Degeneration of Sweetpotato Seed in Tanzania:

Can Cleaned-up, Virus-tested Seed Help?

Kwame Okinyi Ogero

Propositions

1. The term quality seed only applies when talking about formalized seed systems.
   (This thesis)

2. Without advanced vector surveillance and control, managing sweetpotato seed degeneration is impossible in Africa.
   (This thesis)

3. Farmers suffer when scientists hold strong divergent ideologies on technological innovations.

4. Agricultural productivity is a complicated concept oversimplified by scientists.

5. Technology transfer is only successful when there is a holistic understanding of end users' needs.

6. Approval of propositions at Wageningen University should be done by a member of the Academic Board from the same discipline as the candidate submitting the propositions.

7. The tenets of modernity are insufficient to provide an ethical direction for quality of life.

8. The poor prioritize things that make life less boring over food.

Propositions belonging to the thesis, entitled

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Kwame Ogero

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Thesis

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Abstract

Low yields caused by poor phytosanitary quality of seed vines are a major challenge to sweetpotato farming in Tanzania. Poor seed quality is largely caused by viruses, especially the co-infection of sweet potato feathery mottle virus (SPFMV) and sweet potato chlorotic stunt virus (SPCSV). These two viruses are the most devastating ones in East Africa and when combined cause the sweetpotato virus disease (SPVD), often leading to 56 − 100% yield losses. Being vegetatively propagated, sweetpotato accumulates viruses across generations. The use of virus-infected material obtained from the previous crop is the most common source of viruses. The accumulation of viruses in the propagating material leads to progressive yield loss and is called seed degeneration. Use of clean seed disseminated through a formal system is one of the options to address this. However, clean seed can get infected once in the field and it is not known how it will perform following several seasons of on-farm propagation. Moreover, successful use of clean seed requires an understanding of the current practices that farmers use when sourcing sweetpotato seed.

This research started by assessing the performance of clean seed and farmer-sourced seed of varieties that are susceptible or relatively resistant to viruses grown in environments with high or low virus pressure. The results showed that starting propagation with clean seed slows down degeneration especially in susceptible varieties grown in high-virus-pressure environments. The findings also showed yield stability for farmer-sourced material of virus resistant varieties. The yield stability for farmer-sourced material of the relatively virus-resistant variety illustrates why farmers recycle planting material. The findings also showed that regular replacement by clean seed is especially economic in areas with high virus pressure and for susceptible varieties. In addition, it is important to consider cultivar resistance and agroecology when promoting adoption of clean seed.

To understand farmers' experiences with degeneration a 'Small-N' survey was conducted with 37 respondents. We found that female farmers reported degeneration more frequent than male farmers. The main action to address degeneration was to acquire a new variety, mostly from close sources in the farmers' social networks (friends, neighbors and relatives). Planting material was mostly acquired and shared as gifts. Farm seed recycling still dominates. These networks will continue to play an important role in sweetpotato seed flows and therefore it is important to devise innovative ways of linking them with the formal seed system for access to clean seed and improved varieties.

Clean seed is not immune to viruses and can get infected once planted in the open fields. We investigated the efficiency of insect-proof net tunnels in reducing degeneration of clean seed at seed producer level. Results from a 21-month experiment showed that insect-proof net tunnels can prevent infection in an area with high virus pressure for up to

20 months. The plants sourced from net tunnels would still attain more than 60% of the expected yields after 10 generations while those sourced from open fields would not do so after only 4 generations. The findings form a basis for recommending adoption of net-protected structures such as screenhouses in high-virus pressure environments to reduce rapid infection of clean seed. Such structures can be adopted by well-resourced farmers producing at medium or large scale.

Lastly, we assessed the effect of the ratoon cropping technique and replanting technique on vine production in net tunnels and open fields with an aim of optimizing vine production in net tunnels. The ratoon cropping technique, also known as the 'reaping and regrowth system of production' consists of harvesting the crop, with the next crop being the result of the first crop's regrowth. It was shown that ratooning increases vine production. However, the technique leads to higher virus incidences on plants grown in the open. Ratooning is recommended for vine production in net tunnels and screenhouses. If used in the open field, it should be accompanied with virus control measures.

The findings captured in this thesis show that using clean seed can help improve seed quality in sweetpotato across generations. However, it is important to consider other important factors that can influence its adoption. These include farmer seed sourcing strategies and risk of re-infection. Characterizing the farmer seed exchange networks can give a detailed description of and bring to the forefront the value embedded in them and how they can be tapped to disseminate clean seed. It can also help understand access of different types of farmers to different sources.

Key words: Cultivar decline, *Ipomoea batatas*, seed systems, virus-tested seed

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Mr. Masatu Matale, a sweetpotato seed producer in Geita region, Tanzania, inspecting his vines. Geita district, Tanzania. Credit: K. Ogero.

Chapter 1:

General Introduction

1 Seed degeneration

Seed degeneration can be defined as loss of productivity caused by use of diseased or pest-infested planting material over consecutive seasons (Gibson & Kreuze, 2015). The term was first used to describe accumulation of viruses in potato following several on-farm propagation cycles therefore leading to gradual decline in yields (Schultz & Folsom, 1923). Struik and Wiersema (1999) later expanded this to include non-viral pathogens and pests. The most recent definition is by Thomas-Sharma et al. (2016) who described seed degeneration in vegetatively propagated crops as the build-up of diseases and pests in/on the seed over consecutive seasons of on-farm propagation therefore reducing seed health and progeny yield.

Seed degeneration is a common problem in root, tuber, and banana (RTB) crops whose seed systems are largely informal, and propagation is through various vegetative parts (Bentley et al., 2018). Degeneration has been recognized as a threat to productivity in banana, cassava, potato and sweetpotato (Adikini et al., 2015; Jacobsen et al., 2019; Navarrete, 2021; Shirima et al., 2019). In cassava and sweetpotato, degeneration is mainly caused by viral pathogens whereas in banana and potato it is a mix of both pests and pathogens (Adikini et al., 2015; Gibson & Kreuze, 2015; Jacobsen et al., 2019; Navarrete, 2021; Shirima et al., 2019; Thomas-Sharma et al., 2016). Despite the importance of degeneration in RTB crops, most studies have focused on potato with other crops understudied (Almekinders et al., 2019). This informed the need to study sweetpotato seed degeneration in Tanzania and to assess if cleaned-up, virus-tested seed can be used in management. This thesis describes research conducted in the Lake Zone of Tanzania to understand how cleaned-up, virus-tested seed degenerates in high- and low-virus-pressure areas, the effectiveness of insect-proof net tunnels in reducing degeneration, and farmers' experiences with and actions against degeneration.

2 Sweetpotato in Tanzania

Sweetpotato (*Ipomoea batatas*) is the third most important root and tuber crop in Tanzania after cassava (*Manihot esculenta*) and potato (*Solanum tuberosum*) (Adam, 2014). Sweetpotato is most important in the Lake and Eastern Zones, moderately important in Southern Highlands and Northern Zones, and less important in the Southern and Central Zones (Kapinga et al., 1995). The Lake Zone, comprising of the six regions around Lake Victoria, where a third of Tanzania's population lives, leads in production (Lembris & Walsh, 2012). Sweetpotato is a food security crop and is mainly grown by smallholder

farmers, mostly women (Ngailo et al., 2016). There are some areas where commercial production is picking up with farmers targeting fresh root markets in major cities such as Dar es Salaam. The dominant varieties are white-, cream- and yellow-fleshed. However, there is an increasing appreciation and adoption of orange-fleshed varieties because of their high beta-carotene content. These are often promoted by NGOs, the government and research organizations with agriculture for nutrition programs aimed at reducing vitamin A deficiency.

Despite sweetpotato's importance to Tanzania, productivity is very low ranging between 5 and 7.5 t/ha according to FAO statistics (Table 1) (FAOSTAT, 2020).

Table 1. Trend in sweetpotato production in Tanzania, 2016 – 2020.

Year	Production (tonnes)	Area (ha)	Yield (t/ha)
2016	3,984,652	684,812	5.8
2017	5,440,824	733,726	7.4
2018	3,744,093	530,526	7.1
2019	3,921,590	539,513	7.3
2020	4,435,063	611,788	7.2

Source: FAOSTAT, 2022.

Even lower yields than indicated in Table 1 have been reported. Sindi and Wambugu (2012) reported yields of 3.5 t/ha against a potential of above 30 t/ha obtained under experimental conditions. Several factors contribute to this yield gap, including poor agronomic practices, low-yielding varieties, erratic weather patterns with unexpected dry spells, and poor soil fertility. However, the greatest part of yield gap is attributed to virus infections (Tairo et al., 2004). Gibson and Kreuze (2015) reported that virus infection can cause yield losses either through current season infection or infection carried in recycled planting material. The latter quality decline of repeatedly recycled starting material is known as degeneration or cultivar decline (Clark et al., 2002; Ogero et al., 2019). Unfortunately, there is scanty information on how sweetpotato degenerates in Tanzania. It was therefore important to study the process by combining both experimental research and a survey to capture farmers' experiences.

3 Degeneration in sweetpotato

Seed[1] degeneration in sweetpotato is mainly caused by viruses. The most common virus present in the Lake Zone, Tanzania, is sweet potato feathery mottle virus (SPFMV), which

1 In this context, the term 'Seed' refers to quality 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 which is used for breeding.

is mainly asymptomatic (Clark et al., 2012; Tairo et al., 2004). SPFMV belongs to the genus *Potyvirus* and is transmitted non-persistently by aphids (Karyeija et al., 1998). In non-persistent transmission, virus particles are attached to the vector's mouthparts as it probes on virus-infected plants (Dennien & Henderson, 2018; Dietzgen et al., 2016). The vector then transfers the virus particles onto the next plant it probes upon. Non-persistent transmission is very quick, and the vector requires just a few minutes to transmit the virus from one plant to another but does not remain infectious for a long time. In contrast, persistent transmission is slow as it requires the insect vector to feed for prolonged periods (up to 24 hours) on virus-infected plants to acquire the virus. Virus particles are taken into the gut and transferred to the salivary glands from which they are released as the vector feeds on healthy plants, also requiring equally prolonged feeding times. The vector remains viruliferous (able to transmit the virus) for the rest of its life. SPFMV can combine with the symptomatic sweet potato chlorotic stunt virus (SPCSV; genus: *Crinivirus*), which is transmitted persistently by whiteflies (Schaefers & Terry, 1976) to cause sweet potato virus disease (SPVD) (Mukasa et al., 2006). In single infections, SPFMV can cause negligible to 40% loss of root yield (Adikini et al., 2016; Milgram et al., 1996), whereas SPCSV by itself can lead to more than 52% yield reduction (Loebenstein, 2012). However, in combination, the two viruses have a synergistic effect and cause SPVD which is the main 'virus disease' affecting the crop and often leads to 56 – 100% yield losses (Ndunguru & Kapinga, 2007; Ngeve & Bouwkamp, 1991).

Sweetpotato chlorotic stunt virus is the main agent for the development of SPVD. The virus breaks the host's defense mechanism leading to significant increase in SPFMV titers. Sweetpotato has an antivirus defense mechanism based on RNA silencing whereby the host proteins identify the RNA of the virus and cut it into small molecules of 21 – 24 nt (Baulcombe, 2004; Guo et al., 2016). These molecules then trigger a sequence-specific host response that degrades the RNA of the invading virus (Ding & Voinnet, 2007). Sweetpotato chlorotic stunt virus has been reported to encode two proteins, p22 and p26, which are known to suppress the RNA silencing mechanism of the sweetpotato plant (Kreuze et al., 2005). However, it was later discovered that p22 is only found in a few SPCSV isolates, and it was subsequently shown that p26 was the one responsible for breaking antiviral resistance (Cuellar et al., 2009; Cuellar et al., 2008). The p26 protein expresses an RNase III-type of protein (RNase3), which has been found in all SPCSV isolates characterized (Kreuze et al., 2021). RNase3 is a dsRNA specific endonuclease that digests key small interfering (si)RNA molecules involved in viral defense.

Sweetpotato infecting members of the genus *Begomovirus,* known as sweepoviruses, have also been shown to cause significant yield losses. They are called sweepoviruses because they are phylogenetically distinct from the new and old world begomoviruses. They have been recorded in Argentina, Brazil, Italy, Japan, Kenya, Korea, Peru, Taiwan, and the United

States of America. Sweepoviruses have been reported to cause between 10 and 80% yield loss depending on cultivar susceptibility (Clark & Hoy, 2006; Kim et al., 2015; Ling et al., 2010). In Kenya, Wanjala (2020) reported 47% yield losses in a variety considered virus-tolerant following infection with sweet potato leaf curl virus (SPLCV; genus *Begomovirus*). This happened even without clear symptom appearance on the infected plants as is characteristic of most begomoviruses. Other viruses that affect sweetpotato in the Lake Zone, Tanzania, include sweet potato mild mottle virus (SPMMV; genus *Ipomovirus*), sweet potato chlorotic fleck virus (SPCFV; genus *Carlavirus*) and a number of potyviruses related to SPFMV (Ndunguru et al., 2009).

Being vegetatively propagated, sweetpotato accumulates viruses over generations. The use of virus infected material obtained from the previous crop is the most common source of sweetpotato viruses (Valverde et al., 2007). This leads to accumulation of viruses in the propagation material leading to a decline in cultivar performance (Bryan et al., 2003b; Clark et al., 2002). A susceptible variety such as Beauregard has been reported to be re-infected 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). In New Zealand, Lewthwaite (2011) reported a significant yield decline in cvs Beauregard and Okinawa Red with an increasing number of field multiplications. Virus-related degeneration has also been reported in Australia (Okpul et al., 2011) and China (Feng et al., 2000). In Tanzania, the only report on degeneration was based on random sampling and testing of leaves from seed producers' plots (Luambano & Gibson, 2015). There has been no systematic trial to investigate performance of seed recycled over several generations.

4 Influence of agroecology on degeneration

Virus degeneration may vary with location depending on cropping systems and weather. Agroecological conditions such as temperature, rainfall and relative humidity may affect disease development. Sseremba et al. (2017) demonstrated that SPVD develops best under a temperature range of 20 – 29°C. The authors assessed symptom expression in plants grown in a glass house with temperature variation of 30 – 39°C and open field with temperature of 20 – 29°C. Lower severity was recorded in the glass house than in the open field. Virus infection may also be influenced by rainfall patterns. For instance, there are areas with bimodal rainfall patterns and others with unimodal rainfall patterns (Namanda et al., 2011). Farmers in areas with bimodal rainfall patterns grow sweetpotato throughout the year making carry-over of infection from older to younger crops possible. This leads to build-up of virus inoculum and increase in vector populations therefore making these areas high-virus-pressure zones. Farmers with a unimodal system experience a long dry period between two sweetpotato seasons. Most plants including sweetpotato dry out during this

time. Consequently, virus inoculum and vector populations may reduce making such areas low-virus-pressure zones. The extent to which clean planting material degenerates under different agroecologies is not well-studied at the Lake Zone, Tanzania.

