



# Incidence and distribution of Sweetpotato viruses and their implication on sweetpotato seed system in Malawi

Willard Mbewe<sup>1</sup> · Andrew Mtonga<sup>2</sup> · Margret Chiipanthenga<sup>1</sup> · Kennedy Masamba<sup>1</sup> · Gloria Chitedze<sup>1</sup> · Pilirani Pamkomera<sup>2</sup> · Ellen Gondwe<sup>3</sup> · Obed Mwenye<sup>4</sup> · Felistus Chipungu<sup>4</sup>

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## Abstract

A survey was carried out in 19 districts to investigate the prevalence and distribution of sweetpotato virus disease (SPVD) and its implication on the sustainability of clean seed system in Malawi. A total of 166 leaf samples were collected and tested for the presence of 8 viruses using nitrocellulose membrane enzyme-linked immunosorbent assay (NCM-ELISA). SPVD foliar symptoms were observed in 68.42% of the surveyed districts. There were significant variations in disease incidence and severity ( $p < 0.001$ ) among districts, with the highest incidence in Mulanje (28.34%). Average SPVD severity score was 3.05. NCM-ELISA detected sweet potato feathery mottle virus (SPFMV, 30.54%), sweet potato mild mottle virus (SPMMV, 31.14%), sweet potato mild speckling virus (SPMSV, 16.17%), sweet potato C-6 virus (SPC6V, 13.77%), sweet potato chlorotic stunt virus (SPCSV, 22.16%), sweet potato collusive virus (SPCV, 30.54%), sweet potato virus G (SPVG, 11.38%), cucumber mosaic virus (CMV, 7.78%) either in single or mixed infections. Data from this study indicate a significant SPVD occurrence in the country, and the consequence implications towards national sweetpotato seed system.

**Keywords** Sweetpotato · Sweetpotato virus disease · NCM-ELISA · Incidence · Severity

Sweetpotato (*Ipomoea batatas* (L.) Lam.), a herbaceous, perennial plant belonging to the Convolvulaceae family, is the world's seventh most important food crop, after wheat, rice, maize, potato, barley and cassava (Muhammad et al. 2012; Neela and Fanta 2019). More than 133 million tonnes of sweetpotato are produced globally per year, more than 95% in developing countries (FAOSTAT 2019). In Malawi, sweetpotato comes second after cassava as a food security root crop and is more widely grown in the country (Chipungu 2008). Sweetpotato, being a vegetatively propagated crop can lead to

the accumulation of systemic pathogens, especially viruses. Many viruses infect the crop worldwide. A common virus is the aphid-transmitted sweet potato feathery mottle virus (SPFMV; *Potyvirus*, *Potyviridae*) (reviewed by Syller 2014). It causes mainly transient symptoms when infecting alone; it is most damaging when it is synergized by co-infection with a whitefly transmitted, phloem-limited sweetpotato chlorotic stunt virus (SPCSV; *Crinivirus*, *Closteroviridae*) (reviewed by Gibson and Kreuze 2015). The synergistic interaction causes severe Sweetpotato Virus Disease (SPVD; Byamukama et al. 2004). SPCSV is the most damaging virus affecting sweetpotato plants, causing permanent symptoms even when infecting alone. Virus diseases alone can cause yield reductions of up to 98% (Mukasa et al. 2003). Virus infection in sweetpotatoes causes: (i) yield loss by current season infection, and (ii) yield loss by infection carried in the vine cuttings, known also as degeneration (Gibson and Kreuze 2015).

