetpotato producing regions in Argentina. It can result in yield losses of up to 80% (Di Feo 2000).

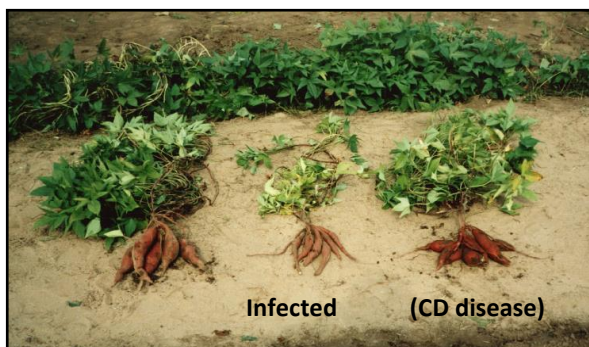


Figure 18. Healthy plants on left and plants infected with CD on right, in Argentina (Fuentes 2014).

Symptoms include stunting, severe mosaic, blisters, leaf distortion and reduced leaf area (Figure 23). CD also causes general chlorosis and vein-clearing. Symptoms increase in severity with propagation (Di Feo 2000). Serological testing using ELISA found SPFMV present in all samples showing symptoms of CD and electron microscopy indicated the additional presence of SPMSV and SPCSV. Use of healthy (PT) planting material is being implemented as a method of controlling CD in the Philippines.

4.3. Sweetpotato severe mosaic disease (SPSMD)

SPSMV results from a dual infection of the Ipomovirus SPMMV, and the Crinivirus SPCSV (Mukasa 2006). Under greenhouse conditions in Uganda, storage root yields were reduced by around 80%, compared to healthy plants (Mukasa 2006). Plants singularly infected with SPMMV showed a non-significant yield reduction, while plants infected with SPCSV alone exhibited a 50% yield reduction. It was shown that SPCSV reduced plant resistance to SPMMV similarly to the reduced plant resistance to SPFMV in SPVD. Due to the synergistic reaction between SPCSV and SPMMV in SPSMD, titres of SPSMMV increased 1000 fold as quantified by qPCR, compared to that in singular infections of SPMMV. Titres of SPCSV involved in SPSMD however reduced twofold indicating an antagonistic reaction (Mukasa 2006).

SPSMD produces severe symptoms such as chlorosis, rugosity, leaf strapping and dark green areas on leaves. Severity of infection and symptoms expresses varied with strains and isolates of SPCSV involved, and also within cultivars, adding to the complexity of this disease and need for further study (Mukasa 2006).

4.4. Camote kulote

Camote Kulot (Figure 24), is a combination of several viruses co-infecting a single plant, SPFMV, C-6, SPCV SPCFV SPCSV, SPLV, SPMMV and SPMSV. Camote Kulot has been reported the most damaging sweetpotato disease in the Philippines, leading to 50% losses in yield.



Figure 19. Sweetpotato plants infected with Camote Kulot in the Philippines (Fuentes 2007).

4.5. Unnamed complexes

- Multiple infections of Cucumber mosaic virus (CMV), SPCSV and SPFMV have been reported from Israel and Egypt, causing reductions in yield up to 80%.
- Multiple infection with SPCSV and SPCV or SPVCV.

- In Australia several mixed infections (Figure 25) have been detected in Queensland germplasm: SPFMV with SPCLV or SPCV; and SPLCV and/or SPFMV with phytoplasma.
- Not all viruses present have been as yet identified, and it is unknown whether synergism is occurring, but symptoms on *I. setosa* suggest this may be the case with severe symptoms typically leading to death of indicator plants 3-4 weeks post grafting.



Figure 20. Co-infections of SPFMV and/or SPCFV and/or, Begomovirus and/or unknown viruses, DAF QLD.

4.6. Other viruses

Some viruses have been reported to be isolated from sweetpotato that normally affect other crops documented in the USA. With the exception of CMV infection, these are isolated incidences and have not been confirmed by serology or herbaceous indexing and do not occur on a regular basis.

- Tobacco mosaic virus (TMV) genus Tobamovirus, family Virgaviridae, found in sweetpotato in USA, transmitted by leafhoppers.
- Cucumber mosaic virus, CMV, genus Cucumovirus, family Bromoviridae. This virus has a worldwide distribution and the widest host range of any known plant virus. CMV is transmitted mechanically by sap, by aphids, in seeds and by parasitic weeds.
- Tobacco streak virus (TSV) found in sweetpotato in USA in a co infection with SPFMV. TSV in the genus Ilarvirus, family Bromoviridae and is transmitted by thrips.

4.7. Sweetpotato Little Leaf (SPLL) (formerly SPLL-V4)

Sweetpotato little leaf (SPLL) previously known as Sweetpotato little leaf variant 4 (SPLL-v4) commonly referred to as witches broom, is a disease caused by the phytoplasma *Candidatus aurantifolia* (Davis *et al.* 2003). Phytoplasmas are phloem-limiting pathogens causing damaging plant diseases in many crops of economic importance in tropical regions of the world (Davis *et al.* 1997; Schneider 1999a; Wilson 2001; Tairo *et al.* 2006a).

SPLL disease was first recorded from Ryukyu Islands, Japan in 1951. Since then it has been reported in China, Indonesia, Korea, Malaysia, New Caledonia, Niue, Palau, Papua New Guinea, Solomon Islands, Taiwan, Tonga and Vanuatu. Severe outbreaks of the disease have occurred in Papua New Guinea and Solomon Islands in areas with low rainfall and a distinct dry season, with

yield losses of 30-90% being reported from these countries (Davis *et al.* 1997; Schneider 1999a; Schneider 1999b; Wilson 2001; Tairo *et al.* 2006a; Hughes undated).

SPLL is transmitted in a persistent manner by leafhoppers of the genus *Orosius* sp. and *Nesophrosyne* sp. *Orosius lotophagorum ryukyuensis* in the Solomon Islands; *Nesophrosyne* (*Orosius*) *ryukyuensis* in Japan. Leafhopper vectors become viruliferous for life. Many crops are hosts for *Candidatus aurantifolia* including wild *Ipomoea* species. Symptoms on sweetpotato in Queensland include, general chlorosis, fine vein clearing and stunting (Figure 26), phyllody (leaf like flowers), virescence (greening of floral tissue), proliferation of stems, leaves and flowers (witches broom) and stunting of the root system leading to few, if any, tuberous roots produced (Wilson 2001).

Phytoplasmas are detected by graft inoculation to *I. setosa* (minimum incubation period of 50 days) in Australia and by PCR. Symptoms on *I. setosa* include stunting, fine vein clearing and production of small leaves (Figure 26). This disease is generally not of economic importance in Australian production regions, as affected plants are easily identified (stunting and chlorosis) and therefore rouged by growers.



Figure 21. SPLL symptoms.

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