Efficiency of insect-proof net tunnels in reducing virus-related seed degeneration in sweet potato

K. O. Ogero, J. F. Kreuze, M. A. McEwan, N. D. Luambano, H. Bachwenkizi, K. A. Garrett, K. F. Andersen, S. Thomas-Sharma and R. A. A. van der Vlugt

Abstract

Virus-related degeneration constrains production of quality sweet potato seed, especially under open field conditions. Once in the open, virus-indexed seed is prone to virus infection leading to decline in performance. Insect-proof net tunnels have been proven to reduce virus infection under researcher management. However, their effectiveness under farmer-multiplier management is not known. This study investigated the ability of net tunnels to reduce degeneration in sweet potato under farmer-multiplier management. Infection and degeneration were assessed for two cultivars, Kabode and Polista, grown in net tunnels and open fields at two sites with varying virus pressures. There was zero virus incidence at both sites during the first five generations. Sweet potato feathery mottle virus and sweet potato chlorotic stunt virus were present in the last three generations, occurring singly or in combination to form sweet potato virus disease. Virus infection increased successively, with higher incidences recorded at the high virus pressure site. Seed degeneration modelling illustrated that for both varieties, degeneration was reduced by the maintenance of vines under net tunnel conditions. The time series of likely degeneration based on a generic model of yield loss suggested that, under the conditions experienced during the experimental period, infection and losses within the net tunnels would be limited. By comparison, in the open field most of the yield could be lost after a small number of generations without the input of seed with lower disease incidence. Adopting the technology at the farmer-multiplier level can increase availability of clean seed, particularly in high virus pressure areas.

Keywords: farmer-multiplier, modelling, net tunnels, seed, sweet potato, virus-related degeneration

Introduction

Sweet potato is an important staple and co-staple food crop in Africa, and orange-fleshed sweet potato varieties are a rich source of vitamin A, especially important for infants and young children. The crop is particularly important in Lake Zone, Tanzania (regions around the Lake Victoria basin) where approximately 15 million inhabitants, a third of Tanzania’s population, live (Lembris & Walsh, 2012). However, sweet potato yields are heavily reduced by viruses, which are carried from generation to generation through recycling of infected cuttings (Gibson & Kreuze, 2015). The most common viruses found infecting sweet potato in Africa are sweet potato feathery mottle virus (SPFMV) and sweet potato chlorotic stunt virus (SPCSV) (Loebenstein, 2015). SPFMV is a potyvirus transmitted nonpersistently by aphids (Karyeja et al., 1998). It can combine with SPCSV, a crinivirus transmitted semipersistently by whiteflies (Schaefers & Terry, 1976), to cause sweet potato virus disease (Mukasa et al., 2006). Individually, SPFMV can cause negligible to 40% loss of root yields (Milgram et al., 1996; Adikini et al., 2016) whereas SPCSV by itself can lead to 50% or more yield reduction (Loebenstein, 2012). However, in combination, the two viruses have a synergistic effect and cause sweet potato virus disease (SPVD) which is the main ‘virus disease’ affecting the crop, often leading to 56–98% yield losses (Ngeve & Bouwkamp, 1991; Ndunguru et al., 2009). Of the two, SPCSV is the most important because it leads to the plant losing any resistance to SPFMV (Nwankwo & Opara, 2015). In addition, a group of geminiviruses, collectively known as sweepoviruses, is increasingly being recognized as damaging and common worldwide, including Africa (Rey et al., 2012). Managing such a complex set of viruses is challenging, especially as many of them show no, or only minor transient symptoms when infecting sweet potato alone, making it difficult to
identify infected plants (Valderde et al., 2007). This is especially true for most African cultivars. Corresponding low titres in plants make their detection by serological methods equally challenging.

There are three major alternatives in managing viruses in sweet potato: (i) deploying resistant cultivars, (ii) using clean (virus-tested) seed, and (iii) employing proper on-farm management practices. Deployment of resistant cultivars is viewed as the most effective strategy in SPVD management (Maule et al., 2007). (In this context, the term ‘seed’ refers to quality (virus-indexed) cuttings or storage roots that have been selected for use in generating new plants; it does not refer to just ‘any vine’, or botanical seed that is used for breeding.) However, whereas landraces and cultivars with higher levels of resistance to SPVD do exist, no immunity to the disease exists, and depending on the virus pressure in the environment all genotypes can become infected in the field (Gibson & Kreuze, 2015). The complex genetics of virus resistance in a hexaploid outcrossing crop additionally make progress through breeding slow (Stephan et al., 2013). The frequency of obtaining SPVD-resistant genotypes in the Ugandan screening schemes at Namulongo is typically less than 0.2% (Mwanga et al., 2002). This has presented a major bottleneck for introducing new orange-fleshed sweet potato varieties high in vitamin A into East Africa, especially in high virus pressure areas.