While SPVD remains the major biotic challenge to sweetpotato production in Malawi and beyond, a clean seed system remains the ultimate goal for sustainable sweetpotato production and is a key component for food security in sub-Saharan Africa (SSA) (MacEwan 2016). A robust

✉ Willard Mbewe  
mbewewillard@yahoo.co.uk

<sup>1</sup> Department of Agricultural Research Services, Bvumbwe Agricultural Research Station, P. O. Box 5748, Limbe, Malawi

<sup>2</sup> Department of Agricultural Research Services, Chitedze Agricultural Research Station, P. O. Box 158, Lilongwe, Malawi

<sup>3</sup> Mathematical Sciences Department, University of Malawi, Chancellor College, P.O. Box 280, Zomba, Malawi

<sup>4</sup> International Potato Centre, P. O. Box 31600, Lilongwe, Malawi

seed system should provide farmers with planting material (i) in sufficient quantities, (ii) at the right time, (iii) of an appropriate physiological state, vigour and health, and (iv) of superior genotypes appropriate to the farmer's purposes among others (Gibson et al. 2009). However, high infestations of SPVD continue to pose a threat to clean seed system in the sweetpotato growing region. Studies have shown that the use of virus-free material could yield 30% greater than normal planting material – with the yield reducing to the same level after five generations (Abidin et al. 2017). Many farmers in Malawi recycle planting material that is inferior and highly susceptible to major pests and diseases due to, among other reasons, shortage of clean planting materials of improved varieties. Use of recycled and/or inferior planting materials, has significant implication on the sustainability of a sound and vibrant seed system. Thus, there is a prevalence of diseases (Chiipanthenga et al. 2015).

In this study, we sought to provide the first nation-wide survey results for sweetpotato viruses and virus-like diseases and offer an insight on how viruses have affected the sweetpotato seed system in Malawi. This knowledge is key and critical, as it will establish the national hotspots for sweetpotato viral pathogens as well as guide decision makers on seed distribution systems for sustainable sweetpotato production.

The survey was conducted in 2019 in nineteen districts that represent the most important sweetpotato-growing areas of the country, viz., Balaka, Chikwawa, Chiradzulo, Chitipa, Dedza, Karonga, Kasungu, Lilongwe, Machinga, Mangochi, Mchinji, Mulanje, Mzimba, NkhataBay, Nsanje, Ntcheu, Phalombe, Salima, and Zomba. The first field in each district was sampled randomly while consecutive fields were sampled at 2–5 km interval along motorable roads traversing each district depending on the availability of sweetpotato fields. Sweetpotato fields 2–4 months old were targeted. All sweetpotato varieties (local and improved), regardless of flesh colour were assessed. In order to track the effect of SPVD on the seed system, vine multipliers (those that get their planting materials from government and international partners such as International Potato Centre (CIP) were also deliberately sampled and their fields assessed. Similarly, local farmers who produce sweetpotato for food, 'the clones' as well as 'tissue-culture material' (which is the genesis of clean seed system in Malawi) were surveyed.

During sampling, plants were selected along representative diagonal transect line (Sseruwagi et al. 2004). To assess and estimate SPVD incidence, a number of visibly diseased plants was counted along two diagonals across each field and expressed as the percentage of the total (30 plants) assessed along the lines. Disease severity, which is defined as the degree of expression of symptoms on individual SPVD-affected plants, was assessed visually using arbitrary scale of 1–5 according to Ndunguru et al. (2009), where 1 represents no disease symptoms and 5 the most severe symptoms,

including leaf distortion, stunting of plants, and in some cases death of plant.

Assessment of adult whitefly (*Bemisia tabaci*; *Aleyrodidae*) involved direct counting of adults on ventral side of five youngest apical leaves of the shoots. Similarly, shoots were examined for the presence of aphids. In each field, plants expressing distinct symptoms and or very mild or severe symptoms were preferentially sampled for laboratory testing. The leaf samples were transported to Bvumbwe and Chitedze Agricultural Research Stations for virus detection.

A disc (1 cm in diameter) was taken from a leaf at the top, middle, and lower part of the stem from each plant and used for serological testing of viruses with nitrocellulose membrane enzyme-linked immunosorbent assay (NCM-ELISA; Gibb and Padovan 1993). Virus-specific polyclonal antibodies to SPFMV, sweet potato mild mottle virus (SPMMV; *Ipomovirus*), sweet potato mild speckling virus (SPMSV; *Potyvirus*, *Potyviridae*), sweetpotato C-6 virus (SPC6V), SPCSV, sweet potato collusive virus (SPCV; *Cavemovirus*, *Caulimoviridae*), sweet potato virus G (SPVG; *Potyvirus*, *Potyviridae*), cucumber mosaic virus (CMV; *Cucumovirus*, *Bromoviridae*), and sweet potato chlorotic fleck virus (SPCFV; *Carlavirus*, *Quinvirinae*, *Betaflexiviridae*), as well as NCM strips spotted with sap from virus-positive and non-infected control plants were used for detection of the viruses. The ELISA kits were supplied by International Potato Centre, Lima, Peru. Visual assessment for the development of a purple color on the sample spots was used to identify virus-positive samples (Gutierrez et al. 2003; Tairo et al. 2004).