5 Farmer practices and degeneration

Various farmer practices influence the rate of virus spread and degeneration in sweetpotato. Practices such as rogueing out diseased plants and positive selection of seed may reduce the rate of virus degeneration or even increase the percentage of healthy plants. On the other hand, recycling of infected planting material leads to virus accumulation and rapid degeneration. Sweetpotato seed recycling is common in East Africa owing to the vegetative nature of the crop (Gibson et al., 2009). Farmer's actions towards addressing degeneration depend on farmer's experience with pests and diseases and loss of productivity. Adam et al. (2015) reported that farmer's knowledge on sweetpotato pests and diseases influenced application of control measures. Farmer practices have also been shown to influence seed degeneration in potato (Navarrete et al., 2022). Some practices, e.g., a rotation of more than 2.5 years, reduced degeneration while others accelerated it, e.g., receiving potato seed as a gift. Preferring seed produced at a different altitude was shown to play a double role i.e., reducing or accelerating degeneration, depending on the cause of degeneration.

6 Reversion of virus-infected plants

Reversion, which is the ability of virus-infected plants to become mostly virus-free, has been shown to prevent the build-up of virus titers in the crop (Gibson & Kreuze, 2015; Gibson & Otim-nape, 1997). Many varieties, especially those that have some level of tolerance to SPVD, have been shown to recover from virus symptoms and produce virus-free branches (Kreuze et al., 2021). Reversion has also been demonstrated in potato where experiments conducted in the Andes showed that not all daughter tubers produced by plants coming from infected mother tubers become infected (Bertschinger et al., 2017). Host recovery is characterized by a scenario whereby plants with severe symptoms produce branches and leaves that have reduced symptoms or are symptomless (Nie & Molen, 2015). It has been shown that RNA silencing, described earlier, contributes to this (Chellappan et al., 2004; Rodríguez-Negrete et al., 2009). Reversion is more pronounced under high temperatures, which provides more evidence that RNA silencing is involved (Ssamula et al., 2019). The anti-virus defense mechanism that is based on RNA silencing is more effective under high temperatures (Szittya et al., 2003). Fertile soil can also increase the rate of reversion. In Uganda, Ssamula et al. (2019) showed that adding NPK fertilizer to the soil increased the rate of reversion in sweetpotato. In the USA, Villordon and Clark (2014) reported 51%

reduction in adventitious root number in plants infected with a combination of SPFMV, SPVC, SPVG and SPV2, and grown without nitrogen as compared to similar plants grown with nitrogen. Nutrients influence plant pathogens both directly and indirectly by controlling the pathogen's rate of germination, penetration, colonization, and reproduction (Barker, 2009).

7 Managing sweetpotato viruses

Three major alternatives exist in managing viruses in sweetpotato: (1) deploying resistant cultivars, (2) using clean virus-tested planting material, and (3) employing good on-farm management practices (Thomas-Sharma et al., 2016). Whereas landraces and cultivars with higher levels of resistance to SPVD do exist, there is no immunity to the disease, and depending on the pressure of the different viruses in the environment, all genotypes can become infected in the field (Gibson & Kreuze, 2015). Resistance can be dissected into different components, including reduced symptom severity, tolerance (manifested by reasonable yield despite being infected and showing symptoms), recovery (ability to recover partially or completely from virus symptoms) and reversion (ability to recover from virus infection). Complementary to the use of genetic resistance could be the production and use of cleaned-up, virus-tested planting material. Virus cleaning is achieved through a combination of thermotherapy and meristem tip culture (Mashilo et al., 2013). This process can eliminate most of the known viruses. The virus-cleaned plants are tested via polymerase chain reaction or other diagnostic methods to confirm that they are virus-free. Although use of clean seed may work well in countries where sweetpotato is grown as a cash crop and large-scale farmers can make the investments necessary to obtain such planting material, it is economically unfeasible for smallholder farmers producing mostly for subsistence. On-farm management strategies such as rogueing, proper isolation and positive selection for clean seed are therefore important (Thomas-Sharma et al., 2016). Additional options such as low-cost insect-proof net tunnels that can enable seed producers to maintain a high sanitary status of planting material are important. The low-cost insect-proof net tunnel can be constructed from locally sourced materials (Ogero et al., 2017). This technology protects planting material against the virus vectors, i.e., whiteflies and aphids (Loebenstein, 2015). Virus-free 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. However, the effectiveness of the net tunnels in reducing virus infection under seed producer management has not been conclusively investigated. Keeping the plants in a healthy status enables several harvests, i.e., the plants can be let to sprout again after harvesting. In sweetpotato, the first harvest of vines can be done 90 days after planting and thereafter harvesting can be done after every 40 – 60 days. This is called ratooning and it consists of harvesting the crop, with the next crop being the result of the first crop's regrowth (Riga, 2008). However, this technique can affect the quality

and quantity of planting material produced because the root stocks age with time. There is need to investigate how ratooning affects vine production in net tunnels.

8 Seed system design and decentralized vine multiplication

A seed system can be defined as the network of stakeholders involved in providing, managing, replacing, and distributing the seed of a particular crop in a certain area (Bentley et al., 2018). These components are regulated by the government seed regulatory authorities in a formal seed system while in the informal seed system they are managed by farmers. The predominant sweetpotato seed system at the Lake Zone, Tanzania, involves farmer-to-farmer exchange of planting material (Adam et al., 2018). This is an informal system that involves recycling of vines and hence is often seen as the main cause for virus perpetuation. The informal system involves conservation of planting material during the dry season, often near water sources e.g., along riverbeds, near the lake, in swampy areas (Namanda et al., 2011). This is usually in lowlands therefore referred to as 'lowland crop'. Lowland vine conservation begins in June when the dry season is setting in and farmers are completing harvesting (Fig. 1). Expansion of the lowland fields is done in September going into October as farmers anticipate the short rains. The lowland crop is transferred to upland farms during the short rain season in October – December. This is the first upland crop. A short dry period occurs in January – February. Harvesting of the upland crop planted during the short rains in October – December starts in February, in time to provide planting material for the next season upland crop. This is the main season and occurs during the long rains in March – May.

Dissemination of quality seed is hindered by the fact that most farmers are not used to buying sweetpotato seed. Those who buy may do so once and revert to recycling. Others opt to borrow from friends, neighbors or relatives who may have already bought or are producing. This exchange may involve some monetary transactions or purely depend on social relations between the buyer and the seed producer. The motivations of farmers underpinning their decisions in exchange of planting material are not well studied. Better understanding of these motivations and decisions can provide entry points for improving availability and access to quality clean planting material. The intricacies of seed acquisition and movement can also influence the epidemiology of sweetpotato viruses depending on factors such as quality and volumes of seed moved, distance, and knowledge exchange between buyers and sellers among others. Understanding the effect of human activities and relationships on seed acquisition and consequently epidemiology of sweetpotato viruses will contribute towards better management of sweetpotato viruses and related decline in cultivar performance.

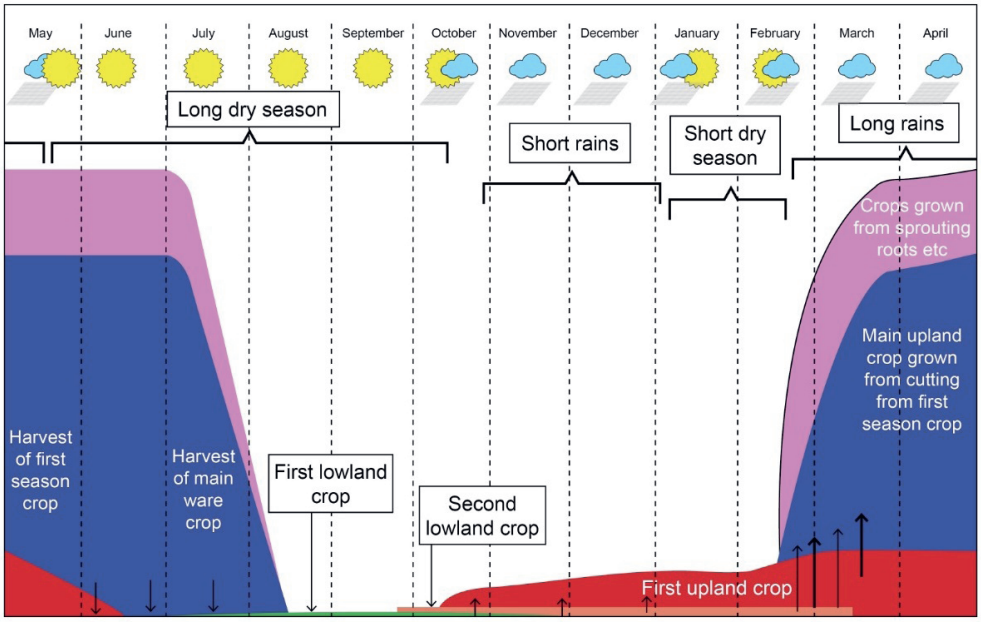

Figure 1. Sweetpotato production cycle in the Lake Zone of Tanzania (Lukonge et al., 2015).

Another seed system is more formal and involves deployment of virus-tested cuttings produced from tissue culture plantlets. This is usually done by agricultural research institutes, local governments and NGOs. Use of virus-tested foundation seed has been reported to boost production in the USA and China (Clark et al., 2010; Fuglie et al., 1999). Recent seed system interventions at the Lake Zone, Tanzania, have also taken this approach. The system is decentralized with research stations producing pre-basic seed which is then supplied to seed producers for production of certified and quality declared seed which is disseminated to root producers (Fig. 2).

9 Problem definition and research questions

As described earlier, seed degeneration caused by viruses is a major constraint to sweetpotato production in Tanzania. Use of clean virus-tested vines disseminated through a formal system is among the proposed strategies to address this constraint. However, clean seed can get infected once exposed in the field leading to cultivar decline (degeneration) over generations, therefore requiring replenishment. In addition, uptake of clean seed from formal seed system is very low given the subsistence nature with which sweetpotato is cultivated in Tanzania. Farmers are used to recycling planting material from their previous crops or obtaining it from neighbors most often without monetary exchange.

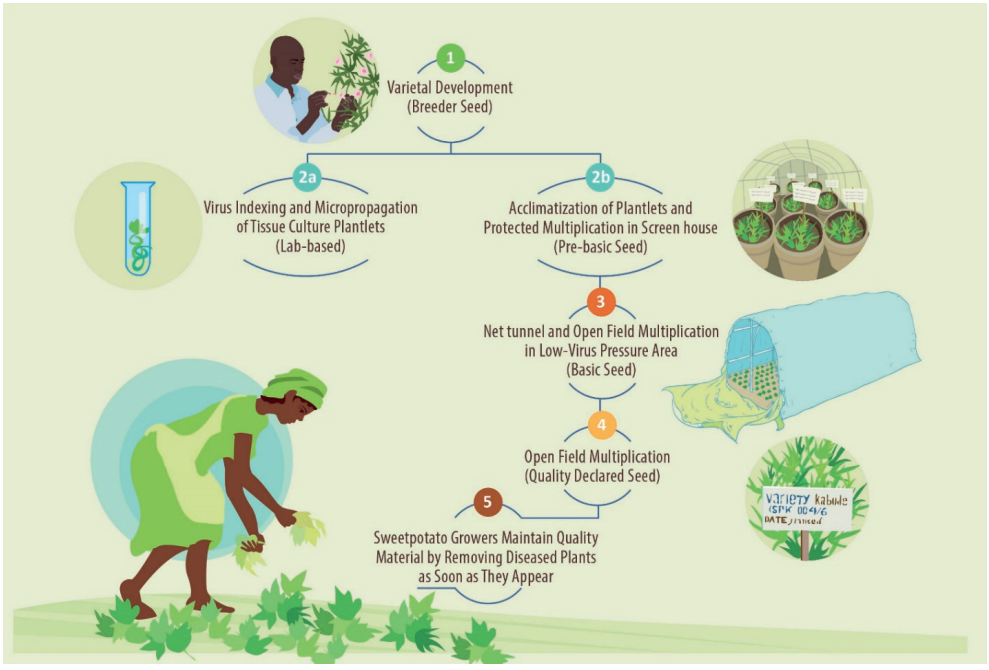

Figure 2. Schematic diagram of the formal seed system for sweetpotato (McEwan, 2015).

On the other hand, sustainable production of clean seed requires that farmers purchase planting material so that seed producers can generate revenue for further production. There is a need to understand more about interactions between viruses and the crop, the environment and farmers' management. This will enable researchers and farmers to know the pattern of degeneration of planting material in different localities, which control measures to use and when to purchase new planting material. Understanding variations in yields of clean planting material after several generations of field propagation defines how seed producers can sustain the supply of quality planting material. Several technologies have been developed to aid on-farm management of sweetpotato viruses during seed production. These include use of insect-proof net tunnels. However, their effectiveness and optimal management for effective protection against viruses need to be investigated. Successful adoption of clean seed also requires an understanding of social aspects of seed acquisition including dominant sources, prices, farmers' knowledge on diseases and control measures, among others. The overall research question addressed in this thesis is how can cleaned-up, virus-tested seed be incorporated into smallholder farming systems to address degeneration in sweetpotato? This overall question led to the following specific questions:

a) How does seed initially sourced from cleaned-up, virus-tested plants perform in different agro-ecologies following several seasons of on-farm propagation?

b) Can insect-proof net tunnels limit virus re-infection of cleaned-up, virus-tested sweetpotato planting material?

c) How does ratooning affect vine production in insect-proof net tunnels?

d) What are farmers' experiences with and actions towards degeneration including seed sourcing strategies?