Complementary to the use of genetic resistance could be the production and use of healthy planting material, largely free of viruses. In developed countries, this is most commonly achieved through formal and centralized certified seed production schemes. However, producing such planting material is expensive. And although this may work well in countries where sweet potato is grown as a cash crop and large-scale farmers can make the investments necessary to obtain such planting material, this has not been economically feasible for smallholder farmers producing mostly for subsistence. On-farm management strategies such as roguing and positive selection for clean seed are therefore important. Roguing is the removal of plants that have virus symptoms whereas positive selection is selection of vigorous healthy-looking plants as planting material/seed for the next season (Muturi et al., 2007). The two approaches reduce virus inoculum and hence disease incidence. A study conducted in Uganda showed that removal of diseased plants within 1 month after planting reduced the spread of SPVD (Gibson et al., 2004). Selection of planting material from symptomless plants has also been reported to reduce virus incidence (Aritua et al., 2003). However, these methods require good farmer knowledge about disease identification. Alternatives that could enable farmers, or specialized local vine multipliers, to maintain a high sanitary status of planting material at low cost and minimum technical input exist. One such technology is a low-cost insect-proof net tunnel that can be constructed from locally sourced materials (Schulte-Geldermann et al., 2012). This technology enables farmers to maintain a nuclear stock of high phytosanitary status vines, by protecting them against the virus vectors such as whiteflies and aphids (Loebenstein, 2015). Vines produced in the net tunnels can be harvested and used either directly, or after one or more cycles of field multiplication for root production and/or sale as quality planting material (Ogero et al., 2015). However, it is important to know how well net tunnels perform in maintaining the phytosanitary status of sweet potato vines under farmer-multiplier management.

This study sought to determine the rate of virus infection and related degeneration in sweet potato planting material maintained in net tunnels and open fields under farmer-multiplier management. In an integrated seed health strategy, choice of seed and on-farm management should be considered together to optimize management of seed degeneration in vegetatively propagated crops (Thomas-Sharma et al., 2016). This study used the observed rates of seed degeneration in the model by Thomas-Sharma et al. (2017) to evaluate the likely long-term patterns of degeneration and when purchase of quality-declared seed would be motivated, as a function of management decisions. Once estimates of the rate of infection for particular system combinations – variety, location and management – are available, these can be used in scenario modelling to understand economic thresholds for purchase of quality-declared seed (Thomas-Sharma et al., 2017). The models include the level of host resistance, environmental conduciveness, vector management, roguing, the amount of previously infected seed material, and rates of reversion to healthy status. Reversion in virus infection is the ability of infected plants to become almost virus-free after several seasons of cultivation and has been demonstrated in several sweet potato cultivars (Gibson & Kreuze, 2015). Modelling can be used to evaluate potential economic thresholds for seed replacement before yield loss becomes too limiting. Simulation models can extrapolate results to larger areas when multi-year and multi-location trials are cost-prohibitive.

Materials and methods

Location and varieties used

This research was conducted at two sites, Mwasonge (02°40′13″ S, 32°54′45″E) and Nyasenga (02°39′40.1″S, 32°44′30.6″E) villages, in Mwanza Region, Lake Zone, Tanzania. Mwasonge is a high virus pressure area owing to high intensity of sweet potato production, whereas Nyasenga is a low virus pressure area due to limited sweet potato cultivation in the area. High cultivation of sweet potato at Mwasonge also translates to higher vector populations due to year-round availability of host plants as compared to Nyasenga. Two sweet potato varieties, Kabode and Polista, were used. Polista is a cream-fleshed local variety, and Kabode is an orange-fleshed Ugandan-bred variety also known as NASPOT 10.