SPVD incidence (I) and severity (S) data were transformed to complementary log–log (CLL) as  $CLL(I) = \ln[-\ln(1 - I)]$  and  $CLL(S) = \ln[-\ln(1 - S)]$ , respectively. This model was selected based on the plots of severity as a function of incidence and because incidence and severity must lie between 0 and 1 (Carrisse et al. 2013). The non-transformed data was used for exploratory analysis while the transformed data was used for other downstream statistical analyses. Analysis of Variance (ANOVA) was used to test variation in SPVD incidence and severity among districts, regions, research stations, sweetpotato flesh colour, aphids and whitefly distribution. Correlation analysis was done to assess the relationship among different variables. The analyses were performed using STATA version 14 and GenStat Discovery (18<sup>th</sup> Edition) (VSN International Ltd, Hemel Hempstead, UK). Maps showing distribution of SPVD, whitefly populations, and aphid infestation were generated using geo-spatial data using Arc View GIS software ([www.arcgis.com](http://www.arcgis.com)).

The study established that SPVD was found in 13 of the 19 surveyed districts, which are Chiradzulo, Dedza, Karonga, Kasungu, Lilongwe, Mangochi, Mulanje, Mzimba, NkhataBay, Nsanje, Phalombe, Salima, and Zomba

(Table 1). The overall mean disease incidence and severity was 11.09% and 3.05 respectively. There were significant variations on SPVD incidence ( $F = 11.67$ ,  $p < 0.001$ ) among the surveyed districts with high incidence (28.34%) recorded in Mulanje district (Table 1). Similarly, significant differences were observed in severity scores ( $F = 9.54$ ,  $p < 0.001$ ) among the districts. Highest severity score (3.36) was, for instance, observed in Mzimba district. Furthermore, the results show that SPVD severity significantly varies among Agricultural Research Stations ( $F = 11.52$ ,  $p < 0.001$ ). There were also significant variations among local varieties, improved varieties and research clones both in disease incidence ( $F = 7.12$ ,  $p < 0.001$ ) and severity ( $F = 5.14$ ,  $p = 0.007$ ). However, disease incidence and severity did not vary significantly ( $F = 0.17$ ,  $p = 0.84$ ) between orange fleshed sweetpotato varieties (OFSP) and white-fleshed sweetpotato varieties (WFSP). SPVD incidence was higher in research test clones (21.39%) than local varieties (18.89%) and improved varieties (16.67%). Average severity scores were also higher in research clones (2.37), while local varieties had a score of 2.33 and improved varieties 2.28. However this did not differ statistically. The differences in SPVD incidence among clones, local varieties and improved differed significantly, while the severity scores among them did not differ significantly ( $F = 8.16$ ,  $p < 0.001$ ). Furthermore, the results show that among the agricultural research stations in Malawi,

Makoka Research Station had the highest SPVD field incidence (100%), while Kandiyani had the lowest (3.65%). The SPVD incidence and severity among research stations also differed significantly ( $F = 11.53$ ,  $p < 0.001$ ). The results also indicated significant variations in SPVD incidence ( $F = 7.12$ ,  $p < 0.001$ ) and severity ( $F = 5.14$ ,  $p < 0.01$ ) among improved varieties and local varieties with disease incidences higher in local varieties than improved varieties. Furthermore, the disease incidence was lower among vine multipliers (5.48%) than local farmers who use local cultivars. However severity did not differ significantly among the vine multipliers and local farmers ( $F = 7.91$ ,  $p = 0.08$ ).

