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Part I (ZA0040):
Identification, characterisation and epidemiological studies of the whitefly-borne component of sweet potato virus disease.

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Part II (ZA0139):
Molecular characterisation of cassava brown streak virus.

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Contents

Executive Summary

This report covers two projects, one on sweet potato viruses and one on cassava brown streak disease. They were linked through both being whitefly-transmitted viruses of root crops. The sweet potato virus disease (SPVD) project identified the whitefly-borne component of this disease in East Africa to be a serologically and genomically distinct strain of sweet potato chlorotic stunt closterovirus (SPCSV). Characteristic symptoms of the two main diseases (SPCSD & SPVD), caused by SPCSV in sweet potato in either the absence or presence of sweet potato feathery mottle virus, have been described and an ELISA-based method for detecting SPCSV has been developed and proven in Africa. Polyclonal antiserum has been developed using bacterially expressed coat protein. The strain of SPCSV found in Uganda has been shown to occur also in Kenya, Tanzania, Zambia and Madagascar. Two serotypes possessing small differences in their coat protein and HSP70 homologue genes were detected and their symptomatologies and distributions in Uganda described. Differences in the incidence of SPVD in Uganda and Tanzania have been demonstrated and mapped in districts located around the perimeter of Lake Victoria. Areas where SPVD was rare tended to have few whiteflies throughout the year or a very SPVD-resistant cultivar predominated. Given the importance of sweet potato to rural livelihoods and the major yield losses incurred annually in sweet potato in many areas of East Africa, the project will contribute substantially to increasing crop yields in East Africa as superior resistant varieties are developed.

The causal agent of cassava brown streak disease was found to be a single virus (CBSV) and a member of the *Potyviridae* family. CBSV is most closely related to the whitefly-transmitted virus, sweet potato mild mottle virus (SPMMV). These two viruses now form the only two sequenced members of a genus called the Ipomoviruses. CBSV was shown to not react with antiserum raised to members of the *Potyviridae* family, including SPMMV. The first reliable test for this disease, a PCR detection method, has been developed and is described here. Three distinct isolates of CBSV have been found in Tanzania and one in Mozambique. These isolates explain the different symptoms reported here and previously with secondary host plants. A construct for expressing the coat protein of the virus has been built and the coat protein of the virus purified. The subsequent polyclonal antiserum proved to be of poor quality and unsuitable for use with infected cassava. A MAP peptide has been made to 15 amino acids of the virus coat protein and polyclonal antiserum to this peptide is being made. Two binary vectors have been built suitable for plant transformation. These constructs deliver identical T-DNA regions to the plant, which express the coat protein of the virus in the antisense orientation under the control of the 35S promoter. Tobacco plants containing these constructs have been generated but further testing for resistance to the virus was not possible due to lack of time.

Part I: Identification, characterisation and epidemiological studies of the whitefly-borne component of sweet potato virus disease.

Background

African sweet potato production is concentrated in E Africa, especially in the countries around the perimeter of Lake Victoria. Uganda has the largest sweet potato production in Africa and the second largest in the World (World Bank, 1993; FAO, 1998). Tanzania has the second largest area of production in Africa (after Uganda), though only the sixth largest production, due to chronically low yields. Sweet potato is an important crop throughout much of East Africa, grown particularly by women around the homestead for daily food and to sell for family needs (Bashaasha *et al.*, 1995; Kapinga *et al.*, 1995). It is also grown extensively in some areas as a cash crop, sold particularly to the urban poor as a staple food. Sweet potato is increasing steadily in importance in most countries in eastern and southern Africa. In several countries, this is as a result of market liberalisation policies resulting in the removal of subsidies from fertilisers required for effective production of other crops notably maize. In a few countries such as Zambia, it is also as a result of the introduction of superior varieties of sweet potato. Sweet potato is particularly valued for its ability to yield in poor soils and to provide food quickly following the onset of the rains, especially when these are unreliable or have largely failed. In East Africa, an epidemic of African cassava mosaic virus has caused widespread destruction of the cassava crop, and sweet potato production has risen locally and dramatically in compensation.

Sweet potato virus disease (SPVD) is the name used commonly in Africa to describe a range of severe symptoms on sweet potato generally attributed to virus infection. Symptoms vary with plant genotype but typically include stunted plants with small distorted leaves, the latter often also being distorted, narrow (strap-like) and crinkled with a chlorotic mosaic and/or vein-clearing, giving affected plants an overall pale appearance. SPVD is the most serious and widespread disease of sweet potato in Africa including the Lake Victoria region (Geddes, 1990) where the bulk of African production occurs. Affected plants commonly yield less than half that of symptomless ones (Mukiibi, 1977; Hahn, 1979).

In Nigeria, SPVD has been associated with the presence of both an aphid-borne potyvirus now known to be sweet potato feathery mottle virus (SPFMV) and a closterovirus transmitted by the whitefly *Bemisia tabaci* (Schaefers & Terry, 1976; Clark & Moyer, 1988). This Nigerian closterovirus was first called sweet potato chlorotic stunt (SPCSV) (Schaefers & Terry, 1976) and later SPVD-associated closterovirus (Winter *et al.*, 1992). A closterovirus isolated in Israel from sweet potato was named sweet potato sunken vein virus (SPSVV) (Cohen *et al.*, 1992) but has since been shown to be serologically similar to the W African closterovirus (Hoyer *et al.*, 1996; Vetten *et al.*, 1996). Consequently, the Closterovirus Study Group of the International Committee for the Taxonomy of Viruses has agreed that the name SPCSV should be retained for this closterovirus (Vetten, personal communication) and this name is used for this virus throughout this report.

In East Africa, Sheffield (1957) identified two viruses commonly infecting sweet potato and distinguished them as sweet potato viruses A and B. Virus A was aphid-borne and seems likely to have been SPFMV. As in West Africa, Virus B was whitefly-borne. Hollings *et al*., (1976) identified the whitefly-borne sweet potato mild mottle ipomovirus (SPMMV) in East Africa; its presence, that of SPFMV and certain other viruses were confirmed by Wambugu (1991). The whitefly-borne SPCSV was not included in either of these studies, partly due to a lack then of suitable diagnostic reagents for SPCSV. The presence of a whitefly-borne component of SPVD was confirmed during the ODA Holdback project R5878 (1994-7). No positive identification of it could be made during the initial phase of R5878 as no antisera available reacted to it, although it was identified as not being SPMMV. This confirmation of the presence of an unidentified whitefly-borne virus in sweet potato in East Africa (where the bulk of the African crop is grown) in plants affected with SPVD, the main disease of the crop, provided the main justification for the R6617. Further evidence of demand was that resistant varieties were (and still are) being sought actively by national sweet potato programmes in Africa, particularly the Ugandan National sweet potato programme (Mwanga & Sengooba, 1996). They are also being sought by the International Potato Center, the CGIAR Institute with the mandate for sweet potato, as resistance is considered to be the most effective means of controlling the disease (International Potato Center, 1995). This required a suitable means of accurately diagnosing the component viruses so another aim of the project was to develop, test and prove a robust method of diagnosing EAWBV. A further important aim of the project was to examine the variability of EAWBV within East Africa by a range of serological and molecular methods so as to assess the likely durability of resistance.

The presence of the whitefly-borne closterovirus SPCSV was eventually demonstrated in a limited number of diseased sweet potato plants obtained from East Africa by Hoyer *et al*. (1996). However, no causal relationship was established and it remained unclear whether SPCSV was generally associated with SPVD throughout East Africa. Polyclonal and monoclonal antibodies (Mabs) raised against this virus (Hoyer *et al*., 1996; Vetten *et al*., 1996) provided the first opportunity to test extensively for this virus in Africa using an enzyme-linked immuno-sorbent assay (ELISA) so enabling the determination of whether SPCSV is generally and causally associated with SPVD.

Project Purpose

SPVD was recognised to be the most serious disease of sweet potato in East Africa, where the bulk of the crop is grown. However, the aetiology of SPVD, particularly the identity of the whitefly-borne component virus, was unresolved. The main aim of the project was therefore to identify the whitefly-borne component of the virus complex causing SPVD in East Africa (*at that time, this virus was called, for purposes of identification in project documentation, the East African whitefly-borne virus (EAWBV) and this acronym has continued to be used in this report when referring to project documentation - although the virus is now known to be SPCSV*). It was considered necessary that a robust and proven detection method should be developed because SPVD is such an economically important disease in Africa and because breeding programmes in Africa, particularly Uganda, are actively seeking

superior resistant genotypes. The latter also required an assessment of the variability of the virus so as to assess the likely durability and geographical efficacy of resistance.

In 1997/8, an Inter-Centers Initiative funded by DANIDA on Whiteflies and Whitefly-borne Viruses was initiated by CIAT, with a sub-project on Rootcrops in Africa managed by IITA. This sub-project largely involved work on the whitefly-borne African cassava mosaic virus (ACMV), but a component on whitefly-borne viruses of sweet potato in Africa was included. This component was managed by CIP from its Nairobi Regional Office and CPP agreed an extension (1998/9) to R6617 to enable a contribution to be made to the Initiative. The extension covered research into the epidemiology of EAWBV in Uganda, the incidence of EAWBV in Uganda and the Lake Zone of Tanzania and the geographical range of EAWBV in eastern and southern Africa.

Dissemination of results and transfer of technologies including diagnostic methods to national programme scientists, particularly those with breeding programmes, were an integral part of the project.

Research Activities

1995-6. The identification of EAWBV was the initial focus of activity. This work was done at two sites: at Namulonge Agricultural and Animal Production Research Institute (NAARI) in Uganda, and in the Department of Biology at Bristol University in UK. Research at NAARI used whiteflies *Bemisia tabaci* and aphids *Myzus persicae* to isolate the whitefly- and aphid-borne components of SPVD. The work was done in purpose-built screenhouses using virus-free sources of sweet potato cultivars, particularly cv Tanzania, supplied by the International Potato Center (CIP) and common indicator plant species, particularly *Ipomoea setosa*. An isolate of EAWBV and samples of naturally SPVD-affected sweet potato plants were supplied to colleagues at Bristol University so that they could use their advanced laboratory facilities to purify and characterise EAWBV.

1996-8. We became aware (Hoyer *et al*., 1996; Vetten *et al*., 1996) of the development in Dr HJ Vetten's laboratory in Braunschweig in Germany of both monoclonal (Mabs) and polyclonal (Pabs) antisera to a closterovirus from sweet potato. "Their" virus had been isolated in Israel and was initially considered to be unique virus called sweet potato sunken vein virus (Cohen *et al*., 1992). This was later found to resemble isolates of SPCSV occurring in West Africa (and elsewhere). Supplies of antisera were provided freely and allowed us to identify EAWBV as an East African strain of SPCSV. This rapid progress in the identification of the virus gave Bristol University opportunity to examine the variability of SPCSV isolates through sequencing the coat protein and the heat shock protein 70 (HSP70) homologue genes of several isolates of SPCSV. Success in this enabled further supplies of polyclonal antiserum to SPCSV to be made using bacteria to express the sequenced coat protein gene. Work at NAARI utilised the Mabs to examine the

serological variability of East African SPCSV in Uganda, to study its occurrence in several other East African countries and for the diagnostic method to be disseminated to national scientists.

The rapid identification of EAWBV as SPCSV also allowed an early start to epidemiological studies in Uganda on the incidence of SPVD and abundance of its whitefly vector *B. tabaci* both in farmers' fields and in field trials done on-station at NAARI and on-farm. The good progress also gave sufficient confidence to include two Makerere University MSc students (Mr T Alicai and Mr V Aritua) in the project work, CIP and USAID providing scholarships.