Experimental set up

Three-node virus-indexed cuttings were planted in two net tunnels and two open beds (control) at each site – one net tunnel and open bed per variety (Fig. 1a).
Each net tunnel and open bed measured $3 \times 1.7$ m and had 270 cuttings planted in nine rows at a spacing of $20 \times 10$ cm. After every 60–80 days, vines were harvested from the net tunnels and planted in the open for a single generation, then destroyed (black arrows in Fig. 1b). The control plants were maintained in the open during the entire period of the study. Visual assessment for virus symptoms was done regularly and any plant that seemed infected removed. Leaf sampling was done after every 60–80 days. Thirty samples were randomly collected from each bed. One leaf from the middle part of each plant was collected into a coffee-filter and put in a zip-lock bag containing 100 g silica gel. Sample collection was done eight times between June 2014 and March 2016 (21 months).

Environmental virus pressure and weather conditions

Local environmental virus pressure at the multiplication sites was assessed eight times by visually surveying fields and weeds in a radius of 250 m surrounding each plot. Whitefly abundance was recorded by counting for 10 s on 10 leaves from 10 different plants and recording the number. Onset HOBO data loggers were used to record daily weather data (rainfall, relative humidity and temperature).

Testing viruses present in the environment at initiation of experiment

At the time of establishment of the experiment, samples were taken from nearby sweet potato plots to determine the viruses present in the environment. This could be done at Mwasonge only, because no sweet potato plots were found near the Nyasenga site. Samples were bulked together and subjected to small RNA sequencing assembly to identify any viruses infecting them, as described previously (Kreuze et al., 2009).

Testing for viruses on samples collected from experimental plots and determination of virus incidence

Leaf samples were analysed in bulks of five, and each bulk was screened for SPLCV, potyviruses (SPFMV, sweet potato virus G, sweet potato virus 2 and sweet potato virus C), and SPCSV using PCR, multiplex reverse transcription PCR (RT-PCR), and reverse transcription quantitative real-time PCR (RT-qPCR), respectively. If a bulk was positive, each sample was analysed separately to determine which one was positive. RNA was extracted using a CTAB method with minor modifications in reagents used (Lodhi et al., 1994). Extracted total RNA was treated with DNase I to remove DNA before measuring purity and concentration of RNA using a NanoDrop 2000 spectrophotometer (Thermo Scientific). Additionally, total RNA was run on a gel containing 1% agarose (Invitrogen) and viewed on a gel documentation machine (Biodoc-H Imaging system with benchtop UV transilluminator) for RNA integrity. Thereafter, total RNA was diluted with nuclease-free water to end up with an estimated concentration of 0.025 ng µL$^{-1}$.

qPCR for SPCSV

The reaction mix for one reaction of qPCR was prepared by mixing 12.5 µL of 2× TaqMan universal master mix (Applied Biosystems), 0.75 µL of each forward (F) and reverse (R) primers (10 µM each; Table 1), 8 µL of nuclease-free water, 0.5 µL of RevertAid reverse transcriptase (Thermo Fisher Scientific), diluted 1/100 (2 U µL$^{-1}$), 0.125 µL of F and R COX (10 µM) primers.

![Figure 1](https://example.com/figure1.png)

**Figure 1** (a) Net tunnels (right) and open fields (left) at the high virus pressure site (Mwasonge). (b) The growing cycles of the experiments: the green line is the intervention (net tunnels, NT) and the red line is the control (open fields, OF). Black arrows indicate vines harvested from the net tunnels and multiplied once in the open field (NT-OF); blue vertical lines indicate points of leaf sampling. G1–G8, generations 1–8.
Multiplex reverse transcription (RT) PCR for potyviruses

Multiplex RT-PCR for sweet potato potyviruses was performed as described by Li et al. (2012) with minor modifications. Total RNA with reverse primer SPFCG2-R (Table 1) was denatured at 65 °C for 10 min and cDNA synthesized using 5× RT buffer, 0.1 μM dTTP, 10 mM dNTPs, RNAase Out inhibitor 40 U μL⁻¹ and M-MLV 200 U μL⁻¹, and the reaction was incubated at 40 °C for 60 min, 95 °C for 5 min. Reaction mix for PCR was prepared using 5× Dream Taq buffer (Invitrogen), 25 mM MgCl₂, 10 mM dNTPs (Invitrogen), primers SPG-F (2.5 μL), SP-F (0.4 μL), SPF-F (2 μL), SP2-F (0.2 μL) and SPFCG2-R (2 μL), each at a concentration of 10 μM, Dream Taq 5 U μL⁻¹ and cDNA. The mixture was incubated in a thermal cycler machine (GenAmp PCR system 9700; Applied Biosystems) using the following conditions: 94 °C for 2 min, 94 °C for 30 s; then 35 cycles of 60 °C for 30 s, 72 °C for 1 min 20 s, 72 °C for 10 min. The gel was prepared using 1.2% agarose in 1× TAE buffer and ethidium bromide was used for staining. This was run at 200 V for 30 min and the product viewed and recorded using a gel documentation machine.