10 Research setting

This research was conducted in the Geita, Mara and Mwanza regions in the Lake Zone of Tanzania (Fig. 3). The Lake Zone comprises of six administrative regions bordering the Lake Victoria (also called Lake Nyanza) basin. The Lake Zone leads in sweetpotato production in Tanzania. Geita, Mara and Mwanza regions have a combined population of about 8.2 million people. Key economic activities include agriculture, fishing and gold mining. Mwanza City, the Capital of Mwanza region, is the second largest city in Tanzania and serves as the main urban market within the Lake Zone and is a key destination for sweetpotato roots. The three regions lead in sweetpotato production at the Lake Zone. Mara and Mwanza have both high- and low-virus-pressure areas whereas most of Geita has a high virus pressure.

11 Thesis outline and summary of the research methods

This thesis comprises of six chapters. **Chapter 1** provides a general introduction to the research problem and lays the context for research. It is based on a literature review of sweetpotato farming in Tanzania, effects of viruses on productivity and their role in degeneration. It also highlights strategies for managing sweetpotato viruses, including use of clean seed programs. **Chapter 2** provides insights from a multi-year study that assessed the rate of degeneration in two agroecologies. It is based on experiments conducted in two locations with varying intensity of sweetpotato cultivation based on amount of rainfall received. The experiments compared virus incidences and storage root yields between clean virus-tested seed and farmer-sourced seed of two varieties recycled over five seasons. The chapter concludes that use of clean, virus-tested seed has a clear advantage on root yields in high-virus-pressure areas and for highly susceptible varieties. **Chapter 3** examines the efficiency of net-protected structures in reducing

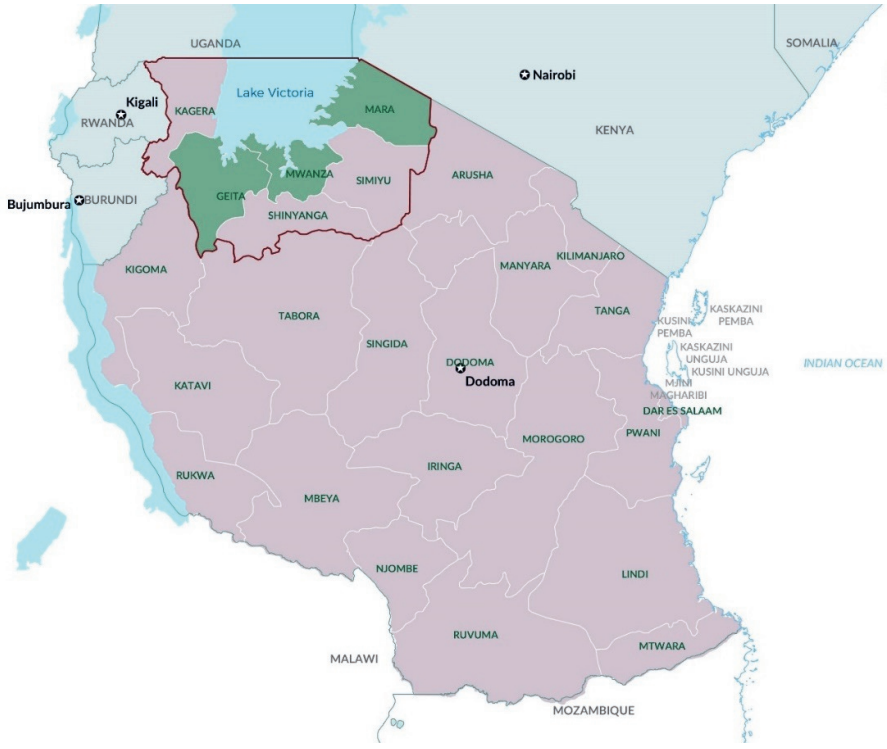

Figure 3. Administrative map of the United Republic of Tanzania with the Lake Zone in a red boundary. Geita, Mara and Mwanza regions where the study was conducted are highlighted in dark green.

seed degeneration in sweetpotato. It is based on findings from a 21-month experiment (June 2014 to March 2016) that assessed virus infection on vines grown in net tunnels and open fields. In addition, a SeedHealth model (see https://tools4seedsystems.org/) was used to model percentages of yield loss over ten seasons in scenario analyses for a high-virus-pressure site. The chapter concludes that insect-proof net tunnels can prevent re-infection in high-virus-pressure area for up to 20 months. Lastly, the chapter shows that plants sourced from net tunnels would still attain more than 60% of the expected yields after 10 generations while those sourced from open fields would not do so after only 4 generations. **Chapter 4** investigates the effect of ratooning on vine production in insect-proof net tunnels. It highlights findings from a 14-month experiment (September 2019 to November 2020) comparing a ratoon cropping technique and a replanting technique. The chapter shows that ratooning increases vine production. However, the technique leads to higher virus incidences on plants grown in the open. **Chapter 5** comprises of a 'small N' survey that investigated farmers' experiences and actions towards degeneration. It also highlights farmers' seed sourcing patterns. It was qualitative in nature and based on

in-depth interviews. The chapter concludes that more women experienced sweetpotato seed degeneration than men. It also shows that the main strategy that farmers use to manage degeneration is to acquire new varieties. **Chapter 6** provides a synthesis of the key findings putting them in a broader context. In putting degeneration in a broader perspective, the chapter also draws some comparisons with seed degeneration in other RTB crops. The chapter notes the importance of getting more information on farmers' experiences with degeneration. Such data can be combined with data from biophysical experiments to come up with better management strategies.

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Healthy (L) and SPVD-infected (R) plants. Bunda district, Tanzania. Credit: K. Ogero.

Chapter 2:

Degeneration of Cleaned-up, Virus-tested Sweetpotato Seed Vines

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Abstract

Viruses pose a major challenge to sweetpotato production in Tanzania. Use of cleaned-up, virus-tested seed vines distributed through a formal seed system is among the proposed strategies to address this challenge. However, virus-tested seed vines can get infected once in the field and it is not known how they will perform following several seasons of on farm propagation. We assessed the performance of virus-tested seed vines and farmer-sourced seed vines of a susceptible variety, Ejumula, and a relatively tolerant variety, Kabode, over five seasons to understand the trend in root yields, vine yields and virus incidences. The experiments were done in high and low virus pressure areas. The most prevalent viruses were sweet potato chlorotic stunt virus (SPCSV) followed by sweet potato feathery mottle virus (SPFMV) and sweet potato leaf curl virus (SPLCV), respectively. Both farmer-sourced and cleaned-up, virus-tested seed of cv. Ejumula were rapidly infected with SPCSV. The incidence of this virus on Ejumula's farmer-sourced material at the high-virus-pressure area reached 100% by the second season. The incidences for all three viruses remained stable for cv. Kabode across the five seasons. Plants generated from cleaned-up, virus-tested seed had lower incidences for all viruses compared to those from farmer-sourced planting material. Virus-tested seed produced significantly higher root yields for cv. Ejumula in the high-virus-pressure site, with a gradual drop across the seasons. The root yields for cv. Kabode for virus-tested and farmer-sourced seed were not significantly different. The findings show that regular replenishment of clean, virus-tested seed is more economical in high-virus-pressure areas and for more susceptible varieties like cv. Ejumula. They also indicate that farmers may be reluctant to invest in cleaned-up, virus-tested seed in cases where they have virus-tolerant varieties such as cv. Kabode due to lack of obvious virus effect on yields.

Key words: Cultivar decline, seed system, sweet potato virus disease

1 Introduction

Sweetpotato (*Ipomoea batatas*) productivity in Tanzania is currently very low, averaging 3 – 6 t/ha, against a potential of over 16 t/ha depending on the cultivar (Sebastiani et al., 2007). Factors contributing to this low yield include limited use of quality seed, poor agronomic practices, low soil fertility, drought and high virus incidences (Ngailo et al., 2016). However, greatest losses in yield have been associated with virus infections (Adam et al., 2015; Ndunguru et al., 2009). Viruses are systemic, hence multiply easily from season to season through vegetative propagation of infected planting material which leads to virus build up over time (across seasons), progressively decreasing yields (Clark et al., 2002). The quality loss caused by this so-called recycling of infected planting material is known as degeneration (Gibson & Kreuze, 2015) or cultivar decline (Bryan et al., 2003; Clark et al., 2002). The viruses contributing to this to a large extent belong to the families of *Closteroviridae, Geminiviridae* and *Potyviridae* (Nakazawa, 2001).

In Tanzania, and East Africa as a whole, sweet potato chlorotic stunt virus (SPCSV; genus *Crinivirus,* family *Closteroviridae*) and sweet potato feathery mottle virus (SPFMV; genus *Potyvirus,* family *Potyviridae*) are the main viruses causing degeneration (Adikini et al., 2016; Mukasa et al., 2003; Tairo et al., 2004). In single infections, SPFMV has been reported to cause up to 40% yield losses while SPCSV has been reported to cause up to 52% yield losses (Adikini et al., 2016). However, co-infection leads to a devastating disease complex known as sweet potato virus disease (SPVD) that can cause more than 90% yield losses (Adikini et al., 2016; Clark et al., 2012; Karyeija et al., 2000). SPCSV makes sweetpotato more susceptible to SPFMV through suppression of its natural virus-defense mechanism. Often plants initiate defense mechanisms when attacked by various pathogens including viruses. One mechanism through which sweetpotato responds to virus infection is based on RNA silencing (Baulcombe, 2004; Guo et al., 2016). Here, host proteins identify the viral RNA and cut it into small molecules of 21–24 nt which then trigger a sequence-specific host response that degrades the RNA of the invader virus (Ding & Voinnet, 2007). Two RNA silencing suppression (RSS) proteins (p22 and p26) have been discovered in SPCSV (Kreuze et al., 2005). The RSS p22 has only been found in SPCSV isolates from Uganda while synergistic effects have been shown even in those isolates not encoding it (Cuellar et al., 2008). This implicates p26 as the protein responsible for breaking antiviral resistance (Cuellar et al., 2009). The p26 protein expresses an RNase III-type of protein (RNase3), which has been found in all SPCSV isolates characterized (Kreuze et al., 2021). This RSS protein suppresses the RNA silencing mechanism in sweetpotato therefore making it more vulnerable to SPFMV and other viruses (Kreuze et al., 2005). SPFMV titers have been shown to increase several 100-folds when SPCSV is present compared to when infecting alone (Cuellar et al., 2008; Mukasa et al., 2006).

Co-infection with SPFMV does not change SPCSV titers. Sweepoviruses belonging to the family *Geminiviridae* have also been reported to cause up to 80% yield losses even without obvious foliar symptoms (Clark & Hoy, 2006). The effect of sweepoviruses on yield loss is now being appreciated and a recent study by Wanjala et al. (2020) reported an average of 47% yield loss from sweet potato leaf curl virus (SPLCV; genus *Begomovirus,* family *Geminiviridae*) on a moderately resistant Kenyan variety, cv. Kakamega.

The extent of virus-related yield losses varies with cultivar and agroecology. Degeneration is likely to be rapid in high-virus-pressure agroecologies. These include locations where sweetpotato is grown year-round, for instance areas with a bimodal rainfall pattern. This means that there is always a crop in the field and therefore there is always a source of virus inoculum. The year-round cultivation also means that vectors are always present. On the other hand, low-virus-pressure areas experience several months of no rain leading to drying up of most sweetpotato crops and alternate virus hosts e.g., other *Ipomoea* spp. This reduces the amount of virus inoculum and vector populations. There is no variety that is one hundred per cent resistant, but there are some varieties that can produce economically acceptable yields even when infected (Mwanga et al., 2013). Some East African landraces have been shown to perform well in high-virus-pressure areas owing to decades of selection by farmers (Bua et al., 2006; Gibson et al., 2000). This means that with time farmers retained more tolerant varieties through selection of healthy-looking planting material. In addition, some varieties have been reported to show signs of reversion whereby the host recovers from disease symptoms. Reversion from virus infection is the ability of plants that are virus-infected to become mostly virus-free (Gibson & Otim-nape, 1997). On the other hand, high-yielding but susceptible improved varieties can perform well if grown in low-virus-pressure areas with good on-farm management practices.

Degeneration in sweetpotato can be managed through use of clean virus-tested seed vines, breeding for resistance and on-farm management of infections (Thomas-Sharma et al., 2016). Use of clean virus-tested seed vines is now widely promoted in sub-Saharan Africa and quality is a value-proposition for formal sweetpotato seed systems (McEwan, 2016). Virus cleaning is achieved through a combination of thermotherapy and meristem tip culture (Mashilo et al., 2013). This process can eliminate most of the known viruses depending on how it is done. After virus elimination, the plants are tested via techniques such as polymerase chain reaction to confirm that they are virus-free, at least of the known viruses. Planting clean virus-tested seed vines has been shown to mitigate losses caused by viruses (Clark et al., 2010; Dennien, 2015; Gibson et al., 2004).

However, clean virus-tested seed is prone to infection once grown in the open where the seed crop is exposed to virus vectors, i.e., whiteflies and aphids. The rate of infection depends on the genotype and environment.

Virus-susceptible varieties can get infected rapidly, especially when grown in high-virus-pressure areas. The risk of infection makes it challenging to promote the use of clean virus-tested seed in sub-Saharan Africa, especially if it leads to a decline in yield. The first-generation seed, which is always virus-free, is expensive due to the cost of clean-up, virus indexing and maintenance and is therefore not recommended for direct planting by farmers to produce storage roots. Therefore, this material must undergo further multiplication on-farm to make it more affordable. Seed systems interventions have taken this into consideration by training seed producers how to minimize infection at farm-level (Adam et al., 2018). Farmers are also advised to avoid too much recycling and buy quality seed from trained seed producers who are multiplying planting material sourced from cleaned-up seed stocks. However, this is challenging because it takes time to change people's mindsets from practices they are accustomed to. Recycling of clean virus-tested seed will eventually lead to yield losses in subsequent plantings (Lewthwaite et al., 2011). Cognizant of the effect of seed recycling, it is important to understand the performance of cleaned-up, virus-tested seed recycled over several seasons in various agroecologies. This will lead to recommendations on its optimal use in addressing degeneration in sweetpotato. This research sought to assess performance of clean virus-tested seed and farmer-sourced seed, recycling it over five seasons, in terms of storage root yield, vine yields and accumulation of SPFMV, SPCSV and SPLCV.

2 Materials and methods

a) Location

The experiments were conducted in Nyamang'uta village, Bunda district and Nungwe village, Geita district at the Lake Zone Tanzania (Fig. 1). Bunda district is in Mara region whereas Geita district is in Geita region. The site at Nyamang'uta village is located at 1° 58' 32" S 33° 59' 03" E and at an altitude of 1254 m asl while the site in Nungwe village is located at 2° 46' 46" S 32° 00' 50" E and at an altitude of 1139 m asl. The two sites are about 310 km apart.