1998-9. A project extension allowed the epidemiological studies in Uganda to be continued for a further annual cycle and for surveys of SPVD to be done in Zambia, Madagascar and Tanzania. This work was done (see Project Purpose) as part of a collaboration with the sub-project on African root crops of the Inter-Centers Initiative on Whiteflies and Whitefly-borne Viruses.

Throughout the project, high priority was given to dissemination of our research results, to international scientists through publication in international refereed journals and at international meetings, and more locally through attendance at African scientific meetings and direct training.

Outputs

Output 1a. Characterisation of EAWBV, development of diagnostics and determination whether it is consistently present in field sweet potato plants with a) the purpling symptom, and b) SPVD.

Virus symptoms observed in field plants

Characteristic symptoms of SPVD observed in sweet potato cultivars at NAARI Farm and in farmers' fields are listed in Table 1. They generally comprised severe stunting, leaves becoming crinkled and distorted (often narrow and strap-like in genotypes such as cv Tanzania having divided leaves), and either a pale green mosaic or clearing of the major veins. Some cultivars with SPVD also had purpling on lower leaves. Another virus symptom commonly observed in field plants comprised a stunting of the whole plant and either a purpling of lower and middle leaves, for example in cv. Tanzania, or a chlorotic yellowing, for example in cv. Wagabolige. No other symptoms characteristic of virus infections have been observed at NAARI Farm or in farmers' fields in these two cultivars over a three year period during which several hundred diseased plants of each cultivar have been examined. In other sweet potato varieties examined at NAARI or sweet potato landraces examined in farmers' fields throughout Uganda, a similar identification of two symptom types could be made, the common one being SPVD and the rarer one a purpling or yellowing of foliage combined with stunting.

Table 1 *Symptoms of SPVD in different Ugandan sweet potato cultivars*

Cultivar	Symptoms of SPVD
Tanzania	Plant stunted, leaves narrow and stunted, crinkled and with a pale-green mosaic
Wagabolige	Plant stunted, leaves small and crinkled, pale green with chlorosis along major veins
Tororo 3	Plant stunted, small puckered leaves with a chlorotic mottle
Sowola	Plant stunted, leaves narrow and stunted with a chlorotic mottle
New Kawogo	Plant stunted, leaves small with chlorosis along major veins
Bwanjule	Plant stunted, leaves narrow with a distorted edge, purple with a pale green mottle
Bitambi	Plant stunted, leaves small, crinkled with chlorosis along major veins.
Kimotoka	Plant stunted, leaves small, crinkled with chlorosis along major veins.

Transmission and serology tests

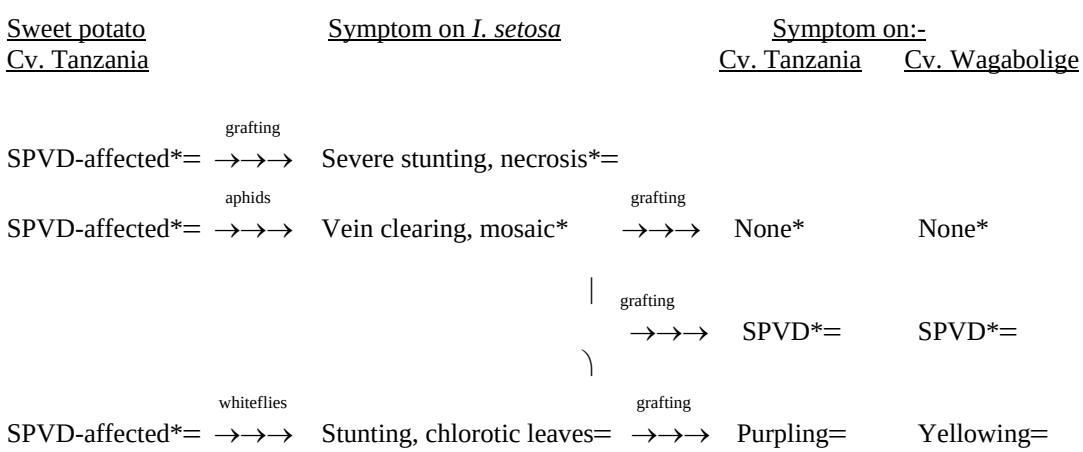
Cuttings of cv. Tanzania established in a screenhouse from a typical SPVD-affected field-grown plant at NAARI were found by ELISA to contain SPFMV and SPCSV, but not SPMMV, SPCFV or SPLV. Grafting scions to *I. setosa* caused within 1-2wks a severe and permanent disease on the latter comprising stunting of the main stem and leaves, general chlorosis of the leaves and necrosis sometimes leading to plant death. Aphid transmissions from the cuttings to *I. setosa* initially induced in the latter a mosaic, then vein-clearing followed eventually by the production of symptomless leaves. These symptoms were quite different from those obtained by the above graft-inoculation but were typical of those reported for SPFMV in this indicator plant. The presence of SPFMV was confirmed by nitro-cellulose membrane enzyme-linked immuno-sorbent assay (NCM-ELISA). When the *I. setosa* infected with SPFMV were grafted to virus-free sweet potato cvs Tanzania or Wagabolige, no symptoms were induced over a period of observation lasting more than 1yr although again back-grafting to *I. setosa* seedlings and testing with NCM-ELISA showed that these plants contained SPFMV. Plants of *I. setosa* inoculated using whiteflies collected from severely diseased field plants at NAARI became stunted and leaves were also brittle, cupped downwards and chlorotic (but not necrotic) and reacted positively in triple antibody-sandwich (TAS)-ELISA in microplates to SPCSV. However, sap samples from these plants did not react in NCM-ELISA with antibodies to the whitefly-borne SPMMV, or to SPFMV, sweet potato chlorotic fleck virus (SPCFV) or sweet potato latent virus (SPLV), all of which had been detected previously in Uganda by the Holdback Project R5878.

Table 2 *The absorbances (A_{405nm})= generated in TAS-ELISA for SPCSV of duplicate sap samples of I. setosa and sweet potato plants cv. Tanzania graft-inoculated with SPCSV and/or SPFMV*

	Inoculated with SPCSV	Not inoculated with SPCSV
I. setosa	1.508; 1.586	0.008; 0.004
I. setosa + SPFMV	0.654, 0.665	0.004, -0.012
Cv. Tanzania	0.688; 0.624	0.013; 0.004
Cv. Tanzania + SPFMV	0.238; 0.246	0.008; 0.005

= Blanked on wells containing substrate only.

Virus-free cuttings of cvs Tanzania or Wagabolige were grown in pots of soil in a screenhouse, four plants of either cultivar to a pot and four pots of each cultivar. In each pot, one cutting was grafted with *I. setosa* infected with SPFMV, one cutting was grafted with *I. setosa* infected with SPCSV, one cutting was grafted with *I. setosa* infected with SPFMV and SPCSV, and one cutting was mock-grafted. After *c.* 4wks, the plants of cv. Tanzania inoculated with SPFMV + SPCSV developed very stunted growth, old lower leaves became purple and new leaves were narrow and strap-like with a pale green mosaic. Similarly inoculated plants of cv. Wagabolige also became very stunted with pale-green, chlorotic leaves which often also had pronounced vein-clearing. These symptoms resembled those observed in the field and described as SPVD in each cultivar. After about 7wks, the plants of cv. Tanzania inoculated with SPCSV became stunted and developed purple middle and lower leaves and the plants of cv. Wagabolige inoculated with SPCSV also became stunted and developed golden yellow leaves, symptoms resembling in each cultivar the other common virus-like symptom observed in crops. TAS-ELISA absorbance values for SPCSV in *I. setosa* and cv. Tanzania plants are given in Table 2. All plants mock-grafted or grafted with SPFMV remained symptomless. The results of all these transmission tests are summarised diagrammatically as follows:-



* SPFMV +ve by NCM-ELISA; = SPCSV +ve by TAS-ELISA

These results:-

- *provide the first positive demonstration that SPFMV + SPCSV are the causal agents of SPVD in sweet potato in East Africa;*
- *demonstrate this under controlled conditions using serologically identified, East African isolates of SPFMV and SPCSV and virus-free sweet potato plants.*
- *provide the first identification of SPCSV as the cause of purpling and stunting in sweet potato.*

Output 2a. Diagnostic test for WBC leading to improved screening for WBC and SPVD resistant genotypes.

Serological detection and strain identification of viruses in diseased field plants. Following the above results, tests were made to determine which part of diseased field plants gave the most reliable detection of SPCSV and which strain of SPCSV was present in Uganda. Monoclonal antibodies provided by Dr HJ Vetten were tested in TAS-ELISA using either a mix of Mabs all of which had previously been found to react only to a Kenyan isolate of SPCSV (Mab Mix-1)(Hoyer *et al.*, 1996), or using a mix of Mabs (Mab Mix-2) all of which reacted only to isolates of SPCSV found in West Africa.

Table 3 *Viruses detected by TAS-ELISA (SPCSV) and NCM-ELISA (SPFMV, SPCFV, SPMMV & SPLV) in SPVD-affected sweet potato obtained from different locations in Uganda.*

Location	SPCSV		SPFMV	SPCFV	SPMMV	SPLV	Number of plants tested
	Mix-1	Mix-2					
Rakai	20	0	20	2	0	0	20
Fort Portal	14	0	14	1	0	0	14
Mpigi	15	0	15	1	0	0	15
Tororo	9	0	9	0	0	0	9
Tororo	10	not tested	11	0	5	0	11
Iganga	37	not tested	37	1	1	0	37
Total	**105**	**0**	**106**	**5**	**6**	**0**	**106**

Samples from middle leaves gave both the highest absorbance values (0.780±0.127 compared to 0.322±0.107 from bottom leaves and only 0.096±0.041 in top leaves) and detected SPCSV in all diseased plants, so all subsequent samples were taken from there. Cuttings were obtained from 106 plants of a range of local cultivars, all with symptoms involving some combination of stunting, chlorosis, mosaic, vein-clearing or purpling, from farmers' fields in several locations in Uganda (Table 3). All samples but one contained both SPFMV and SPCSV, a few were

additionally infected with either SPCFV or SPMMV (but none with all four viruses) and one sample was infected with SPFMV and SPMMV only. SPCSV was detected in all cases only by Mabs Mix-1. Plants infected with SPCFV as well as SPFMV and SPCSV seemed to have typical symptoms of SPVD, although perhaps slightly more severe. Although plants with SPVD are stunted, they typically remain upright. By contrast, the five plants containing SPMMV plus SPFMV and SPCSV were all weak as well as stunted, and had a straggly appearance, leaves had a wavy edge and a chlorotic mottle. The plant containing SPMMV plus only SPFMV was also straggly but had only occasional chlorotic spots on leaves instead of a general mottle.

Tests were also done on field plants of cvs Tanzania and Wagabolige at NAARI which were symptomless, purpled (cv. Tanzania) or yellowed (Wagabolige), or had the symptoms of SPVD typical for each variety (Table 4). SPCSV was detected by Mabs Mix-1, but never by Mix-2 in all plants with SPVD, yellowing or purpling. SPFMV was detected in all plants with SPVD but only in about a third of yellowed or purpled plants and in only one symptomless plant. Similar results were obtained with symptomless and SPVD-affected plants of cvs Tororo 3 and New Kawogo. TAS-ELISA tests for SPCSV using Mix-1 and NCM-ELISA for SPFMV on a further ten plants each of cvs Tanzania, Wagabolige, Sowola, Bwanjule and Tororo 3 all with SPVD were also all positive.