PCR for SPLCV

The reaction mix was prepared using 5× Dream Taq buffer, 25 mM MgCl₂, 10 mM dNTPs, primers SPG1 (0.5 μL) and SPG2 (0.75 μL) at 10 μM concentration described by Li et al. (2004) (Table 1), Dream Taq 5 U μL⁻¹ and cDNA template. The reaction mix was incubated in a thermal cycler machine (Gen-Amp PCR system 9700) using the following conditions (touch down); 94 °C for 40 s, 72 °C for 30 s, 72 °C for 90 s; for 11 cycles (n − 1 °C per cycle); then 94 °C for 40 s, 60 °C for 40 s, 72 °C for 92 s for 24 cycles, followed by 72 °C for 10 min. The gel was prepared, and products viewed as in RT-PCR above.

Calculating virus incidence

The number of samples testing positive for various viruses was established through counting and virus incidence at 95% confidence interval calculated as follows:

\[
\frac{\text{infected samples}}{\text{sample size}} \pm \Phi^{-1}(0.0975) \sqrt{\frac{\text{infected samples/sample size} \times (1 - \text{infected samples/sample size}) \times (\text{population size} - \text{sample size})}{(\text{sample size} \times (\text{population size} - 1))}}
\]

Modelling of seed degeneration

To model the likely influence of the management components evaluated in this study on seed degeneration over the course of 10 seasons, virus incidence data were used to estimate parameters in the model from Thomas-Sharma et al. (2017). Four treatment combinations were compared using data from Mwasonge, the 'high virus' location. Treatments where cv. Polista and cv. Kabode were grown in the net tunnel, with seed continuously replaced from the net tunnel for each season, were compared with treatments where seed for these two cultivars was continuously grown and obtained from the open field. Virus incidence data collected for the last three generations (generations 6, 7 and 8) for each of these four treatment combinations were used to parameterize the proportional change in infection due to the combined effect of environment, host and management (Thomas-Sharma et al., 2017). Simulation experiments were carried out in the R programming environment (R Core Team, 2018) using the custom package seedHealth (https://www.garrettlab.com/softwar e/). Each parameter combination was evaluated in 1000 simulations, over 10 generations. Details of parameters used in simulations for each of the four scenarios are given in Table S1. Note that this analysis is based on applying a simple model of yield loss as a function of disease incidence across all scenarios, so the results should be interpreted in terms of relative loss and not in terms of actionable economic threshold values.

Results

Environmental vector pressure and weather conditions

Only a few (<10) volunteer sweet potato plants were found within a 250 m radius of the two sites at any of
the sampling times between June 2014 and March 2016. Cultivated crops within a 250 m radius of the sweet potato seed plots included cabbage, okra, capsicum, spinach, watermelon, tomatoes, cucumber, amaranth, maize, rice and beans at the Mwasonge site, and pumpkin, beans, maize, rice, cassava, amaranth, cotton, cabbage and spinach at the Nyasenga site. Weeds were also present, but none were *Ipomoea* spp. There was minimal whitefly presence in the sweet potato plots and surrounding crops during most of the experiment. A spike in whitefly populations was seen in October 2014 in surrounding crops at both sites, principally in pumpkins and beans. The population of whiteflies at both sites started to increase in August 2015, this time principally in sweet potato (Fig. 2). Aphids were only observed in October 2014 at Mwasonge on cucumbers and beans (data not shown).

**Virus incidence**

** Viruses present in the environment at initiation of experiment **

Results from small RNA sequence assembly analysis of bulked samples from nearby sweet potato fields at the time of installation of the experiment revealed the following viruses were present in the environment: SPFMV, SPVC, SPCSV-EA strain, SPLCV, sweet potato pakakuy virus and sweet potato symptomless mastrevirus 1. Because the latter two viruses are not known to cause any disease in sweet potato and occur only at extremely low titres in plants (J. Kreuze, unpublished data, International Potato Center, Lima, Peru), only SPFMV, SPVC, SPCSV and begomoviruses were considered relevant for this study.