Both Bunda and Geita have two rainfall peaks in a year, November-December, and March-April. However, the amount of rainfall received varies with Geita receiving more rainfall in both seasons (Fig. 2, left). Bunda receives moderate rains in November-December reducing in January and peaking in March-April. This difference gives farmers in Geita two distinct sweetpotato cropping seasons whereas those in Bunda mostly rely on the March-April rains and therefore on one season of cultivation. This leads to year-round cultivation of sweetpotato in Geita while Bunda has a break during which there is no sweetpotato crop in the field. The continuous cultivation in Geita means high virus inoculum in the

Figure 1. A map indicating Nungwe and Nyamang'uta villages where the experiments were conducted.

environment compared to Bunda where plants desiccate, and the infection cycle is broken during the dry period.

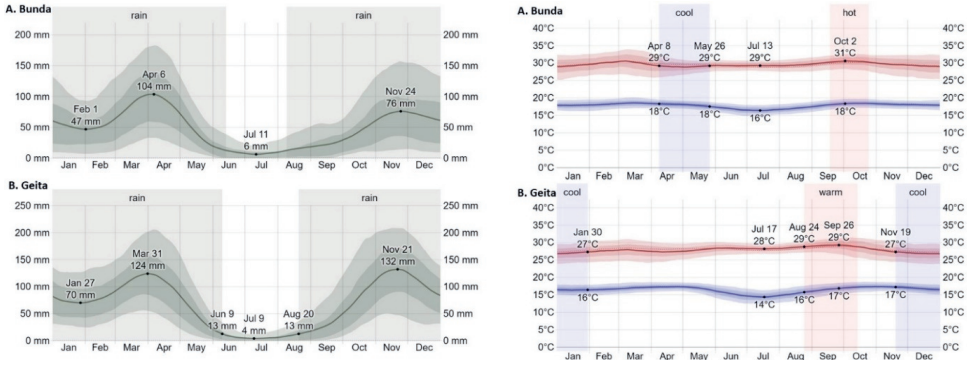

Figure 2. Yearly average rainfall (left) and temperature (right) in Bunda and Geita. © WeatherSpark.com.

There are no major differences in temperature, but Bunda is relatively warmer (by an average of 1°C) compared to Geita.

b) Soil analysis

Six soil samples were collected from each site and analysed for texture and nutrient contents based on standard procedures as stipulated in the Tanzania National Soil Service manual (National Soil Service (NSS), 1990). Eight soil parameters were tested: particle size, pH, total nitrogen, organic carbon, electric conductivity, available phosphorus, exchangeable bases and cation exchange capacity (CEC). The Geita site was dominated by sand particles and grouped as sandy loam (SL) to sand clay loam (SCL) textural class whereas clay (C) soil dominated in Bunda. This indicates that the two sites differed in their physical and mineralogical properties. The soil pH range in Geita site was 5.92 - 6.98 indicating moderately acidic to almost neutral pH. These extreme ends indicate less possibility of aluminium (Al) or manganese (Mn) toxicity. At the Bunda site the soil pH was between 5.73 and 7.08 which also ranges from moderately acidic to neutral. Organic carbon (OC), which is a measure of organic matter in the soil was low to high in Geita, ranging between 0.77% and 2.11%. The organic carbon in Bunda was between 1.09% and 1.93% which is moderate to high. Total nitrogen (N) was at low to medium levels in both sites ranging from 0.08% to 0.18% in Geita and 0.08% to 0.16% in Bunda. The levels of available phosphorus (P) were moderate to very high in both sites ranging from 12 mg/kg to 29 mg/kg in Geita and from 14 mg/kg to 86 mg/kg in Bunda. Exchangeable potassium (K) in both sites was below 0.2 cmol(+)/kg which is very low. Exchangeable calcium (Ca) was between very low (0.89 cmol(+)/kg) to high (19.36 cmol(+)/kg) in Geita and between very low (0.37 cmol(+)/kg) and low (4.68 cmol(+)/kg) in Bunda. In both sites, exchangeable magnesium (Mg) ranged between very low (below 0.3 cmol(+))/kg and low (0.3 – 1.0 cmol(+)/kg. The cation exchange capacity (CEC) in Geita ranged between 11.2 cmol(+)/kg and 24.4 cmol(+)/kg which is low to moderate. Bunda also had a low to moderate CEC ranging from 11.1 cmol(+)/kg to 16 cmol(+)/kg. The carbon/nitrogen ratio was normal at both sites i.e., Geita (11.61) and Bunda (11.71). A carbon/nitrogen ratio of 10 – 12 is normal for arable soils.

c) Sourcing of planting material

Cleaned-up, virus-tested planting material sourced from a pre-basic seed vine production screen house at the Tanzania Agricultural Research Institute (TARI) - Ukiriguru and healthy-looking material obtained from farmers' fields were used in this experiment. The pre-basic seed vines multiplied at TARI had been obtained four months earlier from the Kenya Plant Health Inspectorate Service (KEPHIS) Plant Quarantine Station at Muguga, Kenya following virus cleaning via thermotherapy and meristem tip culture, and virus testing via polymerase chain reaction. The farmer-sourced material of both varieties had been under field propagation for four seasons (information from the farmer who provided the material). Two varieties were selected based on their level of resistance to sweet potato virus disease. Cv. Ejumula is highly susceptible to SPVD and does well in low virus pressure zones whereas cv. Kabode is moderately resistant and adaptable to most areas.

d) Experimental set up and field management

A split-plot design with source of planting material in main plots and the two varieties in subplots was used to minimize the rapid spread of viruses from farmer-sourced to clean virus-tested plants. There were four replications leading to eight main plots and 16 subplots per site. Each subplot measured 3 m × 3 m and comprised of three ridges each measuring 3 m × 1 m. These were planted with thirty cuttings measuring 30 cm long at a spacing of 1 m (between ridges) × 0.3 m (between plants). The subplots were separated by two metres. Harvesting was done at 120 days after planting. The experiment was repeated for five seasons using planting material recycled from the previous crop (Fig. 3).

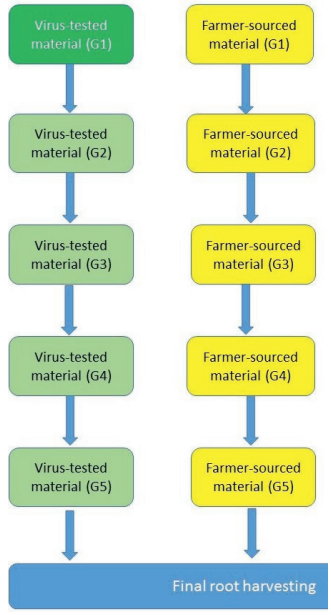

Figure 3. Schematic diagram indicating the five seasons and recycling of planting material at each of the two trial sites.

The experiments followed the local sweetpotato growing seasons meaning that they coincided with the rain seasons therefore not requiring irrigation. However, provisions for irrigation were made to mitigate against erratic rains. No manure or mineral fertilizers were used during the entire experiment. Weeding was done manually by scouting and removing any emerging weeds.

e) Field data collection and leaf sampling

Root harvesting was conducted 120 days after planting and the yield per subplot determined by weighing all the roots using a digital scale. The total vine yield per subplot was also determined by weighing all the vines.

Ten leaf samples per subplot were randomly collected in each season for virus testing via reverse transcriptase polymerase chain reaction (RT-PCR) for potyviruses, RT-qPCR for SPCSV and PCR for begomoviruses. Each sample comprised of three leaves cut from the top, middle and bottom of a plant. These were collected into coffee-filters and put in zip-lock bags containing silica gel to dehydrate them and prevent rotting.

f) Molecular diagnostics for common sweetpotato viruses

The following viruses were targeted in testing: the genus *Potyvirus* - sweet potato feathery mottle virus (SPFMV), sweet potato virus G - SPVG, sweet potato virus C - SPVC, sweet potato virus 2 – (SPV2); genus *Geminivirus* - begomoviruses (generic primers) and genus *Crinivirus* - sweet potato chlorotic stunt virus (SPCSV). Each diagnostic run included three levels of control: positive – for the respective virus; healthy sweetpotato plant RNA/DNA control and non-template control. The primers used for the various viruses are listed in Table 1.

Table 1. Primers used for the detection of common sweetpotato viruses.

Name	Direction	Sequence (5' – 3')	Assay	Target	Fragment size bp
SPG-F	Forward	GTATGAAGACTCTCTGACAAATTTTG	RT-PCR	SPVG	1,191
SPC-F	Sense	GTGAGAAAYCTATGCGCTCTGTT	RT-PCR	SPVC	836
SPF-F	Sense	GGATTAYGGTGTTGACGACACA	RT-PCR	SPFMV	589
SP2-F	Sense	CGTACATTGAAAAGAGAAACAGGATA	RT-PCR	SPV2	369
SPFCG2-R	Reverse	TCGGGACTGAARGAYACGAATTTAA	RT-PCR		
CL43 U	Sense	ATCGGCGTATGTTGGTGGTA	RT-PCR	SPCSV	486
CL43 L	Antisense	GCAGCAGAAGGCTCGTTTAT	RT-PCR	SPCSV	
SPG1*	Sense	CCCCKGTGCGWRAATCCAT	PCR	SPLCV	912
SPG2*	Antisense	ATCCVAAYWTYCAGGGAGCTAA	PCR	SPLCV	
SPCSV-Uni-E	Sense	CGGAGTTTATTCCCACYTGTYT	RT-qPCR	SPCSV	
SPCSV-Uni-E	Antisense	GGGCAGCCYCACCAA	RT-qPCR	SPCSV	
SPCSV-Uni-E-P	Probe	[FAM]-TCTGTCACGGCTACAGGCGACGTG-[TAMRA]	RT-qPCR	SPCSV	

*Primers set SPG1 and SPG2 (Li et al., 2004) can also detect other SPLCV-related sweetpotato begomoviruses (known as 'sweepoviruses') and the sizes of amplified products may be different from 912 bp. All primer sets apart from SPG1 and SPG2 were designed internally within the International Potato Center (CIP).

Total nucleic acid extraction

Isolation of total DNA and RNA was done as described by Doyle and Doyle (1990) with minor modifications. The CTAB extraction buffer comprised of 2% CTAB, 20mM EDTA, 100 mM Tris-HCl, pH8.0, 1.0% Na sulphite and 2.0% PVP-40. Two steel balls were placed in a well labelled 2.0-ml microcentrifuge tube and 0.02 grams of the sample placed inside. One ml of the CTAB buffer was added and the mixture centrifuged at 12000 rpm for 5 minutes at room temperature. Seven hundred (700) µl of the supernatant was transferred into a new set of microfuge tubes.

An equal volume of chloroform/isoamyl alcohol (24:1) was added and mixed thoroughly by shaking. This was then centrifuged at 12000 rpm for 5 minutes and the upper aqueous layer carefully transferred to a new set of microfuge tubes without interfering with the interphase. Ice-cold isopropanol (350 µl) was added, mixed well, and left at room temperature for 15 min. The mixture was then centrifuged at 12000 rpm for 10 minutes and the supernatant removed without disturbing the pellet. Five hundred (500) µl of 70% ethanol were added and the mixture centrifuged at 12000 rpm for 5 minutes. The supernatant was discarded and the pellet air dried for 10-15 mins. The pellet was then resuspended in 100 µl of nuclease-free water (NFW). The purity and concentration of the extracted RNA/DNA were checked using a NanoDrop ND-1000 Spectrophotometer (Thermo Fischer Scientific) followed by a quality check on 2% agarose gel. RNA/DNA concentrations were standardized to 100 ng/µl before use in detection assays.

PCR assays

Simultaneous detection of Potyviruses (SPFMV, SPVC, SPVG, SPV2)

A multiplex One-Step Reverse Transcriptase (RT) PCR was used to detect the potyviruses. The Invitrogen SuperScript™ III One-Step RT-PCR with Platinum Taq kit was used. The quadruplex RT-PCR primers for the potyviruses and their expected sizes are listed in Table 1. The optimal concentrations for the primer sets were: 2.5 µl (1.25 µM) for SPGF, 0.4 µl (0.2 µM) for SPCF, 2.0 µl SPFF (1.0 µM) and 0.2 µl (0.1 µM) for SP2F in a reaction volume of 20 µl. RT-PCR master mix as recommended by the manufacturer was used. Reaction conditions were: cDNA synthesis for 45 minutes at 52°C; initial denaturation at 95°C for 5 minutes and 40 cycles consisting of 30 s at 95°C, 1 min at 55°C, 1 min at 72°C, and final extension 5 min at 72°C. Runs were performed using a GeneAmp 9700 PCR (Applied Biosystems, Foster City, CA, USA). PCR products were analyzed by electrophoresis on a 1.5% agarose gel, 0.5 X TAE buffer; run at 100 V for 1.5 hours, stained with GelRed and visualized using a UV transilluminator. Test samples with a distinct product size corresponding to the respective potyvirus listed in Table 1 were presumed positive.

Geminivirus (SPLCV) testing

Sweet potato leaf curl virus was tested by PCR as described by Li et al., (2004) using sweepovirus-specific primers SPG1 and SPG2, designed to amplify a 912-bp fragment. A 25 µl reaction mix with 1X Dream Taq buffer, 2 µl DNA, 0.3 µM forward and reverse primers, 2.0 mM $MgCl_{2,}$ 0.3 mM dNTPs and 1.0 U Taq polymerase was prepared. The reaction was incubated in a thermocycler machine (GeneAmp 9700 PCR (Applied Biosystems, Foster City, CA, USA) using the following conditions: initial denaturation at 95°C for 5 min and 40 cycles consisting of 30 s at 95°C, 1 min at 55°C, 1 min at 72°C, and final extension 5 min at 72°C. PCR products were analyzed by electrophoresis on a 1.5% agarose gel, 0.5 X TAE; run at 100 V for 1.5 hours, stained with GelRed and visualized using a UV transilluminator.

Samples were scored as positive or negative for begomoviruses if the 912-bp PCR amplicon expected fragment size was produced.