Table 4 *Detection of SPFMV and SPCSV by TAS-ELISA in field plants of sweet potato cvs Tanzania (Tz), Wagabolige (Wb) and Tororo 3 (Tor) affected with SPVD or a purpling or yellowing symptom.*

Plant Sample	SPFMV	SPSVV: MAbs-1		SPSVV: MAbs-2	
	Positives/total sample	Positives/total sample	Absorbance (A_{405nm})	Positives/total sample	Absorbance (A_{405nm})
Tz – SPVD	35/35	35/35	0.967±0.105	0/35	0.096±0.024
Tz – Purpled	14/35	35/35	1.440±0.100	0/35	0.096±0.026
Tz – symptomless	1/35	0/35	0.095±0.003	0/35	0.095±0.002
Virus-free control			0.102±0.006		0.099±0.005
Wb – SPVD	5/5	5/5	0.782±0.167	0/5	0.082±0.010
Wb – yellowed	1/5	5/5	1.059±0.124	0/5	0.093±0.006
Wb –symptomless	0/5	0/5	0.094±0.006	0/5	0.100±0.006
Tor – SPVD	8/8	8/8	0.533±0.063	0/8	0.089±0.004
Tor – symptomless	0/8	0/8	0.094±0.003	0/8	0.093±0.003
Virus-free control			0.085		0.090
Total					
SPVD	**48/48**	**48/48**		**0/48**	
Purpled/yellowed	**15/40**	**40/40**		**0/40**	
Symptomless	**1/48**	**0/48**		**0/48**	

Additional survey results. In Madagascar, Zambia and Tanzania, field plants with symptoms of SPVD were tested for SPCSV by TAS-ELISA using Mabs Mix-1 and for SPFMV using either double antibody-sandwich (DAS)-ELISA or NCM-ELISA. Tests in all cases were positive for both viruses.

The effect on yield of SPCSV

Plants grown from SPCSV-infected cuttings produced about a quarter the foliage of uninfected cuttings; SPVD-affected cuttings from the field scarcely grew and produced *c.*3% of the foliage of uninfected cuttings (Table 5). Storage root yield was poor in the screenhouse for virus-free and diseased plants; however, SPCSV-infected cuttings produced only *c.* 13% and SPVD-affected cuttings produced only *c.*2% of the yield of uninfected cuttings.

Table 5 *The mean yield (fresh weight in kg) in a screenhouse of mounds planted with virus-free, SPCSV-inoculated or SPVD-affected cuttings of sweet potato cv. Tanzania*

	Virus-free	SPCSV-infected	SPVD-affected	L.S.D. (P= 5%)
Foliage	3.6	0.96	0.11	0.87
Storage roots	0.64	0.085	0.012	0.15

These results:-

- *provide the first large-scale demonstration and for a diversity of cultivars growing both in farmers' fields and on-station that SPCSV and SPFMV are consistently present in SPVD-affected, and that apparently no other virus is consistently present;*
- *confirm the presence of SPCSV in purpled field plants of some sweet potato varieties, but also in plants with a yellowing symptom in other cultivars;*
- *provide and prove a method of diagnosing the component viruses of SPVD and that this method is effective in African conditions and on a large scale;*
- *confirm the extremely severe yield losses associated with infection by SPCSV, especially when combined with SPFMV to produce SPVD.*

Output 1b. The ability of Ugandan national scientists to diagnose EAWBV.

The method of diagnosing SPCSV and SPFMV have been transferred to:

- Ugandan national programme staff at NAARI. The methods are currently being used at NAARI by the senior sweet potato breeder, Mr R O.M. Mwanga, as part of his PhD studies (University of North Carolina) on the inheritance of resistance to SPVD in sweet potato;
- Kenyan Agricultural Research Institute staff. CIP paid Mr T Alicai (an MSc student studying within the Project) to train KARI staff at Muguga Research Institute;
- Makerere MSc students.

Output 2b. Knowledge on whether genotypes identified as resistant in one part of Africa are likely to be useful elsewhere.

Tests done in Zambia, Madagascar (Gibson *et al.*, 1998) and Tanzania showed that samples of SPCSV there reacted, as do samples from Uganda, Kenya and Tanzania,

only with Mab Mix-1. This suggests that SPCSV isolates in all of East Africa and perhaps all of southern Africa, belong to a single East African strain of SPCSV and that sweet potato varieties resistant in part of this region will be resistant throughout. This has not been tested systematically because of inadequate resources and phytosanitary difficulties in transferring sweet potato from one country to another. However, the sweet potato variety known as Tanzania in Uganda and SPN/0 in Tanzania appears to be resistant from Uganda through to Zambia (RW Gibson, personal observations). By contrast, Ugandan national programme staff have reported that varieties of sweet potato resistant to SPVD in West Africa (where a different serological strain of SPCSV is known to occur) were susceptible in Uganda (Mwanga *et al.*, 1991). Similar results of unusual susceptibility of West African sweet potato varieties have been reported to me (witnessed in Tanzania) during field trips to Tanzania and Zambia.

These results and observations suggest that SPVD-resistant sweet potato varieties from East Africa will remain resistant within all of East and Southern Africa. This is economically important because it indicates that superior sweet potato genotypes generated by the Ugandan National Programme (the largest and most active breeding programme in East Africa) should retain their virus resistance if transferred to other East and Southern African countries.

Output 2c. Determination of the variability of EAWBV will allow an assessment to be made on the likely durability of resistance.

Occurrence and geographical distribution of serotypes of the East African strain of SPCSV. The isolate of SPCSV obtained by transmission using whiteflies collected from an SPVD-affected sweet potato plant growing at Namulonge Agricultural and Animal Production Research Institute (NAARI) Farm, Mpigi District, Uganda (Fig. 1) was tested using each of the six Mabs coded 2G8, 2G9, 2G11, 6C9, 6C10 and 6D12 which had comprised Mab Mix-1. Although all these Mabs had been reported to react to a Kenyan isolate of SPCSV (Vetten *et al*., 1996), only Mabs 2G8 and 2G9 reacted strongly with the Ugandan isolate. Consequently, leafy cuttings were collected from diseased plants of local cultivars growing in farmers' fields in each of Soroti, Tororo, Iganga, Mpigi, Masaka, Rakai and Kabarole Districts in Uganda (Fig. 1). These were grown in pots of soil in a whitefly-free screenhouse at NAARI before testing with the full panel of Mabs to determine their serotype. Five plants of sweet potato with SPVD were obtained from the Kenyan Germplasm Collection maintained in the field at the Kabete Campus of the University of Nairobi and one SPVD-affected plant from a collection at the Kenyan Agricultural Research Institute, National Agricultural Research Centre at Muguga. Those samples reacting to all Mabs in a manner similar to the original Kenyan isolates tested were classified as serotype East Africa 1 (S_{EA1}) and those reacting strongly to only Mabs 2G8 and 2G9 were classified as serotype East Africa 2 (S_{EA2}). Results from these were used to generate Fig. 2.

Fig.1. *Map of Uganda showing the districts sampled and the location of NAARI.*

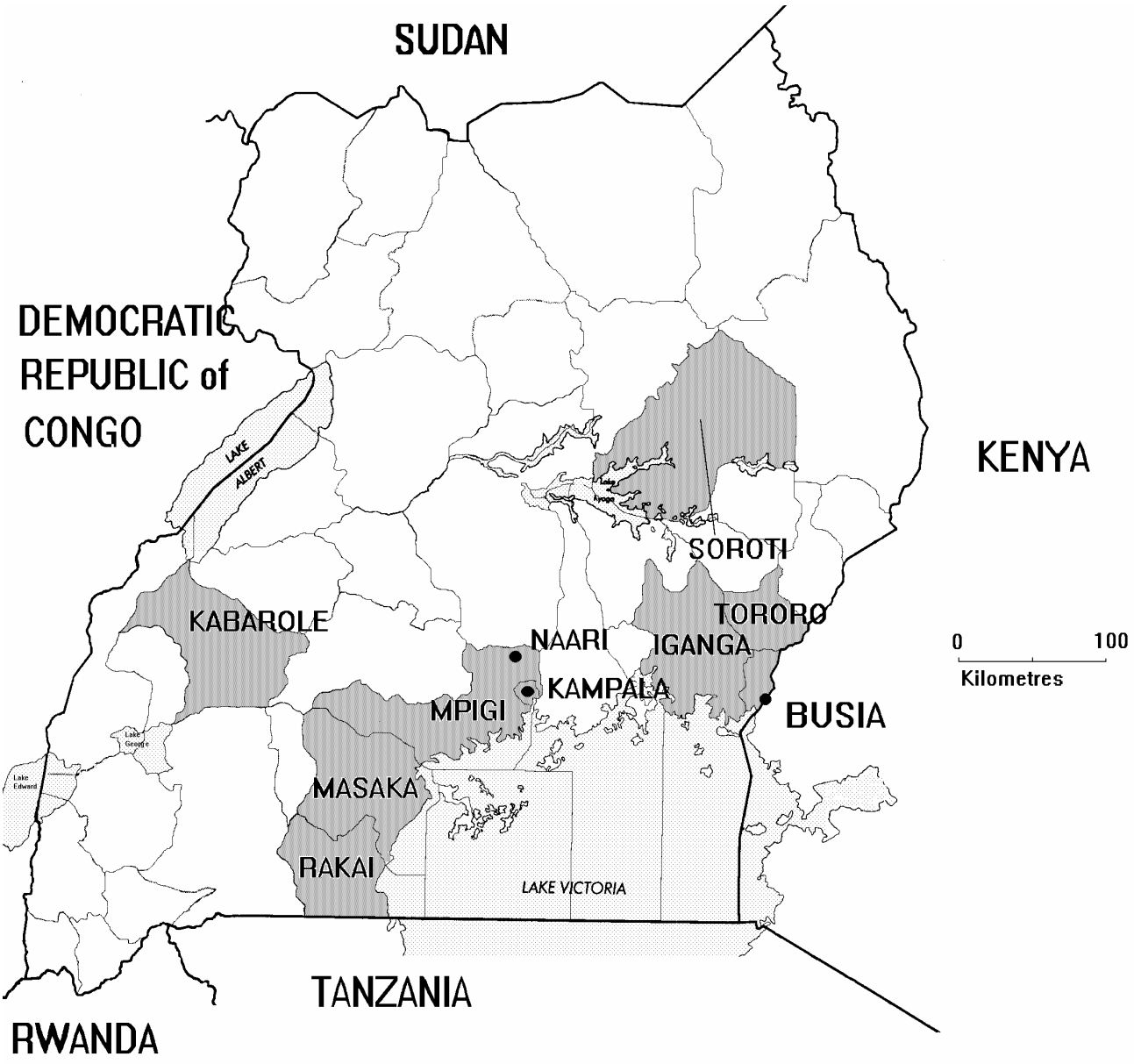
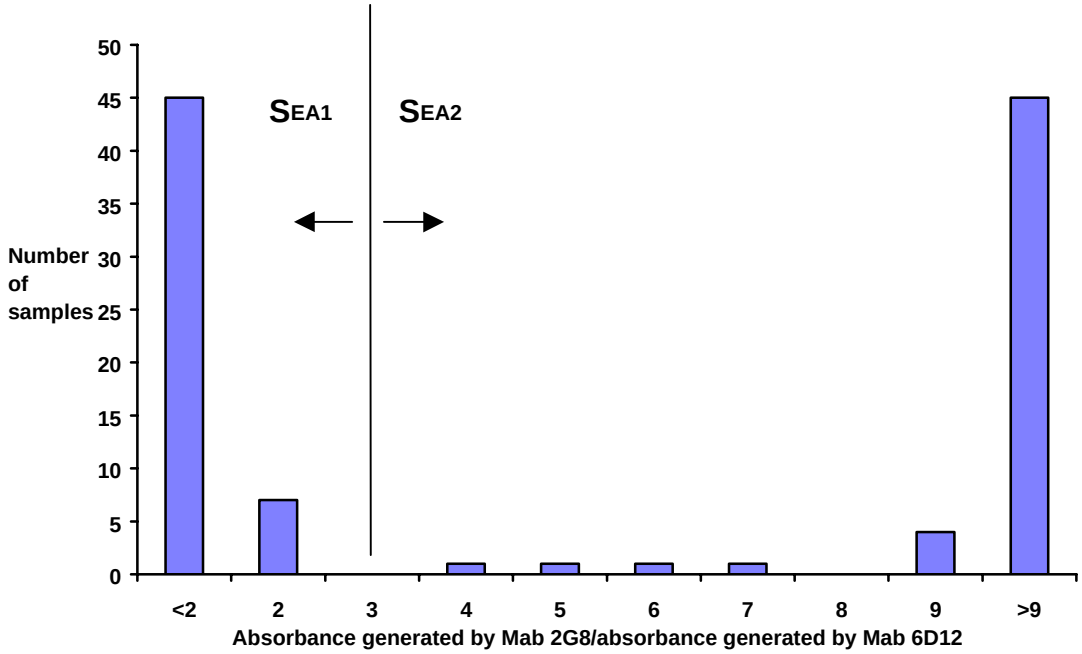