**Viruses in samples collected from the experimental plots**

For the first five generations, all samples collected from vines maintained in net tunnels (Net-tunnel), vines harvested from the net tunnels and planted in the open field for one generation (Net-tunnel-OF) and vines grown in the open throughout (Open field (control)) tested negative for viruses. Virus infection started occurring in generation 6 and increased with successive generations at both sites (Fig. 3). There was higher virus incidence at Mwasonge as compared to Nyasenga. Only marginal changes in virus incidence occurred at Nyasenga while a sequential increase was noted at Mwasonge from generation 6 to 8. Viruses detected at the two sites were SPFMV and SPCSV occurring singly or coinfecting to form SPVD (Fig. 3b). SPFMV was the most prevalent at both sites.

Vines of variety Kabode maintained in the net tunnels at Mwasonge had 7% and 13% virus incidence during generations 7 and 8, respectively (Fig. 4a). Vines of the same variety continuously grown at the open field (control) at Mwasonge had 7%, 10% and 23% virus incidence during generations 6, 7 and 8, respectively. Vines of the variety Polista maintained in the net tunnel at the high virus pressure area were diagnosed with 7% virus incidence in generation 8. Planting material of the same variety continuously grown at the open field had the highest virus incidence (Fig. 4b). Virus incidence was zero for variety Polista at the Nyasenga site (low virus pressure area) from generations 6 to 8.

![Figure 2](image-url) **Figure 2** Weather conditions and whitefly counts during the experiments at the two trial sites.
Seed degeneration modeling

Results from seed degeneration modelling illustrated how, for both Kabode and Polista, yield degeneration was reduced by the maintenance of vines under net tunnel conditions for each growing cycle, when compared to treatments where seed vines were grown in the open field (Fig. 5). This result was somewhat more pronounced for the cream-fleshed variety, Polista. The time series of likely degeneration based on these observations, in a scenario with a generic yield loss model, shows how a potential economic threshold such as 40%, would not be reached in net tunnels for many seasons, while the same threshold might be crossed in the open field after only a couple seasons.

Discussion

Growing sweet potato seed in net tunnels reduced degeneration by limiting virus incidence. Previous on-station research had shown that well-managed net tunnels could be used to maintain virus-free vines for at least 33 months (Schulte-Geldermann et al., 2012). The insect-proof net tunnels block virus vectors (whiteflies and aphids) from accessing the plants. By avoiding physical contact between vectors and sweet potato plants, the crop is protected from virus infection. As indicated in this study, up to 100% protection can be achieved in low virus pressure areas. High degeneration rates in open fields is linked to exposure to vectors that transmit viruses from diseased sweet potato plants or wild Ipomoea spp. to newly established virus-free plants. An increase in virus incidence coincided with increased whitefly population. The population of whiteflies at both sites started to increase in August 2015, corresponding with the increasing virus incidence during the last three cycles of growth. The whiteflies were predominantly on sweet potato plants being established in neighbouring fields, therefore providing a favourable host for reproduction and multiplication of the vectors.