SPCSV testing

One-step quantitative reverse transcription PCR (RT-qPCR) was used for the detection of sweet potato chlorotic stunt virus (SPCSV) – East African strain (EA). TaqMan One Step PCR Master Mix kit (Applied Biosystems) was used. A 25 µl reaction mix containing 2 µl of template RNA, 0.4 µM each of forward and reverse primer, 0.2 µM TaqMan probe, 12.5 µl of the 2× Master Mix (Applied Biosystems), MMLV (2U/ul) and 10.45 µl nuclease free water (NFW) was prepared. The following real-time PCR thermal cycling conditions were used: 42°C for 42 min (cDNA synthesis) and 95°C for 10 min (hot start activation), followed by 40 cycles of denaturation at 95°C for 30 s and annealing/extension at 55°C for 1 min. All samples were run in triplicate. Results for SPCSV-EA reactions were verified after confirming that all the controls were valid. Samples producing a FAM Ct in the range of ≤ 36 were considered positive for SPCSV-EA.

a) Data analysis

All data was analysed in the R program (*version 4.0.5*). The following variables were included in this analysis: root and vine yields, the percentage incidences of SPCSV, SPFMV, SPLCV, and co-infection with SPFMV and SPCSV, i.e., SPVD, simultaneously. We estimated descriptive statistics for each of these variables and disaggregated these estimates by sweet potato variety and sources of vines over a period of 5 planting seasons. The *psych* package was used to estimate descriptive statistics. Figures were created with the package *ggplot2*.

We used an analysis of variance to identify the influence of the varieties, sources of vines, and seasons on root and vine yields, and virus incidences under high and low virus conditions. Assumptions of the analysis of variance were evaluated using QQpplots, and residual plots. We used generalized linear models to estimate the influence of varieties, sources of vines, and seasons on the incidence of viruses and yields (Equation 1). Statistical significance was evaluated at the 0.05 level.

$$Root\ yield, vine\ yield, Virus\ incidence = Variety + Sources\ of\ vines + Season + E$$

Equation 1. *Generic equation to determine the incidence of the variety type, sources of vines and Seasons on the root and vine yield. Variety represents the two variety types used in the experiments. Sources of vines represent cleaned-up, virus-tested seed and farmer-sourced seed. Season is a continuous variable and represent the number of on-farm propagation cycles. E describes the error of the model.*

3 Results

a) Root yields

Cleaned-up, virus-tested seed vines of the susceptible cv. Ejumula planted in the high-virus-pressure site performed better than farmer-sourced material with significant differences in Seasons 3 to 5 (Fig. 4). Across the seasons, there was very minimal reduction in root yield, irrespective of variety, source of vines and agroecology. Season 3 was an outlier with highest root yield for the tolerant cv. Kabode in both sites and the susceptible cv. Ejumula in the low-virus-pressure site. Kabode variety did much better than Ejumula at both sites regardless of the source of vines. In terms of univariate analysis between root yield and source of vines, the clean virus tested (VT) vines outshone those sourced from farmers (FS). There were no major differences in root yields for clean virus-tested seed and farmer-sourced seed of cv. Kabode at both sites. Root yields at the high-virus-pressure site were significantly influenced by variety, the sources of vines, and the on-farm propagation cycles ($p < 0.05$). No interactions were identified. Under the low virus conditions, root yield was affected by the interaction between variety and seasons ($p < 0.01$), and by the sources of vines ($p < 0.01$).

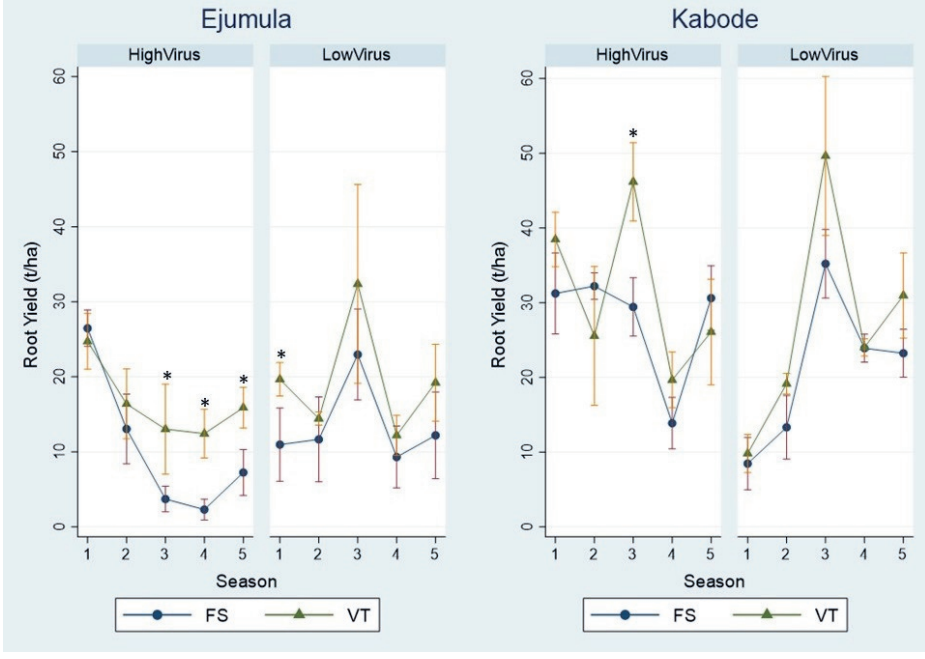

Figure 4. The trend in root yields for farmer-sourced (FS) and virus-tested (VT) planting material of the susceptible cv. Ejumula and the more resistant cv. Kabode, recycled over five seasons in high- and low-virus-pressure environments. *(* means statistically different at P < 0.05).*

b) Vine yields

In general, cleaned-up, virus-tested planting material of the susceptible cv. Ejumula had higher vine yields than farmer-sourced planting material in both the low- and high-virus pressure-environments (Fig. 5). This was significant for Seasons 3 to 5 at the high-virus-pressure site and Seasons 2 to 5 at the low-virus-pressure site. There was a season-to-season decline in the weight of vines produced by farmer-sourced planting material of this variety at both sites. Cv. Kabode recorded no differences in vine yields for both cleaned-up, virus-tested planting material and farmer-sourced planting material at both sites. A season-to-season decline in vine yields was recorded for both farmer-sourced and virus-tested planting material of cv. Kabode at the high-virus-pressure site, with Season 3 being an outlier. An interaction between the varieties and the sources of vines was observed under high virus conditions (p= 0.01). Under low virus conditions, vine yield was affected by the effect of the seasons ($p < 0.01$)

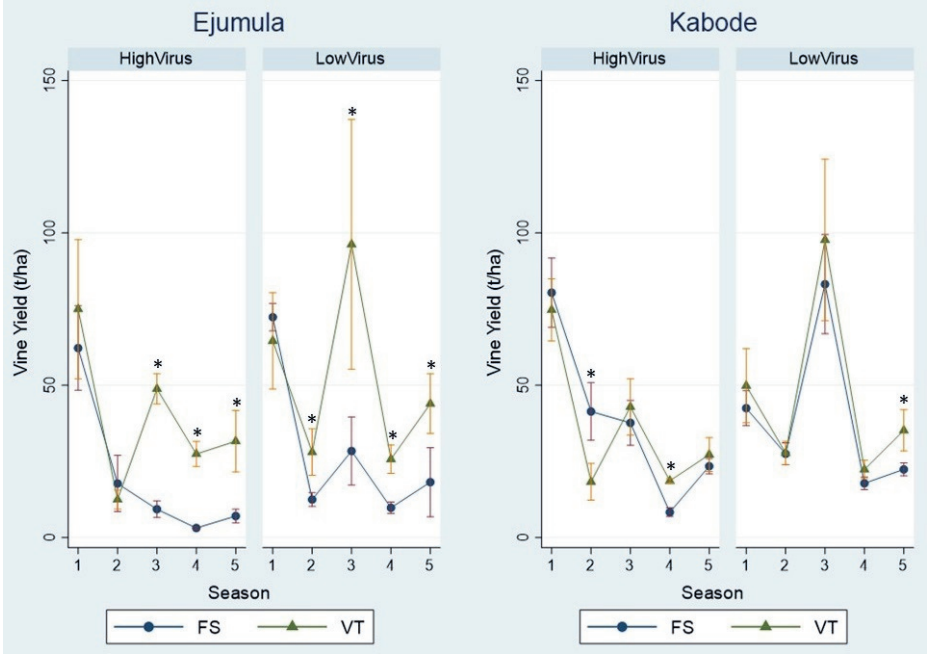

Figure 5. The trend in vine yields for farmer-sourced (FS) and virus-tested (VT) planting material of the susceptible cv. Ejumula and the more resistant cv. Kabode, recycled over five seasons in low- and high-virus-pressure environments. *(* means statistically different at P < 0.05).*

c) Virus incidences

Incidences of sweet potato chlorotic stunt virus

There was rapid infection of both farmer-sourced and clean virus-tested seed of the susceptible cv. Ejumula with sweet potato chlorotic stunt virus (SPCSV) at both high- and low-virus-pressure sites (Fig. 6). This cultivar was infected from the first season, with farmer-sourced vines recording significantly higher incidences compared to clean virus-tested seed. By the third season infection had reached 100% at the high-virus-pressure site for FS vines. The SPCSV incidences increased gradually with seasons for virus-tested seed of cv. Ejumula at both sites and cv. Kabode at the high-virus-pressure site. The more resistant cv. Kabode had lower SPCSV incidences at both sites compared with cv. Ejumula. The incidence of SPCSV was statistically significant between varieties ($p < 0.001$) and sources of vine ($p < 0.05$) for plants grown under high virus conditions. This was also observed under the low virus conditions, with significant differences between varieties ($p < 0.001$) and the sources of vines ($p < 0.01$).

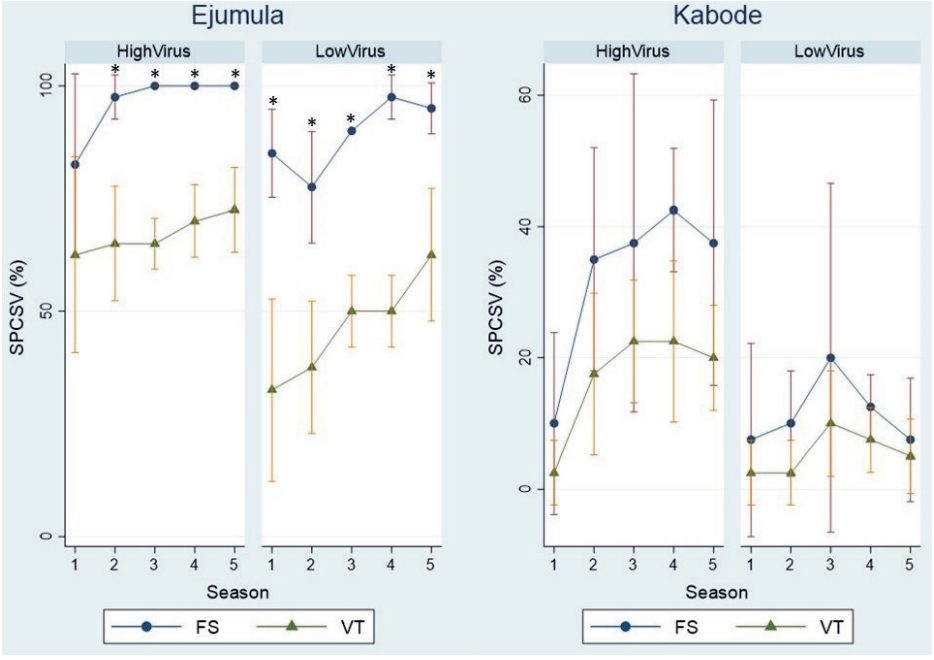

Figure 6. Trend in sweet potato chlorotic stunt virus (SPCSV) incidences for farmer-sourced (FS) and virus-tested (VT) planting material of the susceptible cv. Ejumula and the more resistant cv. Kabode recycled over five seasons in low- and high-virus-pressure environments. *(* means statistically different at P < 0.05).*

Incidences of sweet potato feathery mottle virus

Under high virus conditions, the incidence of SPFMV was influenced by the variety with cv. Ejumula recording higher incidences than cv. Kabode ($p<0.05$) (Fig. 7). Under low virus conditions, the percentage of plants infected with this virus was influenced by the seasons ($p<0.05$). Incidences of sweet potato feathery mottle virus (SPFMV) for cv. Ejumula increased gradually across the seasons. Higher incidences were recorded for farmer-sourced material compared with virus-tested material. Cv. Kabode had lower SPFMV incidences compared to cv. Ejumula at both sites.

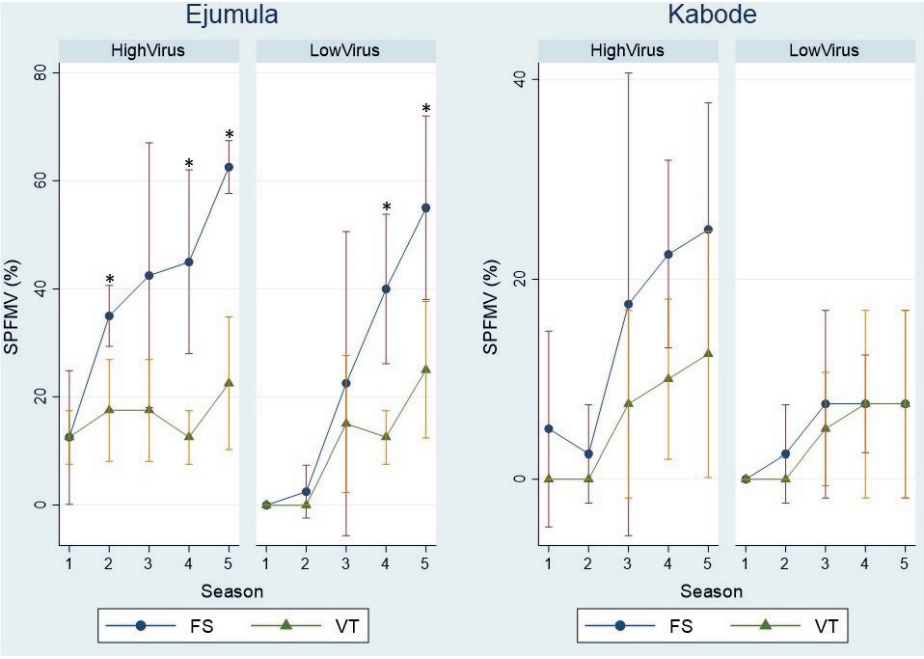

Figure 7. Trend in sweet potato feathery mottle virus (SPFMV) incidences for farmer-sourced (FS) and virus-tested (VT) planting material of the susceptible cv. Ejumula and the more resistant cv. Kabode recycled over five seasons in low- and high-virus-pressure environments. *(* means statistically different at P < 0.05).*

Incidences of sweet potato leaf curl virus

Higher incidences of sweet potato leaf curl virus (SPLCV) were recorded at the high-virus-pressure site for cv. Ejumula compared with the low-virus-pressure site (Fig. 8). Farmer-sourced planting material of both varieties was more infected with SPLCV compared with clean virus tested seed. Virus-tested seed of cv. Ejumula grown at the low virus pressure site was not infected by SPLCV throughout the experiment.