Fig. 2. *The frequency of occurrence of different ratios of the absorbances generated by Mabs 2G8 and 6D12, showing the clear separation of S_{EA1} and S_{EA2}.*



Most SPCSV found in samples of SPVD-affected plants from Tororo District in Eastern Uganda and Iganga was of S_{EA1} (81% and 60% respectively) whereas most SPCSV found in samples of SPVD-affected plants from Kabarole District in western Uganda, Rakai in southern Uganda and Mpigi in central Uganda were of S_{EA2} (89%, 82% and 75% respectively) ($P<0.001$) (Table 6). Three of the Kenyan samples of SPVD-affected plants reacted as S_{EA1} and two as S_{EA2}.

Table 6 *The detection of SPCSV serotypes S_{EA1} and S_{EA2} in different districts in Uganda*

District sampled	**Numbers of S_{EA1} (%)**	**Numbers of S_{EA2} (%)**
Tororo (eastern Uganda)	13 (81%)	3 (19%)
Iganga (eastern Uganda)	21 (60%)	14 (40%)
Mpigi (central Uganda)	21 (25%)	66 (75%)
Rakai (southern Uganda)	3 (18%)	14 (82%)
Kabarole (western Uganda)	1 (11%)	8 (89%)
Total	69	105

Severity of SPVD associated with each serotype. Naturally infected plants cv. Tanzania growing in a field planted *c.*3 months previously with largely disease-free cuttings at NAARI farm were assessed for the severity of SPVD using a 1 to 5 scale

with 1 as just recognisable as diseased and 5 as very severely stunted and chlorotic. A total of 61 leaf samples were collected from plants with different severities of SPVD and tested by TAS-ELISA using Mabs 2G8, 2G11 and 6D12.
Most plants of cv. Tanzania with mild (scores 1 & 2) symptoms were infected with S_{EA1}, and most plants with severe (scores 4 & 5) symptoms were infected with S_{EA2} (Table 7).

Table 7 *The detection of SPCSV serotypes S_{EA1} and S_{EA2} in plants of sweet potato cv. Tanzania with SPVD symptoms of different severities.*

Severity score*	Numbers of S_{EA1}	Numbers of S_{EA2}
1	4	1
2	11	2
3	8	7
4	1	20
5	0	7

*Score 1 = hardly affected increasing to score 5 = severely stunted and chlorotic.

Presence of serotypes in cultivars. Leaf samples were obtained from 10 SPVD-affected plants of each of the nationally released cultivars Tanzania, New Kawogo, Wagabolige, Sowola, Bwanjule or Tororo 3 (Mwanga *et al*., 1995) growing as part of national yield trials in replicated plots at NAARI Farm. Samples were distinguished as S_{EA1} or S_{EA2} by TAS-ELISA using Mabs 2G8, 2G11 and 6D12.
Both S_{EA1} and S_{EA2} serotypes naturally infected all six Ugandan cultivars tested (Table 8).

Table 8 *The detection of SPCSV serotypes S_{EA1} and S_{EA2} in plants of different Ugandan sweet potato cultivars affected by SPVD.*

Cultivar	Numbers of S_{EA1}	Numbers of S_{EA2}
Tororo 3	2	8
Bwanjule	3	7
New Kawogo	7	3
Sowola	8	2
Wagabolige	7	3
Tanzania	8	2

These results demonstrate:-

- *the presence of variation in the coat protein of SPCSV isolates found in Uganda and Kenya;*
- *that two serotypes occur in all districts surveyed and in all sweet potato varieties tested;*
- *that the serotypes differed in the severity of the disease associated with each and in their geographical predominances.*

Genomic variation

It proved difficult to extract RNA from sweet potato and a method had to be developed using the Clontec (USA) RNA extraction kit and Promega (UK) "Wizard" purification kit (Fenby *et al*., 1998). Eight SPVD-affected sweet potato plants, four of which reacted as S_{EA1} and four as S_{EA2}, were obtained from different locations in Uganda: S_{EA1}-11, S_{EA1}-19 and S_{EA1}-22 from Rakai, and S_{EA1}-13 from Tororo; S_{EA2}-4 and S_{EA2}-25 from Rakai, and S_{EA2}-39 and S_{EA2}-41 from Kabarole. Total RNA was extracted from 100 mg leaf material.

Reverse transcription (RT) was performed at 37 ^{o}C for 45 min according to Fenby *et al*. (1998). The RT primer used for the CP (CP3: 5'-AACGCGGAAGTGTAAGGTAT-3') was downstream of the gene and for the HSP70 homologue gene was within the HSP70 coding region (CL43L: 5'-GCAGCAGAAGGCTCGTTTAT-3'). cDNA was precipitated with 2 volumes ethanol, incubated at -20 ^{o}C for 1 hour and then re-suspended in 50 µl distilled water. PCR was performed in a total volume of 20 µl of reaction buffer containing 4 µl cDNA, 1 mM dNTPs, 4.5 mM MgCl, 0.5U *Taq* polymerase, and 0.4 µg of each primer. The primers used for CP gene amplification were position 1 -18 (CP1: 5'-CGTCTAGATTGTTAGAAA-3') and position 1165-1182 (CP2: 5'-TATATGAAAATATAGTTC-3') producing a 1182 bp product. All CP primer position numbers are in relation to the SPCSV sequence (Hoyer *et al*., 1996b), where the CP coding region is between bases 197 and 970. The cycle conditions were 92 ^{o}C (1 min), 50 ^{o}C (1 min), 72 ^{o}C (1.5 min) for 30 cycles with extension at 72 ^{o}C (10 min).

The primers used for amplification of a HSP70 homologue gene fragment were positioned in regions conserved in the genus crinivirus; either CL43U (5'-ATCGGCGTATGTTGGTGGTA-3') and CL43L (5'-GCAGCAGAAGGCTCGTTTAT-3') (Winter *et al.*, 1997) giving a 486 base pair (bp) product (S_{EA1}-11 and –22; S_{EA2}-4 and –41) or H5 (5'-TTGGTGGTACGATGAAGGTCC-3') and CL43L giving a 475 bp product (S_{EA1}-13 and –19; S_{EA2}-25 and -39). H5 is 12 bp internal to the start of CL43U, and was used to avoid non-viral amplification products. The cycle conditions were 92 ^{o}C (1 min), 50 ^{o}C (30 sec), 72 ^{o}C (1 min) for 30 cycles with extension at 72 ^{o}C (10 min).

Molecular biological techniques were according to Sambrook *et al.* (1989) unless stated otherwise. PCR products were isolated from agarose gel using Gene Clean (Bio 101, USA) and ligated into pCR 2.1 vector (Invitrogen, Netherlands) according to the manufacturer's instructions. Recombinant clones were selected using 50 µg/ml ampicillin and 40 µg/ml X-gal and confirmed *Eco*RI digestion colony hybridisation. Sequencing was performed using an ABI 100 prism model 377 automatic sequencer and analysed using the Gene Jockey program (Biosoft, UK). Duplicate clones from each isolate were sequenced.

Coat protein gene variation. Amongst the Ugandan isolates sequenced, there were 11 mutations which were not confirmed in the two independent clones; these have not been included in the following discussion although they are shown for completeness in Table 9. Apart from these, there were differences at 18 nucleotide positions within the CP gene and 5 closely adjacent to it, making a total of 23 confirmed variable nucleotides (Table 9). Eleven of these variable nucleotides lead to changes in the amino acid sequence of the CP.

Table 9 *The nucleotide substitutions identified at different positions within and adjacent to the coat protein (CP) gene of SPCSV isolates from Uganda as compared to the CP gene sequence of a Kenyan isolate (Hoyer et al., 1996).*

Nucle-otide position	Kenyan (= S_{EA1})	S_{EA1} samples				S_{EA2} samples				Amino acid change
		13	22	11	19	4	25	41	39	
38	T	T	C	T	T	T	T	T	T	
78	G	A	A	G	G	G	G	G	G	
110	G	G	G	G	G	G	G/A	G	G	
130	C	C	C	C	C	C	C/T	C	C	
139	G	T	T	T	T	T	T	T	T	
169	G	A/G	G	G	G	G	G	G	G	
173	G	G	G	G	G	G	A/G	G	G	
190	A	A	A	A	G/A	A	A	A	A	
203	G	G	G	G	G	G	G	G	A	D-N
210	A	A	A	A	A	A	G	A	A	D-G
211	C	A	A	C	C	C	C	C	C	D-E
247	A	C	C	C	C	C	C	C	C	
268	G	G	G	A	A	A	G	A	G	
273	T	T	T	A	A	A	A	A	A	V-E
286	A	A	A	A/G	A	A	A	A	A	
311	A	A	A	A	A	A	T	A	A	N-Y
327	A	A	G	G	G	G	A	G	G	N-S
358	T	T	T	T	T	T	T	T	C	
400	A	A	A	A	A/G	A	A	A	A	
427	G	A	A	A	A	A	A	A	A	
496	T	A	A	A	A	A	A	A	A	
515	C	T	T	C	C	C	C	C	C	P-S
530	A	A	T	T	T	T	T	T	T	S-G
594	A	A	A	A/G	A	A	A	A	A	N-S
623	A	G	G	A	A	A	A	A	A	N-D
625	C	T	T	T	T	T	T	T	T	
645	G	A	A	G	G	G	G	G	G	R-K
724	C	C	C	C	C	C	C	C	T	
752	T	A	A	T	T	T	T	T	T	L-M
908	A	A	A	A	A/G	A	A	A	A	N-D
911	A	A	A	A	A	A	A	A/G	A	K-E
988	G	G	G	G	G	G	G	G/A	G	
1067	G	A	A	A	A	A	A	A	A	
1071	C	C	C	C	C	C	T	C	C	

Darkly shaded regions indicate positions at which nucleotide substitutions result in an amino acid change in the CP gene. Lighter shaded regions indicate positions at which only one of the clones showed variation. For the latter, where two nucleotides are represented in one box, the first of the clones contained one base at this position whereas the second clone a different base. Changes from 203 to 911 inclusive are within the CP gene.