Generations 1 to 5, with zero virus incidence observed, experienced a period of limited rainfall leading to low cultivation of sweet potato in areas surrounding the experimental sites and therefore reduction in external disease inoculum. A favourable environment is very critical for plant disease manifestation in addition to pathogen–host interactions (Gergerich & Dolja, 2006). Sufficient rainfall and high humidity favour build-up of sweet potato virus inoculum due to increased cultivation, leading to increased infection rate and rapid deterioration. Weather conditions and disease pressure are therefore important considerations in management of sweet potato viruses and selection of sites for seed production. Thiele (1999) linked better seed potato production at higher altitudes to favourable temperatures and low disease pressure. Results of this study confirmed that Mwasonge village was a higher virus pressure area compared to Nyasenga. The Mwasonge site was by the shores of...
Lake Victoria where sweet potato is usually intensely grown due to favourable conditions. That only SPCSV and SPFMV were detected in the study area reflects previous reports that these two are the most common and important yield-limiting sweet potato viruses in East Africa, especially when occurring together (Ndunguru et al., 2009; Adikini et al., 2016). High prevalence of SPFMV is consistent with findings by Clark et al. (2011) and Taibo et al. (2004) who reported the symptomless potyvirus to be the most common single infection virus at the Lake Zone, Tanzania. Efforts employed to control viruses in sweet potato should consider regional variability of the most significant viruses. This can aid in prioritizing resources, especially in diagnostics. Virus testing has become a major component of clean systems but is limited by high costs, especially when using the more sensitive molecular techniques (Boonham et al., 2014). Focusing on the most important viruses in a country can reduce the costs, therefore making clean seed more affordable to farmers. Differential susceptibility to viruses between cultivars such as that seen in this study between cv. Kabode and cv. Polista is another important consideration in management. Yield losses resulting from single infections in particular vary with variety and virus involved (McEwan, 2016). A susceptible variety like Beauregard has been reported to be reinfected quickly, leading to 80–90% yield losses within a single season in Uganda (Adikini et al., 2015) and up to 40% yield losses in five seasons in the USA (Bryan et al., 2003). On the other hand, landraces cultivated in areas where SPVD is prevalent are more resistant (Bua et al., 2009). In addition, some varieties have the ability to revert to virus-free status after several seasons of cultivation (Gibson & Kreuze, 2015).

Evaluation of the field data in the model from Thomas-Sharma et al. (2017) enabled consideration of the potential optimal time to seed replacement for combinations of variety and management with net tunnels. For some treatment combinations, there was not enough data available to generate estimates. Varieties Kabode and Polista were compared, with and without net tunnels, in an analysis with simplifying assumptions about the relationship between disease incidence and yield. Yield in the scenario analysis is sensitive to changes in management such as roguing rates and positive selection (Thomas-Sharma et al., 2017). Actionable economic thresholds can also be difficult to formulate without good models of yield loss and good information about farmer willingness to pay. In sub-Saharan Africa, the challenge of anticipating likely economic thresholds is compounded by sociocultural values that surround sweet potato seed acquisition (Almekinders et al., 2019). For the farmers in this study, decline in cultivar performance might be tolerated for many seasons due to the subsistence nature of their farming systems. Limited record keeping makes it difficult to notice yield losses and therefore farmers may replace seed only at very high infection levels when the crop cannot produce any bulked roots. However, as
farmers have more information available and more options, they may choose to use a lower economic threshold to get an economic return from higher quality seed. It is also important to note that in this experiment there was greater attention to removing inoculum sources than is likely on most farms in the region. Thus, the typical farmer in the region may experience substantially faster seed degeneration, motivating faster seed replacement.

This research provides evidence supporting use of insect-proof net tunnels among farmer-multipliers to reduce seed degeneration in sweet potato. Given their affordability in both construction and management, they can contribute greatly in the improvement of local seed systems. However, the net tunnels should be combined with other on-farm management options such as positive selection and roguing. Piloting in Ethiopia, Kenya, Mozambique, Nigeria, Rwanda, Tanzania and Uganda has shown that well-resourced, trained farmers are better adopters of the technology, therefore recommending it for basic seed production especially in high virus pressure areas (Ogero et al., 2017). This research also sheds more light on parameters to consider in seed degeneration studies, especially for vegetative crops. The need to consider variety, environmental virus pressure, weather conditions and agronomic practices in seed degeneration modelling was illustrated. This is consistent with examples from Thomas-Sharma et al. (2016) and Bryan et al. (2003). Seed degeneration models can help to inform farmers, and those who advise farmers, about the time to economic seed replacement. To stay in business farmers should operate at an equilibrium whereby cost of production equals revenue. Because cost of production includes other inputs, a farmer might decide to forgo or reduce investments in one input to compensate for the losses associated with the quality of seed. This implies that a farmer is likely to purchase quality seed when yield losses without the use of clean seed are greater than the cost of seed, all other factors constant. Use of net tunnels for sweet potato seed production might increase the cost of seed due to the additional investment. To keep the cost affordable, farmer-multipliers using the net tunnels are advised to sell after two rounds of open field multiplication thereby increasing the quantities.