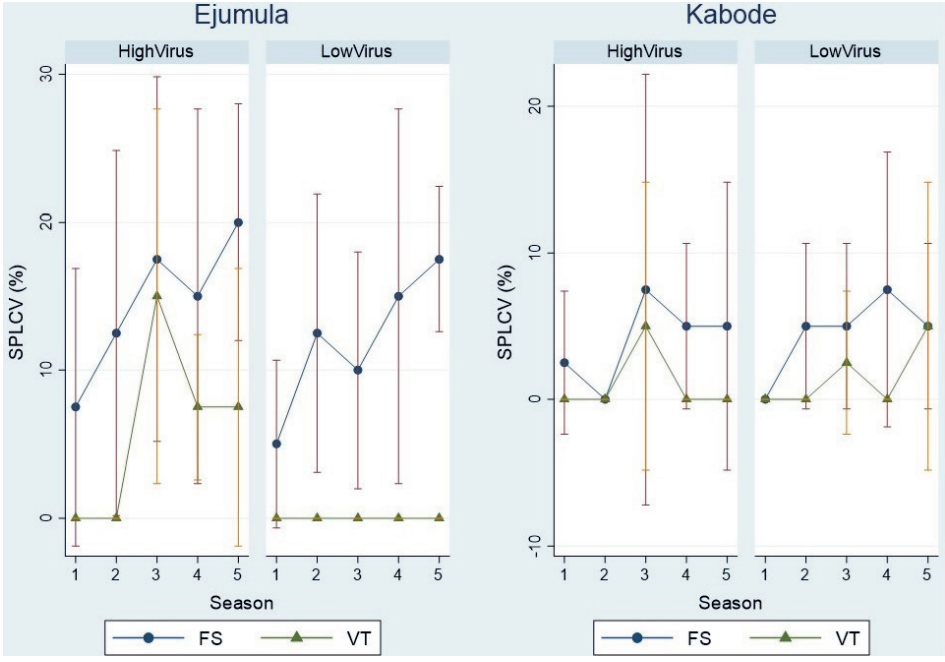

Figure 8. Trend in sweet potato leaf curl virus (SPLCV) incidences for farmer-sourced (FS) and virus-tested (VT) planting material of the susceptible cv. Ejumula and the more resistant cv. Kabode recycled over five seasons in low- and high-virus-pressure environments. *(* means statistically different at P < 0.05).*

Incidences of dual infection with SPFMV and SPCSV

Some plants were infected with both SPFMV and SPCSV. When this happens, the plant is considered to have the disease complex known as sweet potato virus disease (SPVD). Highest incidences of dual infection with SPFMV and SPCSV, i.e., SPVD were recorded at the high-virus-pressure site for farmer-sourced planting material of cv. Ejumula (Fig. 9). This increased gradually with season and was significantly higher compared with SPVD incidences on clean virus-tested planting material of the same variety ($p<0.05$). Kabode variety recorded very low SPVD incidences at both sites regardless of the source of planting material.

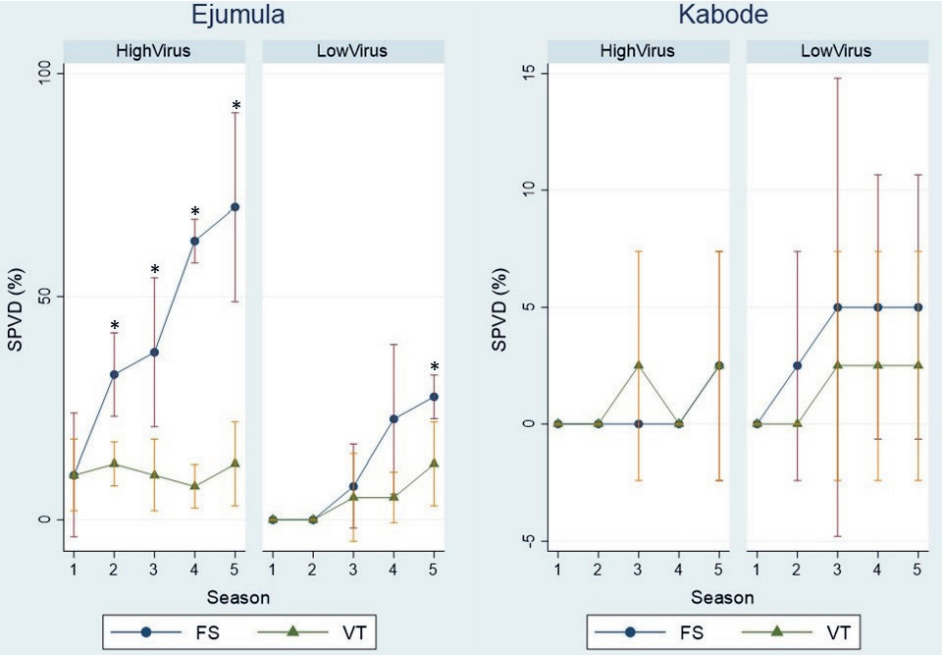

Figure 9. Trend in sweet potato virus disease (SPVD) incidences for farmer-sourced (FS) and virus-tested (VT) planting material of the susceptible cv. Ejumula and the more resistant cv. Kabode recycled over five seasons in low and high-virus-pressure environments. *(* means statistically different at P < 0.05).*

Incidences for the potyviruses SPV2, SPVC and SPVG

Sweet potato virus G (SPVG) was absent at both sites during the entire experiment. Sweet potato virus 2 (SPV2) and sweet potato virus G (SPVG) were also absent at the low-virus-pressure site, Bunda, during the entire period of the experiment. The two viruses (SPV2 and SPVG) were present in very low incidences at the high-virus-pressure site, Geita, in seasons 4 and 5. The incidence for SPV2 was 5% in Season 4 and 8% in Season 5. SPVG was only recorded in Season 4 and at 4% incidence. These viruses were mostly recorded on the susceptible cv. Ejumula.

4 Discussion

Cleaned-up, virus-tested seed as a control strategy against sweetpotato viruses

Use of cleaned-up virus-tested planting material is one of the best strategies in reducing degeneration in sweetpotato and has proven effective in several countries (Bryan et al., 2003; Milgram et al., 1996).

The data from this research has shown that this approach has more advantage for susceptible varieties especially in areas with high virus pressure. Virus-tested seed of the more susceptible variety, Ejumula, had higher vine and root yields compared with farmer-sourced material (Figs. 4 and 5). This directly correlated with virus incidences whereby farmer-sourced planting material of the variety had higher SPCSV, SPFMV and SPLCV incidences compared with virus-tested material. The farmer-sourced material of this variety also had higher incidences of dual infection with SPCSV and SPFMV, i.e., the sweet potato virus disease complex. Okpul et al. (2011) reported that susceptible varieties are more likely to show improved vigour and performance following virus cleaning. They observed a 148% increase in total root yields in some Australian cultivars following virus cleaning. In China, Feng et al. (2000) reported a 224% increase in yields when using cleaned-up, virus-tested seed for susceptible varieties but also noted a gradual decline in yield with successive plantings. A gradual decline in yield was also observed for cv. Ejumula planted in the high-virus-pressure area in this study. This correlated with the virus incidences which increased with progressive cycles of field propagation.

The use of virus-tested seed did not seem to have an advantage over farmer-sourced planting material for the relatively tolerant cv. Kabode. Despite virus-tested material producing higher yields than farmer-sourced material the differences were not significant. The virus incidences recorded on this variety were also lower compared to cv. Ejumula (Figs. 6-9). This confirms that the cv. Kabode is somewhat tolerant to viruses. This differed between the different viruses with the variety recording more SPCSV incidences than SPFMV and SPLCV. Genetic makeup of a variety has been reported to influence susceptibility to viruses (Mwanga et al., 2002). Varieties that have a level of resistance to sweet potato viruses have been reported to have minimal yield increase from clean seed (Valverde et al., 2007). Carey et al. (1999) reported no root yield benefits on the use of virus-tested seed for several Ugandan landraces. They attributed this to the relatively high levels of tolerance to viruses in the local landraces used in their study. The initial farmer-sourced material used in our study was also carefully selected to ensure that only asymptomatic plants were used as seed. The symptomless plants might have been healthy especially for cv. Kabode which had very low levels of virus incidences in the first season. Cultivar resistance and selection of healthy-looking plants (positive selection) as sources of planting material are also important strategies that can complement use of virus-tested seed in limiting degeneration in sweetpotato (Gibson et al., 2004; Gibson & Kreuze, 2015; Thomas-Sharma et al., 2016).

Virus incidences

Contrary to a previous report by Tairo et al. (2004), SPCSV incidences were higher than those of SPFMV at both agroecologies. Farmer-sourced planting material of cv. Ejumula had more than 70% SPCSV incidences at the end of the first season at both sites.

This was significantly higher than virus-tested seed indicating that the farmer-sourced material might have already been infected with the virus. The high incidences of SPCSV on virus-tested seed of cv. Ejumula at the end of Season 1 also indicates the possibility of external sources of virus infection. Isolation of production fields from potential external sources of infection can limit virus infection. Wosula et al. (2013) recommended locating seed beds away from sweetpotato plants of unknown sources to avoid infection from external inoculum. Distances as low as 15 m have been shown to limit infection in Uganda (Gibson et al., 2004). A study conducted by Aritua et al. (1999) also showed that SPVD incidences had a direct relation with proximity to external inoculum sources. In addition, isolating clean planting material using a physical barrier such as insect-proof nets has been shown to reduce virus infections (Ogero et al., 2019).

The begomovirus, SPLCV, had the lowest incidences with higher prevalence in the high-virus-pressure area. Despite the low incidences our results indicate that SPLCV is likely an important virus threat to sweetpotato production at the lake Zone Tanzania. Previous studies did not include SPLCV and related sweepoviruses as threats in the Lake Zone (Ndunguru & Kapinga, 2007; Tairo et al., 2004). This is because they used serological assays which did not include begomoviruses. The challenge posed by sweepoviruses to sweetpotato production is increasingly getting global recognition (Clark & Hoy, 2006; Kim et al., 2015; Wanjala et al., 2020; Wasswa et al., 2011). These viruses can cause considerable yield losses even without symptom expression on the foliage. In addition, SPLCV has been shown to amplify the effect of SPVD resulting in significant losses. Wanjala et al. (2020) reported highest severity scores and low root yields in treatments inoculated with SPVD and SPLCV for cv. Ejumula and cv. Kakamega in Kenya. The lack of symptoms can make on farm management practices such as those suggested by Thomas-Sharma et al. (2016) difficult to implement. The symptomless nature of SPLCV and SPFMV (in tolerant varieties) may lead to spread and prevalence of the viruses because farmers are more likely to use infected material. For efficient control of sweetpotato viruses it is important to come up with affordable diagnostics that can be deployed at all stages of the seed system. Currently seed inspections at the downstream stages are based on visual symptoms therefore mostly effective for SPVD-infected plants. This poses a risk of leaving a high reservoir of symptomless viruses in the seed multiplication plots, which can later cause SPVD (Kreuze et al., 2021). The Loop-mediated Isothermal Amplification (LAMP) is among the technologies that can be deployed easily for field-based detection of SPCSV, SPFMV and SPLCV whose assays have already been developed (Wanjala et al., 2021).

Agroecology and virus incidences

There were higher virus incidences at the high-virus-pressure site compared with the low-virus-pressure site indicating the influence of agroecological conditions and cultivation patterns on virus infections.

Geita, the high-virus-pressure area, has two distinct rainfall seasons leading to continuous sweetpotato cultivation throughout the year. Being a few metres from the lake shore, the experimental site was also in a location where farmers usually conserve their planting material. Continuous sweetpotato cultivation leads to virus accumulation in an area and has also been reported in Uganda for sweetpotato (Adikini et al., 2015) and in Tanzania for cassava (Shirima et al., 2019). Moreover, it is important to consider soil characteristics in a certain area when studying the effect of viruses on root yields. The differences in physical and mineralogical properties and nutrient status of soils can also influence yields. Mineral nutrition in the soil may slow or exacerbate the effect of viruses on yields (Barker, 2009). Adding NPK fertilizer to soil has been shown to increase the rate of reversion in sweetpotato (Ssamula et al., 2019). Better fertility and mineralogical properties of the soil in Geita might have contributed to the higher yields in Season 1 compared with the Bunda site despite the high virus incidences in the former.

Conclusion

This study has shown that using cleaned-up, virus-tested seed is an important strategy in limiting degeneration in sweetpotato especially for susceptible varieties grown in high-virus-pressure areas. In addition, the lack of a clear yield decline when using farmer-sourced planting material of the tolerant cv. Kabode shows why farmers may be reluctant to regularly replace preferred varieties if they are tolerant to viruses. This may have a negative implication on sustainability of seed systems to deliver cleaned-up, virus-tested seed because farmers may opt to recycling after the initial purchase. It is important to continue creating awareness among farmers that different varieties behave differently in different agroecologies and that tolerance to viruses may vary. Our findings also indicate that the prevalence of SPCSV and SPLCV at the Lake Zone Tanzania might be higher than previously thought. Surveys using sensitive diagnostic procedures may help provide more insight on the current prevalence of various sweetpotato viruses in Tanzania. Clear mapping of high- and low-virus-pressure areas can help in targeting control strategies.

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A female farmer sorting out sweetpotato vines produced in net tunnels under drip irrigation. Misungwi district, Tanzania. Photo credit: K. Ogero.

Chapter 3:

Efficiency of Insect-proof Net Tunnels in Reducing Virus-related Seed Degeneration in Sweetpotato

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Abstract

Virus-related degeneration constrains production of quality sweetpotato 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 modeling 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.