The Ugandan isolates could be divided on the basis of their CP nucleotide and amino acid sequences into two groups with isolates 13 and 22 forming an almost identical pair and isolates 4, 11, 19, 25, 39 and 41 forming another largely homologous group (Table 9). At 21 of the 23 variable nucleotide positions present on the CP gene or adjacent to it, S_{EA1}-13 and S_{EA1}-22 were homologous and at 14 of the 23 positions S_{EA1}-11, S_{EA1}-19, S_{EA2}-4, S_{EA2}-25, S_{EA2}-39 and S_{EA2}-41 were homologous. Furthermore, at seven of these 23 positions, S_{EA1}-13 and -22 contained the same substituted nucleotide, and this differed from the one present in S_{EA1}-11, S_{EA1}-19, S_{EA2}-4, S_{EA2}-25, S_{EA2}-39 and S_{EA2}-41. If, at each of these nucleotide positions, the four nucleotides occurred randomly, the chance of such a consistent pattern would be less than one in a million, indicating that these groups represent two distinct populations.

Other relatively major differences amongst the Ugandan populations were in samples 13, 25 and 39. Sample 13 had two nucleotides (positions 327 and 530) which were identical to the Kenyan isolate but differed from sample 22 and most S_{EA2} samples. Sample 13 was isolated from Tororo which is in the east of Uganda bordering Kenya. Sample 25, obtained from Rakai in the south of Uganda, had three unique changes (positions 210, 311 and 1071). Sample 39 had three unique nucleotide changes (positions 203, 358 and 724). This isolate was obtained from Fort Portal, located in the extreme west of Uganda.

HSP70 homologue gene variation. There were no confirmed genomic variations amongst the Ugandan isolates which would cause amino acid substitution, but there were confirmed changes at the RNA level (Table 10). These latter changes closely corresponded with the serological groupings.

Table 10 *The nucleotide substitutions (from replicate clones) identified at different positions within the HSP70 homologue gene of SPCSV isolates from Uganda.*

Sample serotype	Sample designation	Nucleotide position				
		136	187	280	460	471
S_{EA1}	13	G	G/A	A	T/A	C
	11	G	G	A/C	T	C
	19	G	G	A	T	C
	22	G/A	G	A	T	C/T
S_{EA2}	4	A	G	A	T	T
	25	A	G	A	T	T
	41	A	G	A	T	T
	39	A	G	A	T	T

Where two nucleotides are represented (e.g. A/C) the first of the clones contained an A at this position and the second a C.

The nucleotide and deduced amino acid CP sequences of the Ugandan samples distinguished them into two major groups comprising samples 4, 11, 19, 25, 39 and 41 and samples 13 and 22. These groups did not correlate exactly with serological groups, samples 13, 22, 11 and 19 being S_{EA1} serotypes and samples 4, 25,

39 and 41 being S_{EA2} serotypes. Our panel of Mabs did not contain a Mab which reacted with only S_{EA2}, so it seems possible that plants infected with both S_{EA1} and S_{EA2} would be typed as S_{EA1}. If isolates 11 and 19 were such mixed infections of S_{EA1} and S_{EA2}, either S_{EA1} or S_{EA2} isolates could have been cloned prior to sequencing and so lead to an apparent poor correlation between serology and CP amino acid groupings. Duplicate sequencing of the HSP70 homologue gene for sample 22 indicated that at least that plant was infected with both types (Table 10).

The nucleotide and deduced amino acid CP sequences of the Ugandan samples demonstrated small differences within the two major groups of the Ugandan isolates, such as those between S_{EA1}-13 and -22; S_{EA1}-13 substitutions resembling those nucleotides reported in the Kenyan isolate (Table 9), and the apparent uniqueness of isolates S_{EA2}-25 and -39. These differences may reflect geographical variation, in particular, S_{EA2}-25 was obtained from the south of Uganda and S_{EA2}-39 was obtained from the western border of Uganda.

Sequencing of the HSP70 homologue gene also revealed differences at the nucleic acid level (Table 10). These corresponded exactly to the S_{EA1} and S_{EA2} groupings obtained by TAS-ELISA (based on the CP). No amino acid differences were seen in the HSP70 homologue gene.

Additional work on Madagascan and Nigerian SPCSV Opportunity was taken to utilise these methods to examine the sequences of more geographically distant isolates of SPCSV, from Madagascar and from Nigeria. The Madagascan isolates had already

Table 11. Nucleotide differences found in the CP gene (coded between 200 and 973) of Madagascan and Nigerian isolates of SPCSV as compared to Kenyan (Hoyer *et al*., 1996) and/or Ugandan sequences

Nucleotide position	Kenyan isolates	Ugandan isolates	Madagascan isolate	Nigerian isolates		Predicted amino acid alteration from Kenya
				1	2	
291	G	G	G		A?	arg-lys
319	C	C	C		T?	asn-asn
334	A	A	A		G?	
394	G	G	G		A	arg-arg
477	G	G	G	A	A	glu-glu
486	G	G	G	A	A	arg-lys
530	A	A or T	G	G	G	ser-gly
768	A	A	A	G	G	val-val
774	T	T	T	C	C	pro-pro
864	T	T	T	C	C	thr-thr

been determined by serology to be closely related to those from Uganda (dissemination output Gibson, Kaitisha, & Randrianaivoarivony, 1998), and sequence analysis of its coat protein gene (Table 11) and its HSP70 homologue gene (data not shown) confirmed this similarity. The HSP70 homologue gene of the Nigerian isolates differed by a massive 22% from that of the East African isolates but a partial

coat protein gene sequence of it differed by only 2% from that of the East African isolates. This may show how few mutations can be tolerated in the CP as compared to the HSP70 gene.

Output 4. Phytosanitary practices based on knowledge of the presence, or otherwise, of EAWBV reservoirs in sweet potato, other crops and weeds.

Additional outputs agreed for project extension:-

- The causal agent(s) of SPVD in Tanzania identified
- Incidence and damage caused by SPVD in the Lake Victoria shore region of Tanzania assessed
- Causes of low SPVD incidence in E Uganda assessed

Surveys of the incidence of SPVD Fields of sweet potato were examined in districts bordering or close to Lake Victoria in Uganda and Tanzania between 1996 - 1999. The districts included most of the important areas of sweet potato production at mid-altitude in Uganda and Tanzania. Sweet potato fields were chosen whilst travelling along a rural road or path, stopping at c. 1km intervals or at the first field observed thereafter. Numbers of plants with SPVD were counted amongst 30 plants examined along a V-shaped transect stretching from one corner of the field to the opposite side and back to an adjacent corner.

SPVD was relatively rare in the more eastern districts sampled in both Uganda and Tanzania. Indeed, no virus-affected plants were recorded in Soroti during the survey (although they were found subsequently (see next section), and they were seldom recorded in nearby Tororo District (<2%). Furthermore, few farmers in either district recognised SPVD as a major problem. However, mean incidences in Uganda reached 25 - 30% south of Kampala in SW Mpigi, Masaka and Rakai and nearly 70% in Rukungiri District, which lies on the western border of Uganda with the Democratic Republic of Congo. Similarly, in Tanzania, highest incidences of SPVD were recorded in Kagera District, which lies on the western border of Tanzania with Rwanda and Burundi, and lowest incidences were recorded in the more eastern district of Shinyanga (Table 12).

Table 12 *Average incidences of SPVD[a] in different localities in Uganda and Tanzania around Lake Victoria*

	Plants with SPVD	Number of fields
	%	
Uganda		
Soroti District	0	29
Tororo District	1.5 ± 0.1	26

N. Mpigi District	7 ± 1.9	29
SW. Mpigi District	29 ± 6.2	26
Masaka& Rakai Districts	26 ± 5.2	30
Rukungiri District	68 ± 2.8	50
Tanzania		
Mara District	11.0 ± 2.6	21
Shinyanga District	4.7 ± 1.4	21
Mwanza District	10.9 ± 2.4	38
Kagera District		
Biharimulo Sub-district	15.3 ± 3.7	6
Bukoba Sub-district	36.4 ± 4.3	28
Karagwe Sub-district	6.1 ± 2.6	28

The susceptibilty to SPVD of cultivars from a location where diseased plants were rare, and from a location where diseased plants were common. Foliar cuttings of four cultivars local to Busia (where SPVD is rare) and of four cultivars local to Kanoni (where SPVD is common) were collected from farmers in each area. The cultivars from Busia were Musita, Magendo and two unnamed ones coded E1 and E2 (Table 12). The cultivars from SW Mpigi were Kamyufu, Kimotoka, Buliri b'wamesse and Kalebe. The eight cultivars were planted on 9 September 1996, in a field trial at NAARI which comprised five blocks of eight plots, each cultivar being assigned at random to a plot in each block. Each plot was 3x10m and planted with 30 cuttings arranged in three rows of ten plants. SPVD-affected plants recorded during the first month were considered to be derived from infected cuttings but were retained to act as natural infectors. One hundred such SPVD-affected plants and a similar number of symptomless ones were selected at random during the first month of the study and tested for SPFMV, SPMMV, SPCFV and SPLV by NCM-ELISA and for SPCSV by TAS-ELISA. SPVD incidence was otherwise monitored monthly by visual inspection. Plants became overgrown and entangled, and were cut back during March 1997 before making a final recording of disease incidence in April on new growth. Ten diseased plants and ten symptomless plants of each cultivar were again tested by ELISA for SPFMV, SPMMV, SPCFV, SPLV and SPCSV.

Table 13 *The spread of SPVD at NAARI* in *plots of sweet potato cultivars obtained from Busia and from Kanoni*

	Initial disease [a] (17 Oct'96)		"New" [b] disease on mature crop (19 Feb '97)		"New" [b] disease after cutting crop back (4 April '97)	
Cultivars from Busia	%[c]	(††††)	%[c]	([d])	%[c]	([d])
E1 (un-named)	0.0	*(0)*	18.7	*(10)*	81.5	*(98)*
E2 (un-named)	0.0	*(0)*	17.1	*(8)*	67.9	*(86)*
Musita	0.0	*(0)*	11.7	*(4)*	67.9	*(86)*
Magendo	2.1	*(0)*	10.0	*(3)*	61.5	*(77)*
Cultivars from						

Kanoni							
Kalebe	30.6	*(26)*		0.0	*(0)*	52.1	*(62)*
Kimotoka	24.7	*(18)*		15.1	*(6)*	49.5	*(58)*
Kamyufu	23.0	*(15)*		2.4	*(0)*	28.9	*(23)*
Buliri b'wamesse	30.3	*(26)*		20.4	*(12)*	59.9	*(75)*
S. E.	3.27			2.54		4.62	

[a] Percentage infection at first assessment, assumed to derive from the cuttings planted.
[b] "New " disease was obtained by subtracting initial infection.
[c] Arcsine-transformed percentages.
[d] Values in parentheses and italicised are back-transformed percentages.

Virus symptoms were initially rare (one plant of *c.*1,200) amongst plants derived from cuttings obtained from Busia and grown at NAARI but, by the final assessment, *c.* 90% of the plants of the Busia cultivars had SPVD. By contrast, although *c.* 20% of plants derived from cuttings from Kanoni initially had SPVD, it spread more slowly in the Kanoni cultivars, so only about half the initially symptomless plants were diseased by the final assessment (Table 13). Cv. Kamyufu was particularly resistant, only about a third of plants becoming diseased. SPFMV was detected in all 100 diseased plants sampled at the beginning and the end of the NAARI trial. SPCSV was detected in all except four diseased plants sampled at the beginning of the trial and in all plants sampled at the end of the trial. SPMMV was detected in one and three plants sampled at the beginning and end, respectively, of the trial. SPCFV was detected in eight and one plant sampled at the beginning and end, respectively, of the trial. SPLV was not detected at all; no virus was detected in any symptomless plant tested.