Acknowledgements

This research was funded by the Tanzania Agricultural Research Institute (TARI), Ukiriguru Center (then known as the Lake Zone Agricultural Research and Development Institute (LZARDI)) and conducted as a component of the ‘Keeping Disease-free Sweetpotato Vines Closer to Farmers’ project. Additional funds were provided by the Sweetpotato Action for Security and Health in Africa (SASHA) phase 2 project and the CGIAR Research Program on Roots, Tubers and Bananas (RTB). The authors appreciate assistance offered by Dr Raul Eyzaguirre (CIP) and Israel Navarette (CIP) in data analysis and Obadia Mayanja (TARI) in field work. Professor Paul Struik (WUR) and Dr Peter Kromann (CIP) provided useful reviews that helped improve this paper.

References

Adikini S, Mukasa SB, Mwanga ROM, Gibson RW, 2015. Sweet potato cultivar degeneration rate under high and low sweet potato virus disease pressure zones in Uganda. Canadian Journal of Plant Pathology 37, 136–47.
Adikini S, Mukasa SB, Mwanga ROM, Gibson RW, 2016. Effects of Sweet potato feathery mottle virus and sweet potato chlorotic stunt virus on the yield of sweet potato in Uganda. *Journal of Phytopathology* 164, 242–54.

Almekinders CJM, Walsh S, Jacobsen KS et al., 2019. Why interventions in the seed systems of roots, tubers and bananas do not reach their full potential. *Food Security* 11, 23–42.

Aritua V, Bua B, Gibson RW, Mwanga ROM, Adipala E, 2003. Toward integrated management of sweet potato virus disease: lessons from Uganda. *African Crop Science Conference Proceedings* 6, 307–14.

Boonham N, Kreuze J, Winter S et al., 2014. Methods in virus diagnostics: from ELISA to next generation sequencing. *Virus Research* 186, 20–31.

Bryan AD, Pesci-VanEsbroeck Z, Schultheis JR, Pecota KV, Swallow WH, Yencho GC, 2003. Cultivar decline in sweet potato: I. Impact of micropropagation on yield, storage root quality, and virus incidence in ‘Beauregard’. *Journal of the American Society for Horticultural Science* 128, 846–55.

Bua B, Adipala E, Gibson RW, 2009. Reaction of sweet potato landraces to sweet potato virus disease in Uganda. *African Crop Science Journal* 14, 197–205.

Clark CA, Davis JA, Abad JA et al., 2011. Sweet potato viruses: 15 years of progress on understanding and managing complex diseases. *Plant Disease* 96, 168–85.

Ggergerich RC, Dolja VV, 2006. Introduction to plant viruses, the invisible foe. *The Plant Health Instructor*. https://doi.org/10.1094/PHI-1-2006-0414-01.

Gibson RW, Kreuze JF, 2015. Degeneration in sweet potato due to viruses, virus-cleaned planting material and reversion: a review. *Plant Pathology* 64, 1–15.

Gibson RW, Aritua V, Byamukama E, Mpembe I, Kayongo J, 2004. Control strategies for sweet potato virus disease in Africa. *Virus Research* 100, 115–22.

Kariyeya RF, Gibson RW, Valkonen JPT, 1998. The significance of sweet potato feathery mottle virus in subsistence sweet potato production in Africa. *Plant Disease* 82, 4–15.

Kreuze JF, Perez A, Untiveros M et al., 2009. Complete viral genome sequence and discovery of novel viruses by deep sequencing of small RNAs: a generic method for diagnosis, discovery and sequencing of viruses. *Virology* 388, 1–7.

Lembris L, Walsh S, 2012. Seed System Lessons Learned from Marando Bora in Lake Zone, Tanzania. Dar es Salaam, Tanzania: Catholic Relief Services.

Li R, Salah S, Hurrt S, 2004. Detection of geminiviruses in sweet potato by polymerase chain reaction. *Plant Disease* 88, 1347–51.

Li F, Zuo R, Abad J, Xu D, Bao G, Li R, 2012. Simultaneous detection and differentiation of four closely related sweet potato potyviruses by a multiplex one-step RT-PCR. *Journal of Virological Methods* 186, 161–6.

Lodhi MA, Ye G-N, Weeden NF, Reisch BI, 1994. A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. *Plant Molecular Biology Reporter* 12, 6–13.

Loebenstein G, 2012. Viruses in sweet potato. *Advances in Virus Research* 84, 325–43.

Loebenstein G, 2015. Control of sweet potato virus diseases. *Advances in Virus Research* 91, 33–45.

Maule AJ, Caranta C, Boulton MI, 2007. Sources of natural resistance to plant viruses: status and prospects. *Molecular Plant Pathology* 8, 223–31.