Key words: Virus-related degeneration, sweetpotato, seed, net tunnels, farmer-multiplier, modeling

1 Introduction

Sweetpotato is an important staple and co-staple food crop in Africa, and orange-fleshed sweetpotato (OFSP) 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 and Walsh, 2012). However, sweetpotato yields are heavily reduced by viruses, which are carried from generation to generation through recycling of infected cuttings (Gibson and Kreuze, 2015). The most common viruses found infecting sweetpotato in Africa are sweet potato feathery mottle virus and sweet potato chlorotic stunt virus (Loebenstein, 2015). Sweet potato feathery mottle virus is a *Potyvirus* transmitted non-persistently by aphids (Karyeija et al., 1998). It can combine with sweet potato chlorotic stunt virus, a *Crinivirus* transmitted semi-persistently by whiteflies (Schaefers and Terry, 1976) to cause sweet potato virus disease (Mukasa et al., 2006). Individually, sweet potato feathery mottle virus (SPFMV) can cause negligible to 40% loss of root yields (Adikini et al., 2016; Milgram et al., 1996) whereas sweet potato chlorotic stunt virus (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 which is the main 'virus disease' affecting the crop and often leading to 56 – 100% yield losses (Ndunguru et al., 2009; Ngeve and Bouwkamp, 1991). Of the two, SPCSV is the most important since it leads to the plant losing any resistance to SPFMV (Opara and Nwankwo, 2015). In addition, a group of geminiviruses, collectively known as sweepoviruses, is increasingly being recognized as damaging and being common worldwide, including Africa (Rey et al., 2012). Managing such a complex set of viruses is challenging, especially since many of them show no, or only minor transient symptoms when infecting sweetpotato alone, making it difficult to identify infected plants (Valderde et al., 2007). This is especially true for most African cultivars. Corresponding low titers in plants make their detection by serological methods equally challenging.

There are three major alternatives in managing viruses in sweetpotato: (1) deploying resistant cultivars, (2) using clean (virus-tested) seed, and (3) employing proper on-farm management practices. Deployment of resistant cultivars is viewed as the most effective strategy in sweet potato virus disease (SPVD) management (Maule et al., 2007) . However, whereas land-races 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 and 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 Namulonge is typically less than 0.2% (Mwanga et al., 2002). This has presented a major bottleneck for introducing new OFSP 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 sweetpotato 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 rogueing 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 one 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 white flies 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). It is however important to know how well net-tunnels perform in maintaining the phytosanitary status of sweetpotato vines under farmer-multiplier management.

This study sought to determine the rate of virus infection and related degeneration in sweetpotato 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). We 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 modeling 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, rogueing, 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 status after several seasons of cultivation and has been demonstrated in several sweetpotato cultivars (Gibson and Kreuze, 2015). Modeling 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.

2 Materials and methods

a) Location and varieties used

This research was conducted at two sites, Mwasonge (2° 40´ 13´´ S 32° 54´ 45´´E) and Nyasenga (2° 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 sweetpotato production, whereas Nyasenga is a low-virus-pressure area due to limited sweetpotato cultivation in the area. High cultivation of sweetpotato at Mwasonge also translates to higher vector populations due to year-round availability of host plants as compared to Nyasenga. Two sweetpotato 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.

b) 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 (Figure 1A).

Each net tunnel and open bed measured 3 m × 1.7 m and had 270 cuttings planted on 9 rows at a spacing of 20 cm by 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 Figure 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 of silica gel. Sample collection was done eight times between June 2014 and March 2016 (21 months).

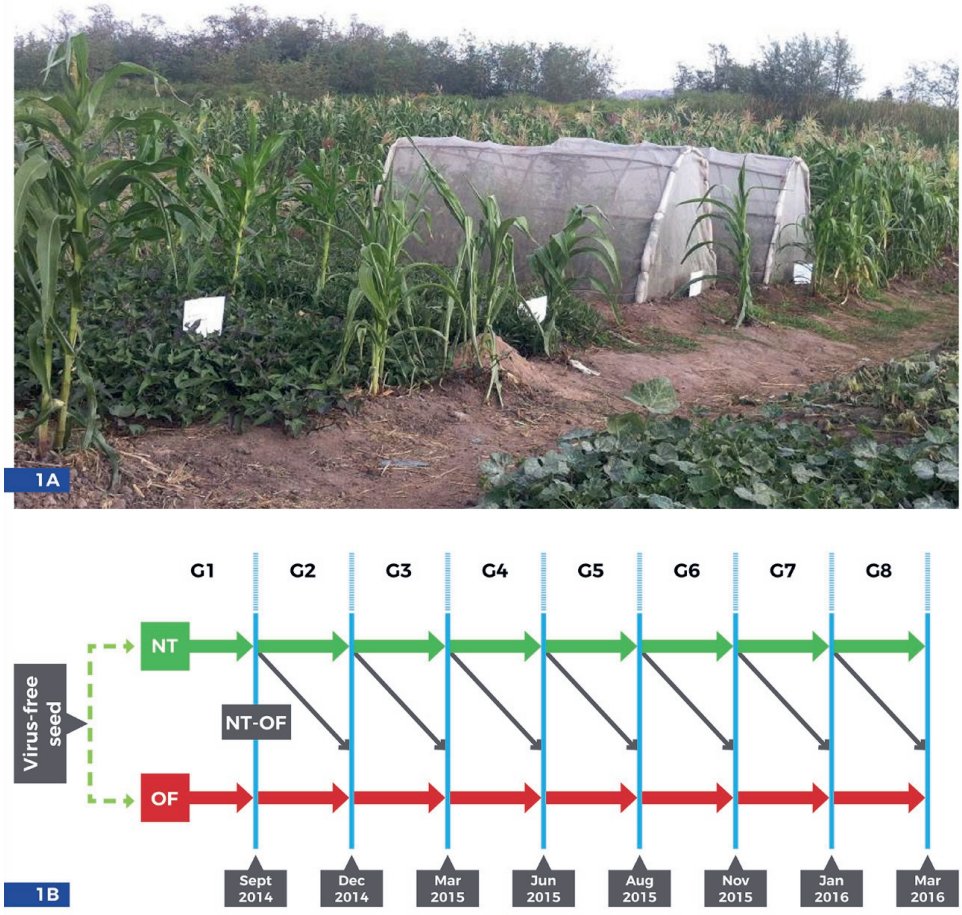

Figure 1. (1A) Net tunnels (Right) and open fields (Left) at the high-virus-pressure site (Mwasonge). (1B) The growing cycles of the experiments: The green line is the intervention (net tunnels) and the red line is the control (open fields). Black arrows indicate vines harvested from the net tunnels and multiplied once in the open field (Net tunnel-OF); blue vertical lines indicate points of leaf sampling.

c) 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 seconds on 10 leaves from 10 different plants and recording the number. Onset® data loggers were used to record daily weather data (rainfall, relative humidity, and temperature).

d) Testing viruses present in the environment at initiation of experiment

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

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

Leaf samples were analyzed in bulks of five, and each bulk was screened for sweet potato leaf curl virus (SPLCV), potyviruses (SPFMV, sweet potato virus G, sweet potato virus 2 and sweet potato virus C), and SPCSV using PCR, multiplex reverse transcriptase PCR (RT - PCR), and reverse transcription quantitative real-time PCR (RT - qPCR), respectively. If a bulk was positive, each sample was analyzed 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 Nano drop machine (Nano drop 2000 spectrophotometer, Thermo Scientific). Additionally, total RNA was run on a gel containing 1% agarose (UltraPure Agarose, Invitrogen by Life Technologies) and viewed on a gel documentation machine (Biodoc-H™ Imaging system with Benchtop UV Transilluminator, Cambridge UK) for RNA integrity. Thereafter, total RNA was diluted with nuclease-free water to end up with estimated concentration of 0.025ng /µl.

qPCR for SPCSV

The reaction mix for one reaction of qPCR was prepared by mixing; 12.5 µl of TaqMan universal master mix (2x) (Applied Biosystems by Life Technologies Kingstand Grange-woolston), 0.75 µl of each forward (F) and reverse (R) primers (10 µM each) (Table 1), 8 µl of nucleuse-free water, 0.5 µl of Revert aid Reverse Transcriptase (Thermo Fisher Scientific), diluted 1/100 (2U/µl), 0.125 µl of F and R COX (10 µM) (Applied Biosystems), 0.25 µl of probe (5 µM) (Applied Biosystems), and 0.25 µl of probe for COX (5 µM) , 1 µl of total RNA. Real-time PCR machine (Strata Gene MX 3000P, Agilent Technology 76337, Woldbronn) were programmed on the following conditions; 30 minutes for 42˚C, 10 minutes for 95˚C followed by 40 cycles of 15 seconds for 95˚C, and 1 minute for 60˚C. Results were viewed and recorded using Stratagene Mx3000P software.

Reverse-transcriptase (RT) multiplex PCR for Potyviruses

Multiplex RT-PCR for sweetpotato potyviruses was performed as described by Li et al., (2012) with minor modifications.

Total RNA with reverse primer (SPFCG2-R) (Table 1) were denatured at 65˚C for 10 minutes and cDNA synthesized using 5xRT buffer 0.1 M, DTT, 10 mM dNTPS, RNAse out Inh.40U/µl, M-MLV 200 U/µl, and the reaction was incubated at 40˚C for 60 minutes, 95˚C for 5 minutes.

Table 1. Primers used for PCR assays

Primer	Sequence	Expected size
Primers for SPCSV		
SPCSV-Uni-E-F	5'-CGGAGTTTATTCCCACYTGTYT-3'	
SPCSV-Uni-E-R	5'-GGGCAGCCYCACCAA-3	
COX-F	5'-CGTCGCATTCCAGATTATCCA-3'	
COX-R	5'-CAACTACGGATATATAAGAGCCAAAACTG-3	
Probe	**Sequence**	
SPCSV-Uni-E-P	5'-[FAM]-TCTGTCACGGCTACAGGCGACGTG-[TAMRA]-3'	
COX-P	5'-[VIC]-TGCTTACGCTGGATGGAATGCCCT-[TAMRA]-3'	
Primers for Potyviruses (SPFMV, SPVC, SPVG, SPV2)		
SPG-F	5' GTATGAAGACTCTCTGACAAATTTTG 3'	1,191 bp
SPC-F	5' GTGAGAAAYCTATGCGCTCTGTT 3'	836 bp
SPF-F	5' GGATTAYGGTGTTGACGACACA 3'	589 bp
SP2-F	5' CGTACATTGAAAAGAGAAACAGGATA 3'	369 bp
SPFCG2-R	5' TCGGGACTGAARGAYACGAATTTAA 3'	
Primers for Begomoviruses (including sweet potato leaf curl virus (SPLCV))		
SPG1	5' CCCCKGTGCGWRAATCCAT 3'	912 bp
SPG2	5' ATCCVAAYWTYCAGGGAGCTAA 3'	

Reaction mix for PCR was prepared using 5x Dream Taq buffer (Invitrogen by Life Technologies), 25 mM $MgCl_2$, 10 mM dNTPs (Invitrogen by Life Technologies), primers (SPG-F (2.5µl), SPC-F (0.4µl), SPF-F(2µl), SP2-F(0.2µl), and SPFCGG2-R(2µl), each with a concentration of 10 µM)), Dream Taq buffer 5U/µl and cDNA. The mixture was incubated in a PCR machine (GenAmp® PCR system 9700, Applied Biosystems) using the following conditions: 94˚C for 2 minutes, 94˚C for 30 seconds with 35 cycles, 60˚C for 30 seconds, 72˚C for 1 minute, and 20 seconds, 72˚C for 10 minutes. The gel was prepared using 1.2% of agarose (UltraPure Agarose, Invitrogen by Life Technologies) in 1x TAE buffer and ethidium bromide was used for staining. This was run at 200 V for 30 minutes and the product viewed and recorded using gel documentation machine (Biodoc-H™ Imaging System Benchtop UV Transilluminator, Cambridge, UK).

PCR for SPLCV

The reaction mix was prepared using 5x Dream Taq buffer, 25 mM $MgCl_2$, 10 mM dNTPs, primers SPG1 (0.5µl) and SPG2 (0.75µl) of 10 µM concentration described by Li et al., (2004) (Table 1), Dream Taq 5U/µl and DNA template. The reaction mix was incubated in a PCR machine (Applied Biosystems GenAmp PCR System 9700) using the following PCR conditions (touch down): 94˚C for 40 seconds, 72˚C for 30 seconds, 72˚C for 90 seconds, with 11 cycles (n-1 ˚C per cycle), 94˚C for 40 seconds, 60˚C for 40 seconds, 72˚C for 92 seconds, and 72˚C for 10 minutes with 24 cycles. 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:

$$\frac{\text{infected samples}}{\text{sample size}} \pm \Phi^{1}\left(0.0975\sqrt{\frac{\text{infected samples/sample size} * (1-\text{infected samples/sample size}) * (\text{population size} - \text{sample size})}{(\text{sample size} * (\text{population size} - 1))}}\right)$$

f) Modeling 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 (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/software/). Each parameter combination was evaluated in 1000 simulations, over 10 generations. 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.

3 Results

a) Environmental vector pressure and weather conditions

Only a few (<10) volunteer sweetpotato 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 250m radius of the sweetpotato 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 sweetpotato 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 sweetpotato (Figure 2). Aphids were only observed in October 2014 at Mwasonge on cucumbers and beans (not shown).

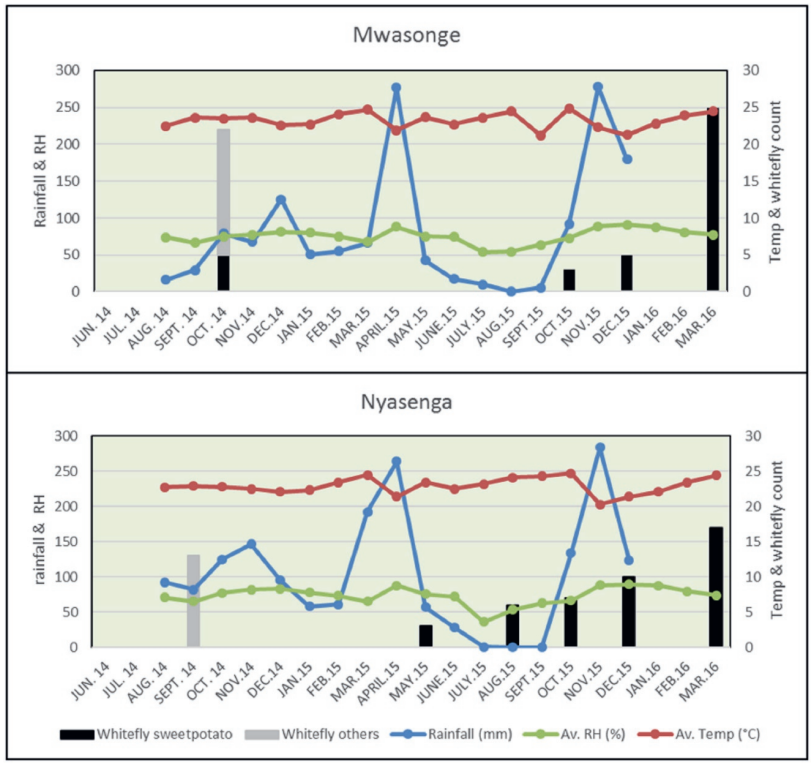

Figure 2. Weather conditions and whitefly counts during the experiment.

b) Virus incidence

Viruses present in the environment at initiation of experiment

Results from sRSA analysis of bulked samples from nearby sweetpotato 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 sweetpotato and occur only at extremely low titres in plants (Kreuze, unpublished), 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 six and increased with successive generations at both sites (Figure 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 six to eight. Viruses detected at the two sites were SPFMV and SPSCV occurring singly or coinfecting to form SPVD (Figure 3B). SPFMV was the most prevalent at both sites.