Landraces were more resistant to SPVD in areas where SPVD is prevalent so unusual susceptibility of local sweet potatoes to SPVD does not explain prevalence of SPVD.

Insect vector populations in farmers' crops during an annual cycle Vector numbers and SPVD incidence were monitored on farmers' sweet potato crops located near a) Busia which is a town in Tororo District straddling the Uganda/Kenya border, b) near Namulonge Agricultural and Animal Production Research Institute (NAARI) in N Mpigi and c) near Kanoni, a trading centre in SW Mpigi (Fig. 1). Ten fields were sampled monthly in each of the three chosen areas from December 1996 to November 1997. SPVD incidence was estimated visually in each field. Vector numbers were assessed by turning over leaves and counting aphids and adult whiteflies for one minute in ten different areas of each field. Only fields 3-6mths old were sampled. Fields sampled varied over the course of the study as some fields were harvested and others were planted.

Fig. 3. *Numbers of adult whiteflies during 1996 and 1997 in farmers' fields around Busia, Namulonge and Kanoni.*

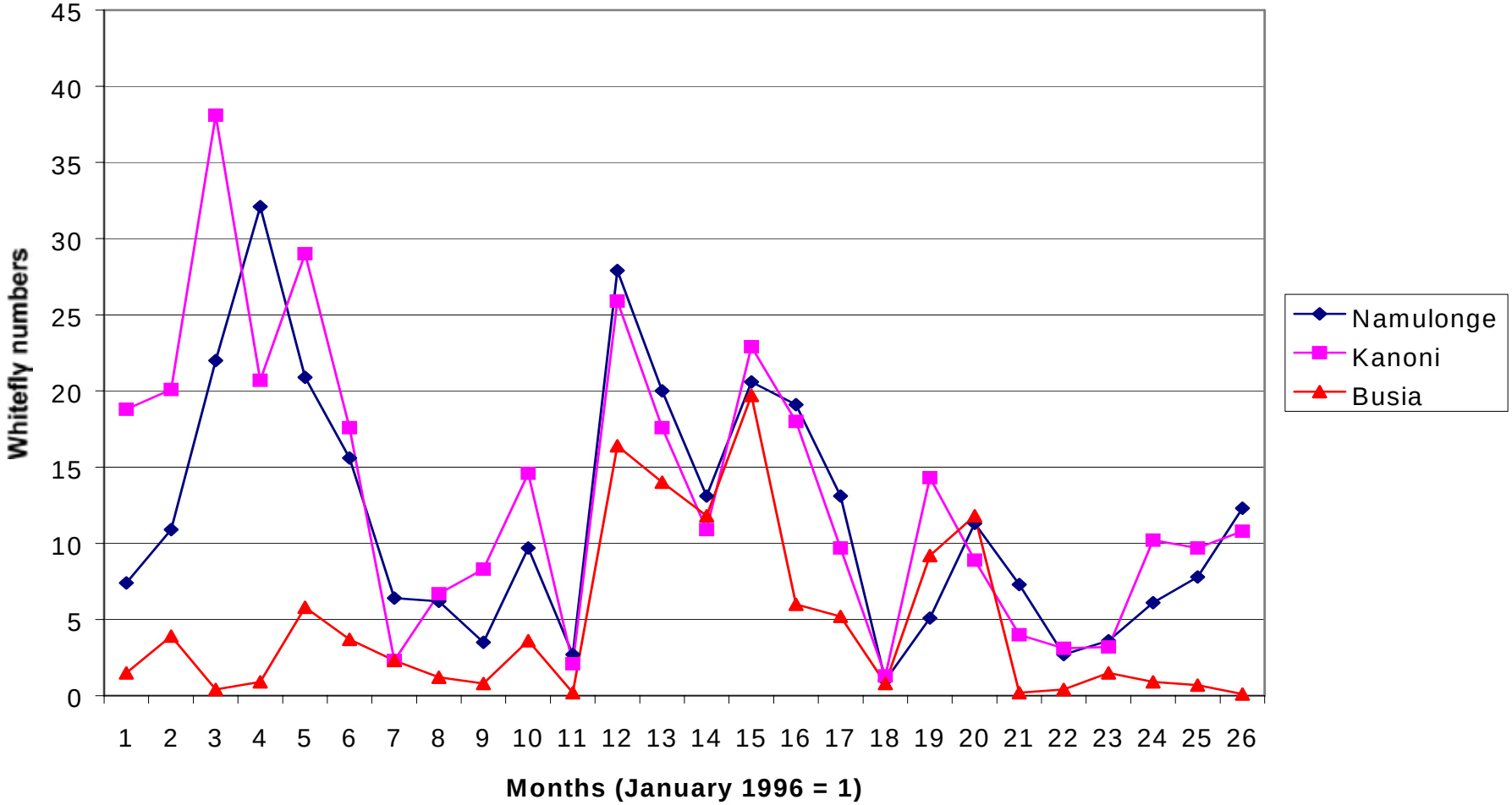
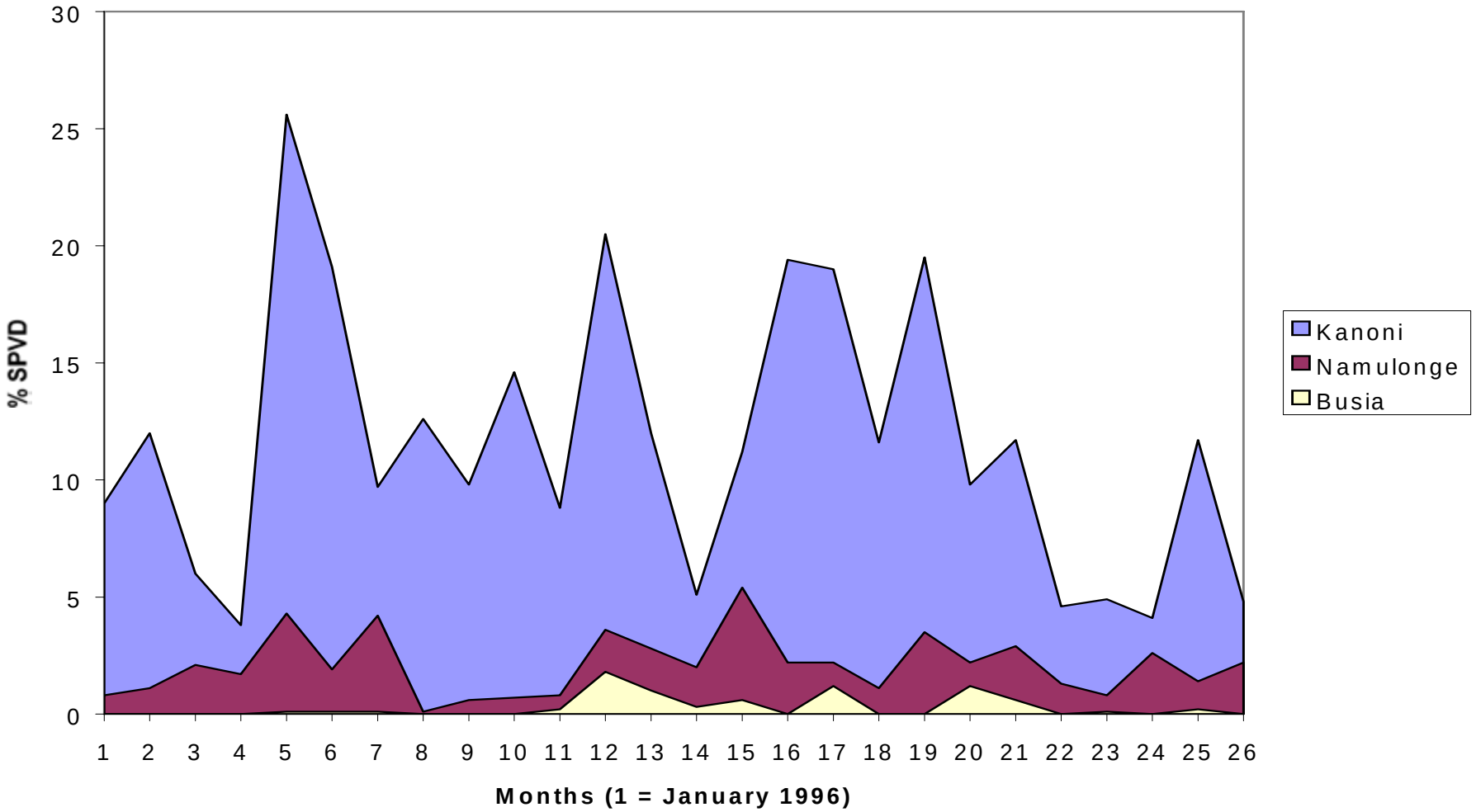


Fig. 4. *The incidences of SPVD during 1996 and 1997 in farmers' fields around Kanoni, Namulonge and Busia*



SPVD was common only at Kanoni and was rarely found at Busia. Incidences even at Kanoni varied considerably during the year (Fig 4). Much of this variation was probably associated with sampling different fields in different months but there was some evidence of incidence increasing with the onset of the first rains in March/April.

Whiteflies were rare on crops around Busia throughout 1996 and in the latter part of 1997 (Fig 3), explaining why SPVD is rare there. However, whiteflies became abundant during February to May, 1997. Intriguingly, this coincided with unusually an unusually wet "hot, dry season" associated with an *El Nino* event. This dry season is usually prolonged in eastern Uganda and may normally be responsible for preventing the upsurge in whitefly numbers found elsewhere in Uganda. In addition, this hot dry season together with associated frequent bush fires results in the natural vegetation being sparse and low-growing. Field records showed that most fields in eastern Uganda and Tanzania were exposed whereas most fields in Mpigi District of Uganda, and also Kagera District in the west of Tanzania, where whiteflies and SPVD are common, were sheltered. Exposed fields may also be unsuitable for the weak-flying, fragile *B. tabaci*.

Whiteflies were abundant on crops around both Kanoni and Namulonge in Mpigi, especially in 1996, about ten times more whiteflies being recorded there than in crops around Busia (Fig 3). They were particularly abundant during December to May, which is the hot, dry season (although Mpigi generally continues to receive some rain), and this is consistent with SPVD incidence increasing when the onset of the first rains causes renewed crop growth.

No aphids were observed on any sweet potato crops during the study period.

Although small population sizes of whitefly seem to explain why SPVD is rare in eastern Uganda, this does not explain the how SPVD remains relatively rare in northern Mpigi in comparison with southwest Mpigi.

Importance of local sources of SPVD inoculum Three on-farm trials were planted at each of the three most contrasting sites, at Soroti, Namulonge and Kanoni, using planting material from NAARI Farm. Whitefly numbers and SPVD incidence were monitored monthly. Whiteflies were rare at Soroti, as previously found in neighbouring Busia (Fig 5) and SPVD was not found on trial plants (results not shown). In contrast, SPVD was found at both Kanoni and Namulonge although it spread much more rapidly at the three trials planted at Kanoni than at the three trials planted at Namulonge. Despite this, whitefly numbers were again similar at the two sites and there was generally no correlation between whitefly numbers and SPVD incidence (Table 14. However, there was a close correlation between the final incidence of SPVD in the different trials and the amount of inoculum of SPVD present within 100m of each trial, suggesting that the greater spread of SPVD at Kanoni was due to the greater incidence of SPVD in nearby farmers' fields (Table 15).