*Bemisia tabaci* adults were observed in all the sampled districts of the country, averaging 0.33 insects per plant (Table 1, Fig. 2), but their numbers did not differ significantly between districts. Highest number of whiteflies observed per plant was 25 and was encountered in a field in Karonga. Overall, most abundant whiteflies were found in NkhataBay with an average of 5.9 whiteflies per plant. On the other hand aphid populations were very low (Fig. 3) with a highest of 0.2 per field. The mean distribution of aphids varied significantly among districts ( $F = 5.84$ ,  $P < 0.001$ ), agricultural research stations ( $F = 3.98$ ,  $P < 0.01$ ), flesh colour ( $F = 11.65$ ,  $P < 0.01$ ), or type of variety ( $F = 13.76$ ,  $P < 0.001$ ). Although the aphid observed were not identified to species, the green peach aphids, *Myzus persicae* (Hemiptera: Aphididae) has been predominantly associated with sweetpotato in southern Africa (CABI 2016) Fig. 1.

Out of 166 sweetpotato leaf samples collected, eight virus species were detected (Table 2) and they include: SPFMV (30.54%), SPMMV (31.14%), SPMSV (16.17%), SPC6V (13.77%), SPCSV (22.16%), SPCV (30.54%), SPVG (11.38%), and CMV (7.78%). A total of 78 samples had mixed infections with 2 or more virus combinations representing 46.71% of total positive samples.

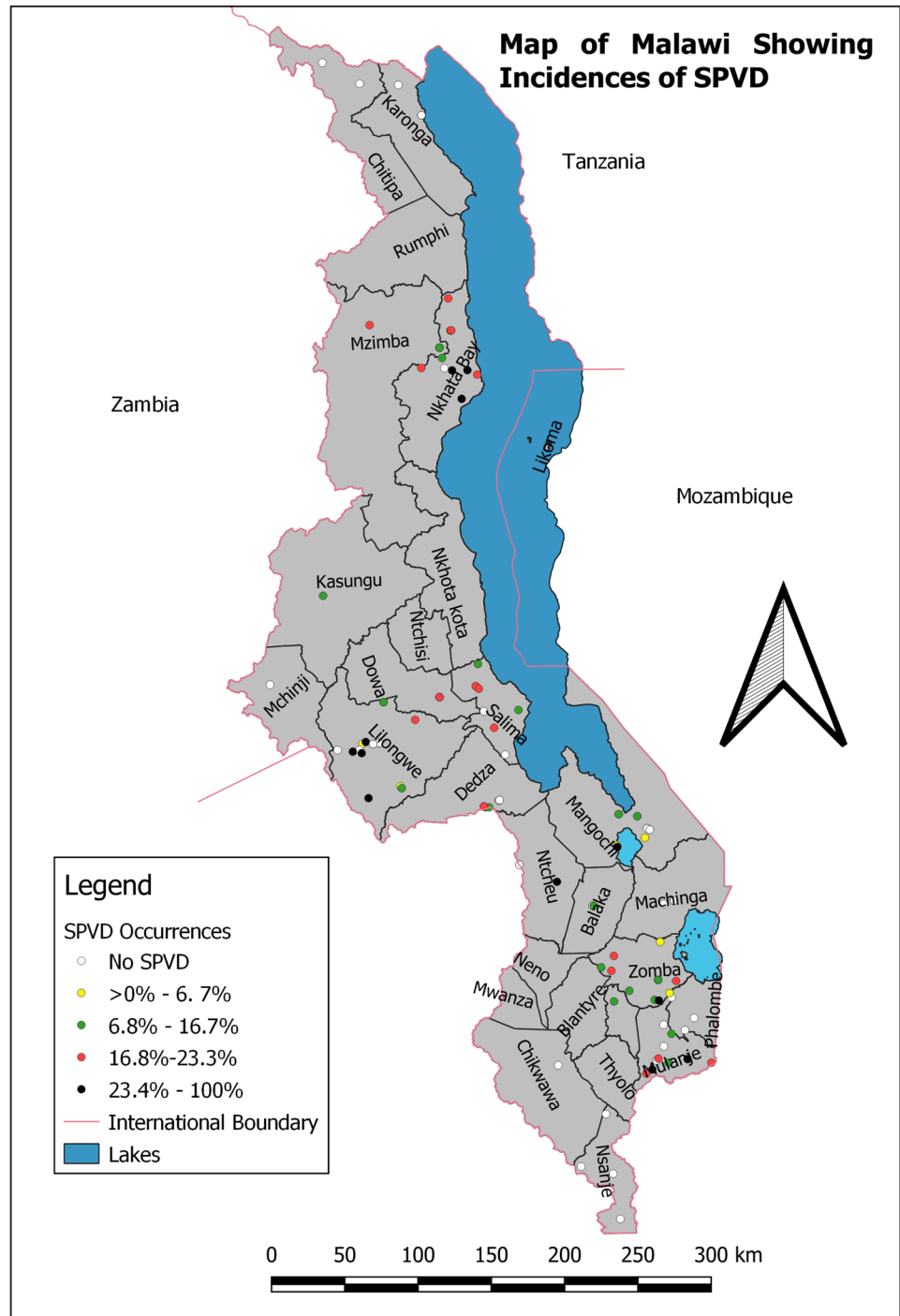
Pearson correlation coefficient tests results showed strong positive correlation between SPVD foliar incidence and SPVD severity ( $r = 0.96$ ,  $p < 0.001$ ). There is also weak positive correlation between aphids and severity ( $r = 0.19$ ,  $p < 0.05$ ). There is strong positive correlation between whiteflies and aphids ( $r = 0.4$ ,  $p < 0.001$ ). However, there was weak positive correlation between aphid populations and SPVD incidence as well as between whitefly abundance and SPVD incidence or severity (Table 3).