McEwan M, 2016. Sweet potato seed systems in sub-Saharan Africa: a literature review to contribute to the preparation of conceptual frameworks to guide practical interventions for root, tuber, and banana seed systems. RTB Working Papers No. 2016–4. CGIAR Research Program on Roots, Tubers and Bananas. Lima, Peru: CGIAR.

Milgram M, Cohen J, Loebenstein G, 1996. Effects of sweet potato feathery mottle virus and sweet potato sunken vein virus on sweet potato yields and rates of reinfection of virus-free planting material in Israel. *Phytoparasitica* 24, 189–93.

Mukasa SB, Rubaihayo PR, Valkonen JPT, 2006. Interactions between a crinivirus, an ipomovirus and a potyvirus in cofounded sweet potato plants. *Plant Pathology* 55, 455–67.

Mutter PW, Mwololo JK, Mburu MWK et al., 2007. Strategies of maintaining sweet potato nurseries free from insect vectors that spread sweet potato virus disease. *African Crop Science Conference Proceedings* 8, 2071–4.

Mwanga ROM, Moyer JW, Zhang DP, Carey EE, Yencho GC, 2002. Nature of resistance of sweet potato to sweet potato virus disease. *Acta Horticulturae* 583, 113–9.

Ndunguru J, Kapenga R, Sseruwagi P et al., 2009. Assessing the sweet potato virus disease and its associated vectors in northwestern Tanzania and central Uganda. *African Journal of Agricultural Science* 4, 334–43.

Ngwe JM, Bouwkamp JC, 1991. Effects of sweet potato virus disease (SPVD) on the yield of sweet potato genotypes in Cameroon. *Experimental Agriculture* 27, 221–5.

Nwankwo EU, Opara IIM, 2015. The role of virus vectors in orange fleshed sweet potato genotypes infection – a case study. *Global Journal of Science Frontier Research* 15, 2–D.

Ogero K, McEwan M, Jeremiah S, 2015. Towards Improved Seed System Management: Use of Affordable Net Tunnels and Decentralized Inspection Schemes. Nairobi, Kenya: International Potato Center.

Ogero K, Njoku J, McEwan M, 2017. Protecting Sweetpotato Planting Material from Viruses Using Insect Proof Net Tunnels: A Guide to Construct and Use Net Tunnels for Quality Seed Production. 2nd edn. Lima, Peru: International Potato Center.

R Core Team, 2018. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.

Rey MEC, Ndunguru J, Berrie LC et al., 2012. Diversity of dicotyledonous-infecting geminiviruses and their associated DNA molecules in southern Africa, including the south-west Indian Ocean islands. *Viruses* 4, 1753–91.

Schaefers GA, Terry ER, 1976. Insect transmission of sweet potato virus disease agents in Nigeria. *Phytopathology* 66, 642–5.

Schulte-Geldermann E, Agili S, Ndolo P, Low J, 2012. *Net Tunnels to Protect Sweet potato Planting Material from Disease: A Guide to Construct and Maintain Tunnels*. Lima, Peru: International Potato Center.

Stephan N, Hussein S, Julia S, Mutanda K, 2013. Sweet potato breeding for resistance to sweet potato virus disease and improved yield: progress and challenges. *African Journal of Agricultural Research* 8, 3202–15.

Tairo F, Kullaya A, Valkonen JPT, 2004. Incidence of viruses infecting sweet potato in Tanzania. *Plant Disease* 88, 916–20.

Thiele G, 1999. Informal potato seed systems in the Andes: why are they important and what should we do with them? *World Development* 27, 83–99.

Thomas-Sharma S, Abdurahman A, Ali S et al., 2016. Seed degeneration in potato: the need for an integrated seed health strategy to mitigate the problem in developing countries. *Plant Pathology* 65, 3–16.

Thomas-Sharma S, Andrade-Piedra J, Carvajal Yepes M et al., 2017. A risk assessment framework for seed degeneration: informing an integrated seed health strategy for vegetatively propagated crops. *Phytopathology* 107, 1123–35.

Valderde RA, Clark CA, Valkonen JPT, 2007. Viruses and virus disease complexes of sweet potato. *Plant Viruses* 1, 116–26.

**Supporting Information**

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site.

Table S1. Parameters used in seed degeneration risk assessment.

---

*Plant Pathology* (2019) 68, 1472–1480