Fig. 5. *Increases in the percentage of SPVD-affected plants in trials at farms at Namulonge (Nam-A, Nam-B, Nam-C) and Kanoni (Kan-D, Kan-E, Kan-F).*

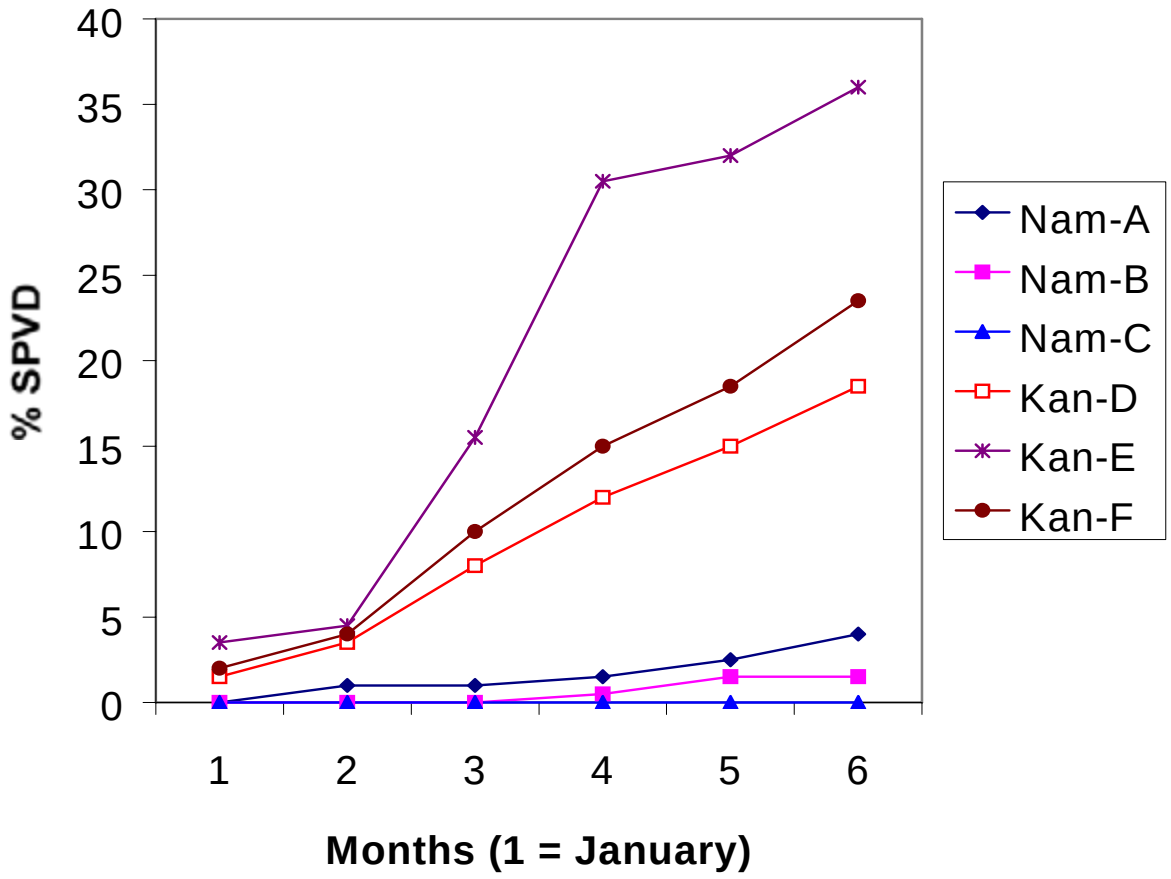


Table 14 *Average numbers of whitefly adults counted in one minute on sweet potato plants of cvs Tanzania and Tororo 3*.*

Location	Farm	Jan	Feb	March	April	May	June	Mean
Namulonge	A	7.7	11.4	16.3	14.8	9.9	8.0	11.4
	B	19.3	15.8	15.4	8.6	10.9	9.2	13.2
	C	28.6	14.6	16.6	10.1	7.6	6.3	14.0
Kanoni	D	4.6	14.6	17.4	14.9	5.1	6.5	10.5
	E	12.1	22.8	20.9	10.6	8.1	5.7	13.4
	F	9.3	14.3	18.0	9.3	2.9	2.6	9.4
Mean		13.6	15.6	17.4	11.4	7.4	6.4	
Correlation**		-0.524	0.705	0.935	-0.013	-0.506	-0.618	-0.284
Probability		N.S.	N.S.	0.01	N.S.	N.S.	N.S.	N.S.

Analysis of Variance Source of variation	d.f.	Mean Square	F ratio	Probability
Cultivar	1	6.9	0.4	N.S.
Farm	5	198.6	10.9	0.001
Month	5	1174.2	64.5	0.001
Farm x Month	25	29.8	18.1	0.001
Residual	329	18.2		

* Average values for all cultivars are presented because differences between cultivars are not significant (P>0.05) in on-farm trials planted at Kanoni and Namulonge.

** Correlation coefficient of final (June) SPVD incidence (Table 2) at each site and whitefly numbers at each site for each month.

Table 15. *Relationship between SPVD incidence and a) whitefly numbers b) local inoculum and c) whitefly numbers x local inoculum at on-farm trials at Kanoni and Namulonge.*

Farm	SPVD incidence[a]	Whiteflies[b]	Local inoculum[c]	Whiteflies x inoculum
Namulonge A	4.0	11.4	0	0
Namulonge B	1.5	13.2	100	1,320
Namulonge C	0.0	14.0	120	1,680
Kanoni D	18.5	10.5	1,043	10,951.5
Kanoni E	36.0	13.4	2,298	30,793.2
Kanoni F	23.5	9.4	1,519	14,278.6
Corr.[d]		-0.284	0.990	0.969
P		N.S.	0.001	0.001

a = Final SPVD incidence (June) in each on-farm trial.
b = Mean monthly (January - June) whitefly numbers on each on-farm trial.
c = Local inoculum calculated from SPVD incidence x area of field ÷ distance from field.
d = correlation of SPVD incidence with data in corresponding column

Examination of farmers' crops in the two localities revealed that a variety called New Kawogo predominated only at Namulonge and the surrounding villages but was absent at Kanoni. This variety is very resistant to SPVD (Table 16) and this appears to explain why SPVD inoculum was rare in the farmers' fields there. In this way, the predominance of New Kawogo in northern Mpigi also explains how SPVD also spreads slowly to susceptible cultivars there (Table 18). At Kanoni, more susceptible varieties predominated in farmers' fields and consequently afforded little protection to other susceptible varieties.

Table 16. *The incidence of SPVD-affected plants in different cultivars of sweet potato in 44 farmers' fields in N Mpigi*

Cultivar	Numbers of affected plants/total number	Numbers of fields in which the cultivar occurred	Cultivar	Numbers of affected plants/total number	Numbers of fields in which the cultivar occurred
New Kawogo	4/949 *(0.4%)*[a]	37 *(84%)*[a]	Nailon	0/47 *(0%)*[a]	2 *(5%)*[a]
Kimotoka	31/187 *(16.6%)*	9 *(20%)*	Munyeera	2/39 *((5.1%)*	6 *(14%)*
Kimese	13/143 *(9.1%)*	10 *(23%)*	Kyebandula	4/39 *(10.3%)*	3 *(7%)*
Bitambi	4/43 *(9.3%)*	2 *(5%)*	Others	14/181 *(7.7%)*	16 *(36%)*

[a] Data in italics and parentheses are percentages.

The basis of the resistance to SPVD in cv New Kawogo On-station field trials comparing the numbers of whiteflies on New Kawogo and a more SPVD-susceptible cv Tanzania indicated that the numbers of whiteflies were similar (P>0.05) on both cultivars despite fewer New Kawogo plants being affected by SPVD (Table 17). These results indicate that New Kawogo is resistant to the viruses causing SPVD (SPFMV + SPCSV) rather than resistant to the whitefly vector.

Table 17 *Mean numbers of aphid and whitefly vectors counted on the three sweet potato clones in weekly assessments during wks 11-23 after planting*

Sweet potato cultivar	First rainy season		Second rainy season	
	Whiteflies/m^2	Aphids/m^2	Whiteflies/m^2	Aphids/m^2
Tanzania	1.6	0	10.3	0
New Kawogo	1.7	0	11.7	0
Standard error	0.2		0.4	

Table 18 *A comparison of the spread of SPVD in plots of cvs Tanzania and New Kawogo planted 9 Sept., 1996*

Cultivar	Percentage SPVD-affected plants			
	19 Feb., 1997		4 April, 1997	
Tanzania	7	*(15.6)* †	39	*(38.6)* †
New Kawogo	0	*(0)*	13	*(21.2)*
Standard Error		*(2.5)*		*(4.6)*

† Values in italics and parentheses are arcsine-transformed mean percentages.

These results show that:

- *SPVD varies in incidence in Uganda and the Lake Zone of Tanzania, generally being more damaging in the west and rare in the east;*
- *The whitefly vector of SPCSV was also rare throughout most of the year in eastern Uganda, explaining the rarity of SPVD;*
- *Elsewhere, differences in the incidence of SPVD were caused largely by differences in the resistance of the predominant cultivar(s) to SPVD;*
- *The widespread cultivation of a resistant cultivar such as New Kawogo can protect other susceptible varieties grown nearby.*

Dissemination outputs

Output 3 Publications describing the role of EAWBV in SPVD, the effect of EAWBV on SPFMV infection and *vice-versa,* and the symptomatology of EAWBV.

Research papers published in peer-reviewed journals (A)

GIBSON, R.W., MPEMBE, I., ALICAI, T., CAREY, E.E., MWANGA, R.O.M., SEAL, S.E. and VETTEN, H.J. (1998) Symptoms, aetiology and serological analysis of sweet potato virus disease in Uganda. *Plant Pathology,* 47: 95-102.

KARYEIJA, R.F., GIBSON, R.W. and VALKONEN, J.P.T. (1998) The significance of sweetpotato feathery mottle virus in subsistence sweetpotato production in Africa. *Plant Disease,* 82: 4-15..

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Papers read at international meetings with published proceedings (B1)

FENBY, N.S., FOSTER, G.D., SEAL, S.E. and GIBSON, R.W. (1997) The whitefly-borne component of sweet potato virus disease in East Africa. *Society for Experimental BotanyAnnual Meeting (Canterbury) Abstract in Journal of Experimental Botany May Supplement*, 48: p19.

CAREY, E.E., GICHUKI, S.T., MWANGA, R.O.M., KASULE, S., FUENTES, S., MACHARIA, C. and GIBSON, R.W. (1998). Sweet potato viruses in Uganda and Kenya: results of a survey. *Proceedings of the Sixth Triennial Symposium of the International Society of Tropical Root Crops - Africa Branch (ISTRC-AB) on Root Crops and Poverty Alleviation. 22-28 October 1995, Lilongwe, Malawi*, pp457-461.