This first national survey has provided novel information on the status and distribution of sweetpotato viruses in Malawi. Most importantly, we have established here the effect that the viruses have on the sustainability of clean seed system. Nitrocellulose Membrane Enzyme-link immunosorbent Assay (NCM-ELISA) detected eight virus species, namely SPFMV, SPMMV, SPMSV, SPC6V, SPCSV, SPCV, SPVG, and CMV. The wide distribution of SPVD in Malawi might be due to the use of infected sweetpotato planting

**Table 1** SPVD incidence, severity and distribution of whiteflies and aphids among sampled districts of Malawi

Districts	Whiteflies	Aphids	SPVD incidence (%)	SPVD severity (1 – 5)
Balaka	0.00	0.00	0.00	1.00
Chikwawa	0.00	0.00	0.00	1.00
Chiradzulo	0.07	0.00	16.67	2.41
Chitipa	0.02	0.00	0.00	1.00
Dedza	0.15	0.07	10.00	2.40
Karonga	4.08	0.00	14.89	2.40
Kasungu	0.00	0.00	3.62	2.30
Lilongwe	0.01	0.002	15.00	2.27
Machinga	0.00	0.00	0.00	1.00
Mangochi	0.07	0.08	17.09	2.32
Mchinji	0.07	0.00	0.00	1.00
Mulanje	0.11	0.005	28.34	2.73
Mzimba	0.03	0.00	20.45	3.36
NkhataBay	1.44	0.02	25.23	2.47
Nsanje	0.26	0.00	0.00	1.00
Ntcheu	0.03	0.00	12.47	2.08
Phalombe	0.00	0.00	0.09	1.00
Salima	0.001	0.006	21.73	2.45
Zomba	0.02	0.01	25.07	2.43
Means	0.35	0.01	11.09	3.05

**Fig. 1** Map of Malawi showing incidence and distribution of SPVD

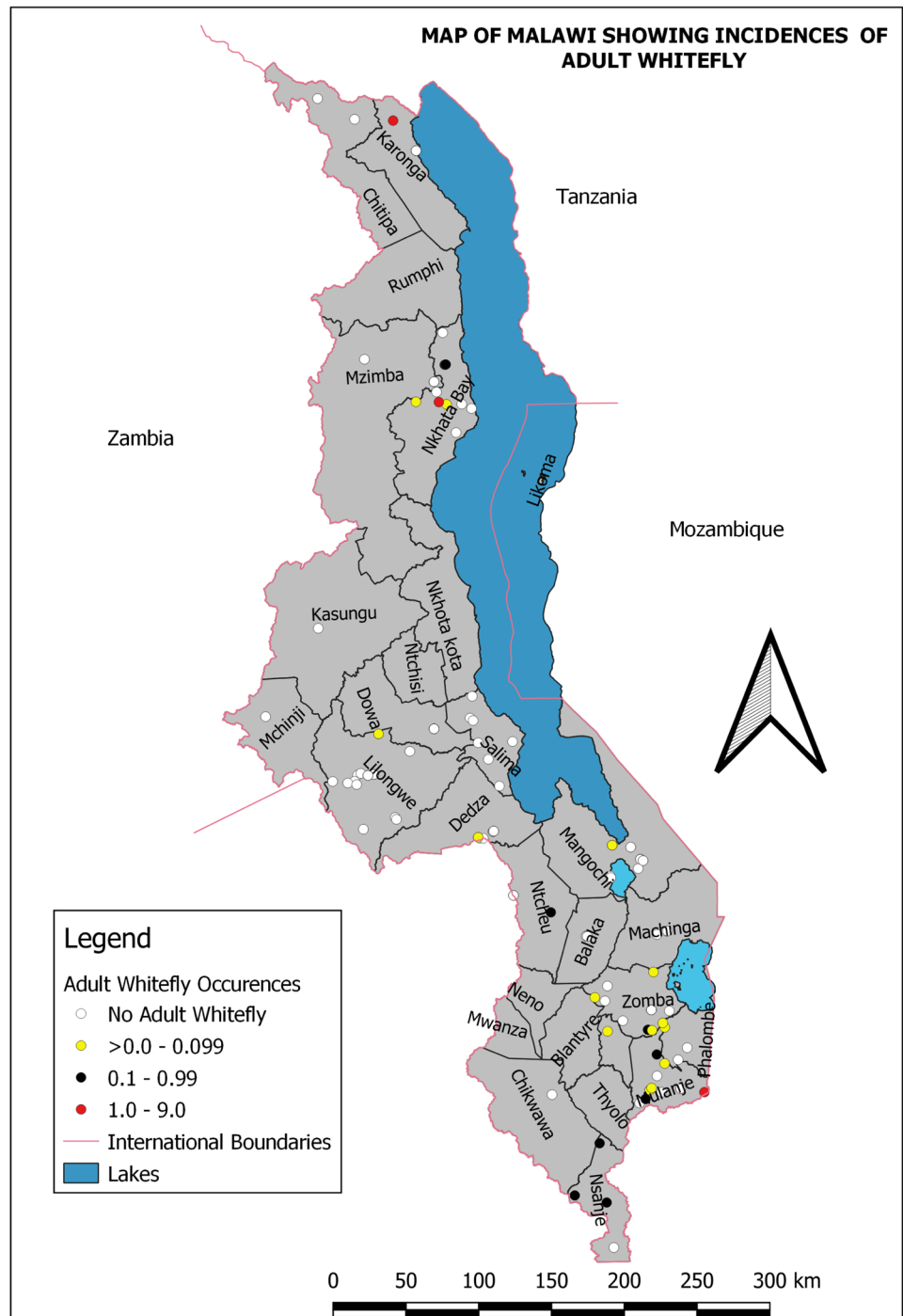


materials. This is so because of low populations of insect vectors as well as no correlation between insect vectors and disease incidences. This is not surprising because in Malawi, farmers recycle planting materials and share freely without sanitary control measures (Chiipanthenga et al. 2015). Furthermore, this is confirmed with the low disease incidences and severities in vine multipliers' fields. These are commercial and registered vine multipliers who access clean

planting materials from government and CGIAR partners like CIP.