GIBSON, R.W., MWANGA, R.O.M., ARITUA, V., & CAREY, E.E. 1998. The causes and incidence of sweet potato virus disease in Uganda. *Poster and abstract 4..7.5 in Proceedings of the 7th International Congress of Plant Pathology, 9-16th Aug., 1998 Edinburgh, UK.*

KARYEIJA, R.F., KREUZE, J.F., GIBSON,R.W. & VALKONEN, J.P.T. 1998. Variability of sweet potato feathery mottle potyvirus (SPFMV) in East Africa. *Poster and abstract 1.11.73 in Proceedings of the 7th International Congress of Plant Pathology, 9-16th Aug., 1998 Edinburgh, UK.*

KAITISHA, G.C. & GIBSON, R.W.. Identification of sweet potato feathery mottle and sweet potato chlorotic stunt viruses in sweet potato in Zambia. *Read at the EARRNET/SARRNET Workshop Aug 17-21, 1998, Lusaka, Zambia & to be published as a full proceedings paper.*

JEREMIAH, S.C., MSABAHA, R.P. & GIBSON, R.W. Virus diseases of sweet potato and cassava in the Lake Zone of Tanzania. *Read at the 7th Triennial Symposium of the International Society for Tropical Root Crops - Africa Branch, Cotoneau, Benin 11-17 Oct 1998 & to be published as a full proceedings paper.*

Papers read at conferences with no proceedings (B2)

GIBSON, R. W. (1999) Sweet potato virus disease: a huge or negligible constraint - it all depends how you look at it. *Paper presented to the Virology Offered Papers Meeting in York, UK on 8-9 April, 1999.*

FENBY, N. S., GIBSON, R. W., SEAL, S. E. and FOSTER, G. (1997) Molecular aspects of the Ugandan isolates of sweet potato chlorotic stunt virus - the whitefly-borne component of sweet potato virus disease. *Paper presented to the Virology Offered Papers Meeting of the Association of Applied Virologists, 30 September - 1 October 1997.*

GIBSON, R. W., ALICAI, T. and VETTEN, H. J. (1997) The aetiology of the whitefly-borne component of sweet potato virus disease in Uganda. *Paper presented to the Virology Offered Papers Meeting of the Association of Applied Virologists, 30 September - 1 October 1997..*

Contribution of Outputs

Sweet potato is a major source of food in Africa, especially in the countries neighbouring Lake Victoria. It is particularly important for the poorer sectors of both rural and urban populations. It is grown particularly by women for daily food but also as a source of cash for their families' needs. SPVD is considered to be the most damaging disease of the crop in the region; our data supports this with average incidences of 30% and greater being found (Table 11), many crops having much greater disease levels. Our yield data confirms that SPVD more than halves yield of affected plants (Table 5).

The Project work has identified the whitefly-borne component of SPVD as SPCSV both by showing its general association with the disease and inducing SPVD by controlled virus inoculations. It has developed and proved an ELISA-based method of detection. This method has been taught locally to Ugandan and Kenyan national programme staff and to Makerere University students, and demonstrated at SARRNET meetings held at NAARI and to national programme staff in Tanzania, Zambia and Madagascar. CIP has been closely involved with all the work (funding students and training programmes) and has provided a key means of disseminating the method. The method is currently being used at NAARI, for example, by the senior sweet potato breeder, contributing to an understanding of the inheritance and mechanism of resistance in sweet potato to SPVD. By facilitating the development of superior sweet potato varieties, it is indirectly making a major contribution to the improvement of livelihoods in Africa.

The Project has investigated the variability of SPCSV. There seems to be a single serologically-identifiable strain of SPCSV throughout eastern and perhaps all of southern Africa. This strain is distinct, and appears to be more severe, than a strain found in West Africa. Small serological and nucleotide sequence differences have been found within this East African strain. This provides scientific support to NAARI providing superior sweet potato genotypes and seedstocks to all East African sweet potato programmes. The small differences found within the East African strain of SPCSV also supports NAARI's policy of using broad quantitative sources of resistance rather than single gene sources (although this latter is largely because no single gene sources of resistance seem available). This result suggests that improvements in livelihoods generated by developing superior resistant varieties will be sustainable.

The Project has provided clear evidence that SPVD-resistant varieties can provide effective control where they can be grown. The results also show that resistant varieties additionally protect nearby crops of susceptible varieties, by limiting local inoculum levels of SPVD. This indirectly suggests also that local phytosanitation may also provide useful methods of control. However, preliminary results have also shown that:-

- sweet potato varieties may have limited adaptability;

- the areas where most of the superior resistant varieties are currently being grown are largely areas where SPVD is relatively unimportant (New Kawogo in N Mpigi is the main exception).

Follow-up Planned

These observations suggest that key additional work that is required to fully exploit these results is to test SPVD-resistant sweet potato varieties in areas where SPVD remains a serious problem in order to confirm:-

- that resistance is maintained;

- that the available SPVD-resistant varieties are locally adapted to yield well there.

On-farm trials done within the Project have revealed that, whilst resistance is generally maintained, the yield of the NAARI varieties is often not superior to local landraces. Table 19 shows how the resistance of the NAARI varieties was superior to that of the local varieties but their yield was relatively poor. However, the local farmers have continued to grow cv Sowola and cv Wagabolige; in particular, the farmers valued the early yield of Sowola. Since this trial, NAARI has released a further six superior resistant varieties and another six or seven are likely to be released in the near future. Furthermore, in high disease areas of both Uganda (Rukungiriri) and Tanzania (Bukoba), farmers gave top priority to receiving superior resistant varieties (Table 20).

The extension of R6617 was done as an integral part of the First Phase of the Inter-Centers Initiative on Whiteflies and Whitefly-borne Viruses. This provided an excellent "umbrella" organisation within which CPP-funded research could be done both in Uganda and in neighbouring countries. Collaboration was further facilitated by RW Gibson also leading the NRI component on sweet potato viruses of this project. The Second Phase of the Inter-Centers Initiative on Whiteflies and Whitefly-borne Viruses is planned to start in 2000. The component on sweet potatoes in Africa will again be managed by CIP and will have the following three main components:-

Dissemination of information on the importance, identification and control (particularly by phytosanitation and the use of resistant varities) of SPVD by:-

- in-country training workshops for national and NGO extension staff;

- distribution of information leaflets (eg handout already prepared by R6617);

- preparation of a video and its distribution to national TV channels.

Evaluation and distribution of superior, SPVD-resistant sweet potato varieties. The Ugandan national programme has recently released 12 resistant varieties; the Tanzanian national programme has also identified landraces resistant to SPVD. It was proposed to:-

- evaluate resistant varieties regionally and search for others in areas of Uganda and Tanzania (the two politically stable, major sweet potato producers in the region) where Phase I had identified high levels of SPVD;

- develop facilities to support increased international exchange of pathogen-tested germplasm.

- **Local phytosanitation**. Most farmers carefully select cuttings from only disease-free parents but there is little evidence of other phytosanitary practices being followed as few farmers rogued out diseased plants and new crops were often planted near old ones. However, experiments and observations made under Phase I and R6617 suggest that phytosanitation may work effectively to control SPVD. A PhD project examining the local movement of whiteflies and SPVD, and the efficacy of local phytosanitation is therefore also proposed. The costs, for example, yield losses caused by roguing, and acceptability of local phytosanitary practices will also be examined.

Dr Gibson has also again been invited to take a leading role. **It is therefore proposed that further work funded by CPP should continue to be done within the Inter-Centers Initiative.** Both IITA and CIP also are keen that NRI should continue to work within the project. Proposed target areas are:-

- Further disseminations including leading the write-up of the work on whiteflies and whitefly-borne viruses of sweet potato in Africa (ICI Phase I), and organising a session on these viruses at the African Potato Association Conference (May, 2,000).

- To evaluate resistant varieties for regional use and search for others in areas of Uganda and Tanzania (the two politically stable, major sweet potato producers in the region) where ICI Phase I had identified high levels of SPVD (email E Carey, 14 Jan);

- To supervise the PhD project on local epidemiology of SPVD in Uganda;

- To re-evaluate likely (whiteflies, eriophyid mites, soil-borne fungus) vectors of SPMMV.

The evaluation of resistant varieties has already received the support of the Ugandan and Tanzanian National Programmes.

Table 19 *SPVD incidence and storage root yields of nationally-released (NAARI) and local sweet potato cultivars in an on-farm trial planted on 15 October 1996 at Kanoni, Uganda*

Date	**New Kawogo†**	**Bwanjule†**	**Sowola†**	**Tororo 3†**	**Wagabolige †**	Old Kawogo	Kalebe	Buliri b'wamesse	Kimotoka	Kiriya	S.E.D. (d.f =50)
Arcsine-transformed monthly SPVD incidence (%) (back-transformed values are in italics and parentheses)											
Dec., '96	**3.2**	**0**	**0**	**1.6**	**0**	5.5	3.9	7.7	14.0	0	3.24
	(0.3)	***(0)***	***(0)***	***(0.1)***	***(0)***	*(0.9)*	*(0.5)*	*(1.8)*	*(5.9)*	*(0)*	
Jan., '97	**3.3**	**0**	**0**	**1.6**	**0**	5.5	6.1	6.1	15.5	0	3.38
	(0.3)	***(0)***	***(0)***	***(0.1)***	***(0)***	*(0.9)*	*(1.1)*	*(1.1)*	*(7.1)*	*(0)*	
Feb., '97	**3.4**	**0**	**0**	**1.6**	**1.6**	7.1	6.1	7.2	19.7	0	3.82
	(0.4)	***(0)***	***(0)***	***(0.1)***	***(0.1)***	*(1.5)*	*(1.1)*	*(1.6)*	*(11.4)*	*(0)*	
Mar., '97	**4.3**	**1.6**	**0**	**1.6**	**0**	7.1	6.1	7.2	18.1	0	3.96
	(0.6)	***(0.1)***	***(0)***	***(0.1)***	***(0)***	*(1.5)*	*(1.1)*	*(1.6)*	*(9.7)*	*(0)*	
Apr., '97	**7.8**	**5.6**	**7.5**	**9.1**	**3.2**	9.0	30.0	29.2	25.0	16.0	6.70
	(1.8)	***(1.0)***	***(1.7)***	***(2.5)***	***(0.3)***	*(2.4)*	*(25.1)*	*(23.8)*	*(17.9)*	*(7.6)*	
May, '97	**8.7**	**14.4**	**6.9**	**29.1**	**20.0**	28.9	32.5	41.7	51.3	27.9	6.53
	(2.3)	***(6.2)***	***(1.4)***	***(23.6)***	***(11.7)***	*(23.4)*	*(28.9)*	*(44.2)*	*(60.8)*	*(21.9)*	
SPVD incidence (%) in cuttings grown, one from each plant.											
May, '97	***(1.3)***	***(9.7)***	**No result**	**No result**	***(7.8)***	*(10)*	*(29)*	*(28)*	*(43)*	No result	
SPVD incidence (%) in farmer-selected cuttings											
May, '97	**2.6**	**2.6**	**3.7**	**4.6**	**11.8**	11.8	14.3	11.6	26.4	14.2	6.09
	(0.2)	***(0.2)***	***(0.4)***	***(0.6)***	***(4.2)***	*(4.2)*	*(7.8)*	*(4.0)*	*(19.7)*	*(6.0)*	
Yield (g/heap)											
May, '97	**52**	**152**	**274**	**62**	**201**	168	231	503	263	153	55.9

† Values for nationally released cultivars are shown in bold.

Table 20 *Help requested by farmers to combat SPVD in the most affected localities*

Locality	Percentage of farmers[a] giving a particular rank to a requested control measure									Number of farmers	
	Superior, resistant cultivars			Technical information			Chemical control			a) requesting	b) interviewed
	1	2	3	1	2	3	1	2	3		
Bukoba	100	0	0	0	0	0	0	0	0	14 (70%[b])	20
Rukungiri	60	21	0	23	6	2	17	15	0	48 (96%)	50
Overall	**69**	**16**	**0**	**18**	**5**	**2**	**13**	**11**	**0**	**62 (89%)**	**70**

[a] Amongst those requesting specific measures to control SPVD
[b] Percentage of farmers requesting assistance with control of SPVD amongst those interviewed.

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