The widespread distribution of SPVD and presence of as many as eight virus species in single and mixed infections pose a serious threat to the seed system in the country. Although not documented in this study (through transmission studies), the high incidence of SPVD is reportedly associated with a common occurrence of SPCSV that suppresses host resistance

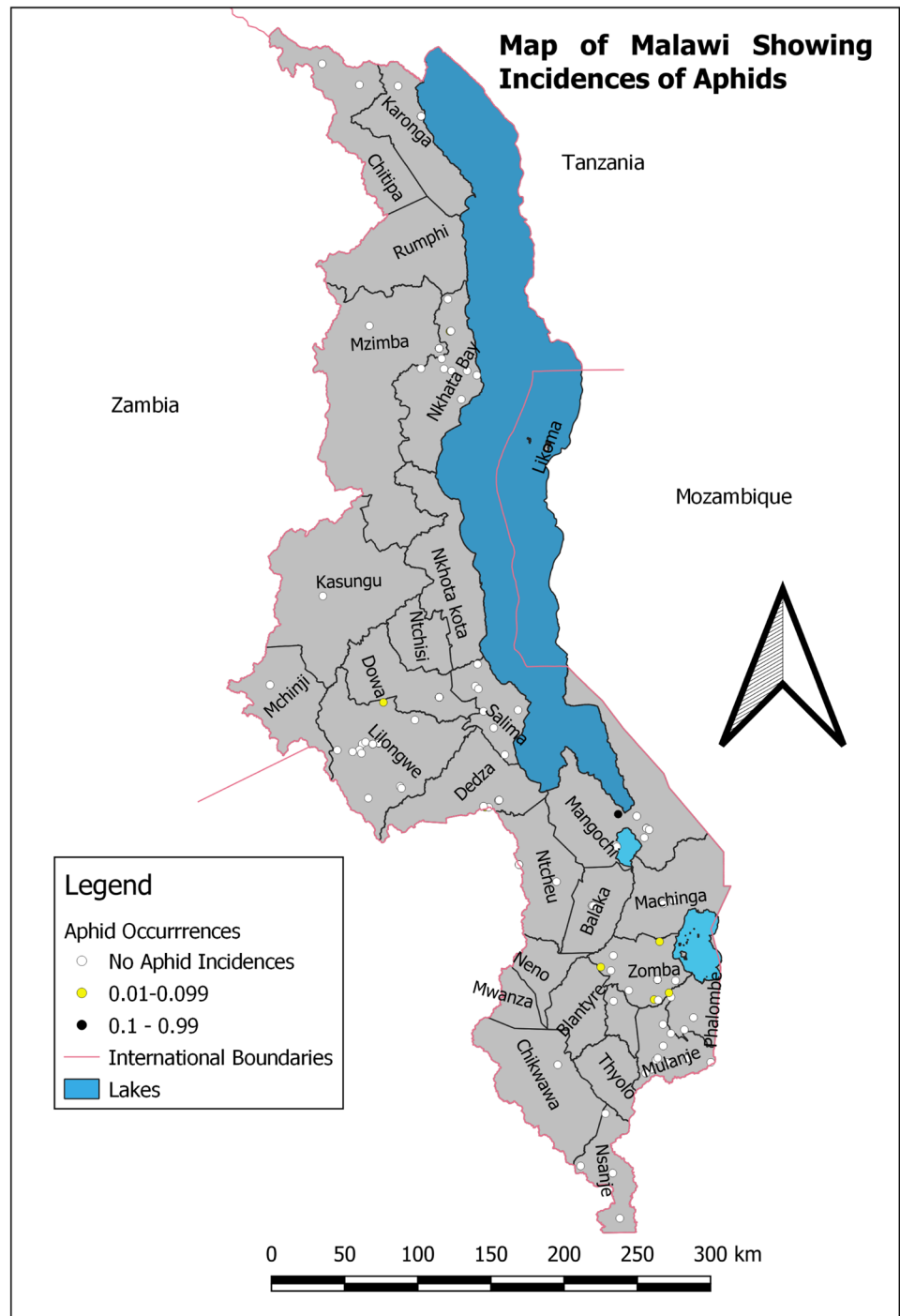
**Fig. 2** Map of Malawi showing distribution of adult whiteflies



and diminishes tolerance to co-infecting, unrelated sweetpotato viruses such as SPFMV (Karyeija et al. 2000; Mukasa et al. 2003; Tairo et al. 2004). Thus, the presence of SPCSV in Malawi is a great concern to sweetpotato seed system and production. Unlike other species like SPMMV and SPFMV that are relatively less virulent, SPCSV causes severe symptoms that are, in most cases, associated with visual identification of SPVD (Gutierrez et al. 2003; Tairo et al. 2004; Tairo 2006).

The study has revealed more frequent occurrence of whitefly in the fields than aphids. However, there was no clear correlation between virus occurrence and whitefly and aphid abundance. Similarly aphids have also been reported to be very low in farmer's fields in Uganda (Karyeija et al. 2000; Ndunguru et al. 2009) and Tanzania (Tairo et al. 2004; Mukasa et al. 2006; Ndunguru et al. 2009).

**Fig. 3** Map of Malawi showing distribution of adult aphids



Low incidences of SPVD in registered seed multipliers including at Kandiyani Agricultural Research Station, which is a seed multiplication site, indicate the viability of the current functional clean seed system in Malawi. This shows that current seed system (starting from tissue culture) has a potential in managing the viruses and consequently help in realizing the full potential of sweetpotato to increase the food security.

Nitrocellulose Membrane Enzyme-link immunosorbent Assay (NCM-ELISA) detected eight virus species, namely SPFMV, SPMMV, SPMSV, SPC6V, SPCSV, SPCV, SPVG, and CMV in single or mixed infections. The presence of SPCSV is of great concern because in co-infection with SPFMV, it causes the SPVD, which can result in devastating yield loss (Tairo et al. 2004; Mukasa et al. 2006; Cuellar et al. 2008; Krueze et al. 2008). Furthermore, the



**Table 2** Distribution of sweetpotato viruses and number of positive samples as detected in Tissue-culture, Improved varieties, local varieties and research clones

Viruses	Tissue-culture	Improved varieties	Local varieties	Research clones
SPFMV	0 (0.00%)	25 (32.05%)	2 (20.00%)	24 (30.77%)
SPMMV	0 (0.00%)	26 (33.33%)	3 (30.00%)	22 (28.21%)
SPMSV	0 (0.00%)	10 (12.82%)	3 (30.00%)	16 (20.51%)
SPC6V	0 (0.00%)	10 (12.82%)	0 (0.00%)	13 (16.67%)
SPCSV	0 (0.00%)	17 (21.79%)	2 (20.00%)	18 (23.08%)
SPCV	0 (0.00%)	21 (26.92%)	3 (30.00%)	27 (34.62%)
SPVG	0 (0.00%)	7 (8.97%)	0 (0.00%)	12 (15.38%)
CMV	0 (0.00%)	5 (6.41%)	0 (0.00%)	8 (10.26%)

**Table 3** Correlation analysis for SPVD incidence, SPVD severity, aphids, and whiteflies

	SPVD incidence	SPVD severity	Aphids	Whiteflies
SPVD incidence	–			
SPVD severity	0.96***	–		
Aphids	0.15	0.19*	–	
Whiteflies	0.02	0.03	0.40***	–

\*Significant at  $p < 0.05$  level; \*\*Significant at  $p < 0.01$  level; \*\*\*Significant at  $p < 0.001$  level

synergistic interactions induced by mixed infections of common viruses pose a serious challenge to controlling virus diseases in sweetpotato using virus-resistant cultivars (Tairo 2006).

The detection and wide distribution of eight sweetpotato viruses (SPFMV, SPMMV, SPMSV, SPC6V, SPCSV, SPCV, SPVG and CMV) in single and mixed infections depicts the threat of SPVD on sustainable sweetpotato production in Malawi. The fact that the viruses were more prevalent in local varieties and research test clones than improved varieties, and that there was no correlation between aphids or whiteflies on SPVD incidence, provides a hypothesis that SPVD transmission in Malawi is more due to the use of infected planting materials than through insect vectors.

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## Declarations

**Conflict of interest** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Furthermore, we declare that no animals were used in this research. All authors have consented to the submission of this paper.

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