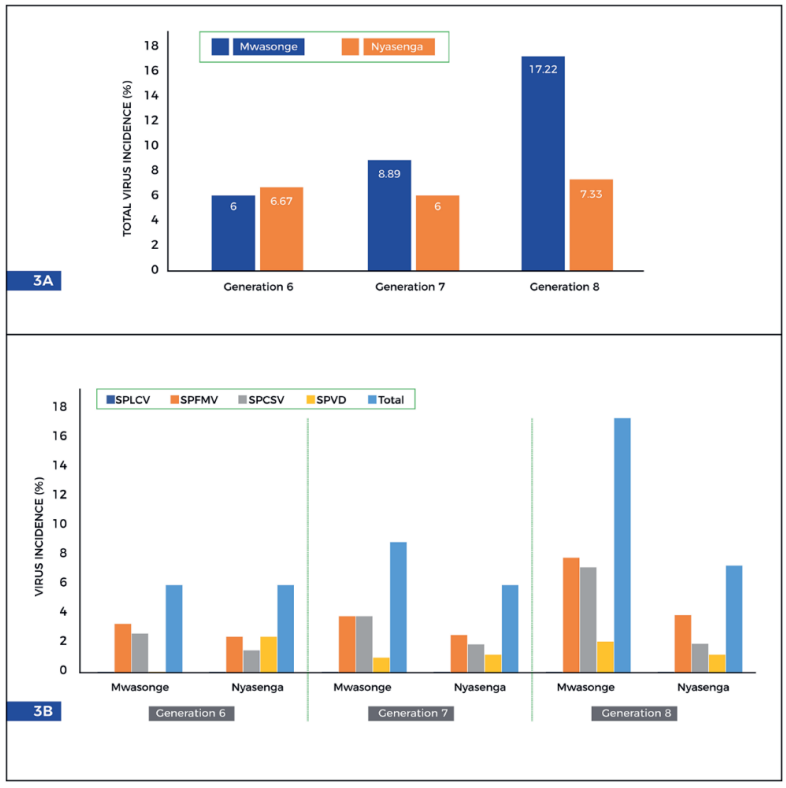

Figure 3. Virus incidence as detected by PCR assays at the high-virus-pressure area (Mwasonge) and the low-virus-pressure area (Nyasenga). 3A shows total virus incidence while 3B shows incidence of specific viruses. Virus incidence was zero in the first five generations.

Vines of variety Kabode maintained in the net tunnels at Mwasonge had 7% and 13% virus incidence during generations seven and eight, respectively (Figure 4A). Vines of the same variety continuously grown at the open field (control) at Mwasonge had 7%, 10% and 23% virus incidence during generations six, seven and eight, 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 eight. Planting material of the same variety continuously grown at the open field had the highest virus incidence (Figure 4B). Virus incidence was zero for variety Polista at the Nyasenga site (low-virus-pressure area) for the entire period of study, both for open fields and net tunnels. Vines of cv. Kabode grown in the net tunnel at this site were not infected by viruses throughout the study. On the other hand, vines of the same variety grown continuously in open fields at the same site had 13%, 13% and 17% virus incidence during generations six, seven and eight, respectively (Figure 4C).

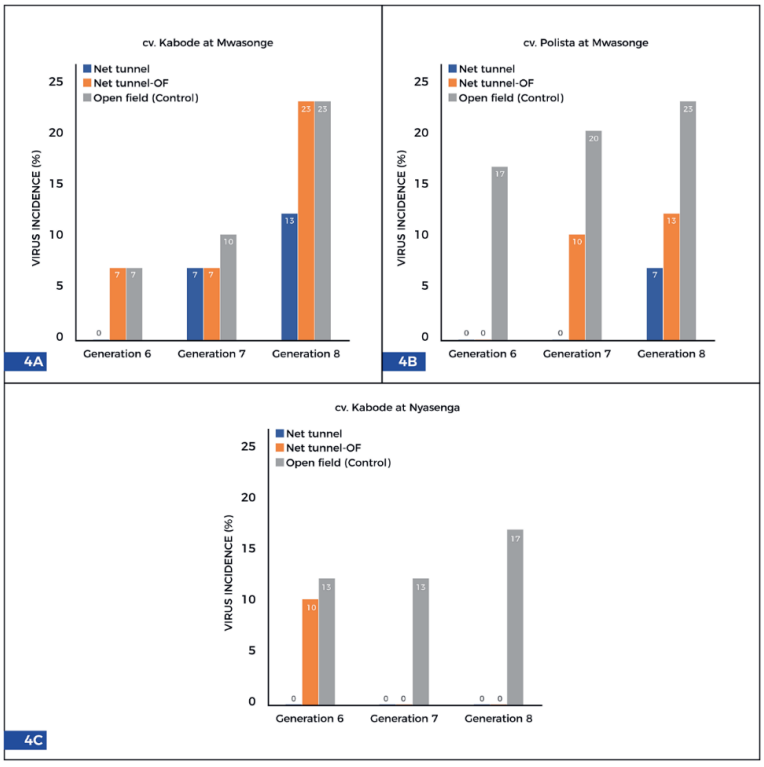

Figure 4. Incidence of viruses detected by PCR assays in cv. Kabode and cv. Polista during the last three generations.

Seed degeneration modeling

Results from seed degeneration modeling 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 (Figure 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.

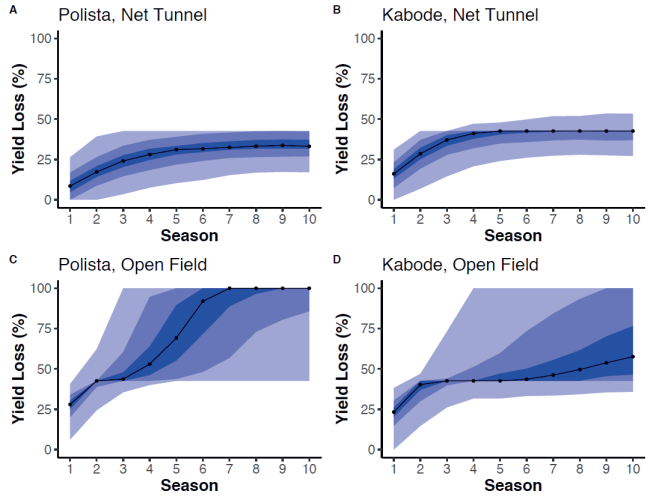

Figure 5. Modeled percentages of yield loss over ten seasons in scenario analyses for the high virus pressure site (Mwasonge), in extrapolations based on estimates of infection rates from the experiment combined with a generic model of yield loss. The yield loss percentages are the results of 1000 simulations, where each simulation started with seed with no infection so that the initial infections came from the surrounding area (Thomas-Sharma et al., 2017). Black lines indicate the median (0.50 quantile) yield loss across the 1000 simulations, and the shading indicates quantiles starting at 0.05, with 0.25, 0.40, 0.60, 0.75, and 0.95 indicated.

4 Discussion

Growing sweetpotato 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 sweetpotato 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 which transmit viruses from diseased sweetpotato 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 sweetpotato plants being established in neighboring fields therefore providing a favorable host for reproduction and multiplication of the vectors.

Generations one to five, with zero virus incidence observed, experienced a period of limited rainfall leading to low cultivation of sweetpotato in areas surrounding the experimental sites and therefore reduction in external disease inoculum. Favorable environment is very critical for plant disease manifestation in addition to pathogen-host interactions (Gergerich and Dolja, 2006). Sufficient rainfall and high humidity favor build-up of sweetpotato 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 sweetpotato viruses and selection of sites for seed production. Thiele (1999) linked better seed potato production at higher altitudes to favorable 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 sweetpotato is usually intensely grown due to favorable conditions.

That only SPCSV and SPFMV were detected in the study area reflects previous reports that the two are the most common and important yield-limiting sweetpotato viruses in East Africa especially when occurring together (Adikini et al., 2016; Ndunguru et al., 2009). High prevalence of SPFMV is consistent with findings by Clark et al.(2011) and Tairo et al. (2004) who reported the asymptomatic potyvirus to be the most common single infection virus at the Lake Zone, Tanzania. Efforts employed to control viruses in sweetpotato 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 varies with variety and virus involved (McEwan, 2016). A susceptible variety like Beauregard has been reported to be re-infected 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 and 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.

We compared varieties Kabode and Polista, 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 socio-cultural values that surround sweetpotato 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's 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 adoption of insect-proof net tunnels among farmer-multipliers to reduce seed degeneration in sweetpotato. Given their affordability both in construction and management, they can contribute greatly in the improvement of local seed systems. The net tunnels should, however, be combined with other on-farm management options such as positive selection and rogueing. 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 modeling 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 sweetpotato seed production might increase the cost of seed due to the additional investment. To keep the cost affordable, farmer-multipliers utilizing the net tunnels are advised to sell after two rounds of open field multiplication thereby increasing the quantities.

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A female farmer harvesting sweetpotato vines. Ukerewe district, Tanzania. Credit: K. Ogero.

Chapter 4:

Ratooning increases production of sweetpotato seed vines multiplied in insect-proof net tunnels in Tanzania

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Abstract

Insect-proof net tunnels can help reduce virus reinfection of clean virus-tested sweetpotato seed produced by decentralized seed producers. However, optimal management is required to maintain both quality and quantity of seed produced. This study investigated the effect of the ratoon cropping technique on vine production in net tunnels and open fields. Virus-tested planting material of two varieties, Kabode and Mataya, were grown in net tunnels and open fields. Each variety had 80 plants per plot, with 40 following the ratooning technique and 40 a replanting technique. The ratooned crop was harvested six times, comprising the initial harvest and five re-growths. This covered 14 months representing 6 generations of vine production. The number of vines, number of nodes per vine and vine length were recorded. The number of plants showing virus symptoms was also recorded. The ratoon cropping technique produced more vines compared with the replanting technique in both net tunnels and open fields. Cv. Kabode produced more vines in open fields compared with net tunnels regardless of cropping technique. On the other hand, cv. Mataya produced relatively equal number of vines in net tunnels and open fields for both ratooned and replanted crops. Despite ratooning leading to more vine production compared with replanting, the technique led to higher virus incidences on plants grown in the open. This also varied with variety with the highest virus incidences being recorded on cv. Mataya. We recommend the ratoon cropping technique for sweetpotato vine production in both net tunnels and open fields. However, open field production should be accompanied with a suite of virus management practices to avoid reinfection.

Key words: Cropping technique, *Ipomoea batatas*, regrowth, reinfection, replanting, virus

1 Introduction

Farmers in Tanzania are yet to realize the full potential of the sweetpotato (*Ipomoea batatas*) crop. The main yield constraint is virus infection of propagation material. Virus spread is aided by vegetative propagation of planting material i.e., seed vine recycling from season to season and by farmer-to-farmer exchange of planting material. More than ninety per cent of sweetpotato seed vines is sourced from an informal farmer-based seed system characterized by free exchange of local landraces (McEwan, 2016; Namanda et al., 2011; Thiele et al., 2021). Very little clean virus-tested seed and very few improved varieties are disseminated through this system. The farmer-based system is made possible by the vegetative nature of the crop which makes it easier for farmers to propagate their own planting material.

This challenge to control virus diseases is not unique to sweetpotato and affects the seed systems of other vegetatively propagated crops such as banana, cassava and potato. Vegetative propagation in root, tuber and banana crops allows farmers to reproduce the same genetic material for many seasons but can lead to degeneration of the crop whereby yields decline from season to season due to accumulation of pathogens in the plant (Almekinders et al., 2019; Thomas-Sharma et al., 2016).

In sweetpotato, synergistic reactions resulting from co-infection with sweet potato feathery mottle virus (SPFMV; *Potyvirus*, transmitted by aphids) and sweet potato chlorotic stunt virus (SPCSV; *Crinivirus*, transmitted by whiteflies) have been particularly identified as a key yield-limiting factor (Clark et al., 2012). Infection with the two viruses causes a disease complex known as sweet potato virus disease (SPVD) which leads to 56 – 100% yield losses (Adikini et al., 2016; Mukasa et al., 2003). Therefore, the focus of seed system interventions has been to disseminate and promote the use of clean, virus-tested seed vines (further referred to as seed) of market-preferred landraces and improved varieties.

Use of cleaned-up, virus-tested sweetpotato seed is recognized as one of the strategies to address virus-related yield losses in sweetpotato in sub-Saharan Africa (McEwan, 2016). This approach has been reported to be successful with immense economic benefits across the world. Clark et al. (2010) reported that farmers in the USA realized better yields by incorporating virus-tested foundation seed into their production schemes. In China, use of virus-free seed in Shandong province led to an internal rate of return of 202% due to increased yields (Fuglie et al., 1999). However, virus-free sweetpotato seed can get reinfected once it is grown in farmers' fields. The rate of reinfection may vary depending on management, proximity to other sweetpotato fields or alternate virus hosts, virus vector densities, and weather conditions.

Often, dissemination of virus-free sweetpotato seed in Eastern Africa is accompanied with farmer training on on-farm seed management strategies to delay or reduce the rate of reinfection. Most target farmers want to become specialized seed producers. In addition to human capacity building, the specialized seed producers are also capacitated to acquire technologies that can help protect clean planting material from virus vectors. This includes the use of low-cost insect-proof net tunnels which have demonstrated to reduce virus reinfection in sub-Sahara Africa (SSA) countries including Tanzania (Ogero et al., 2019).

Whereas use of insect-protected structures is common at research institutions in SSA, they are rarely used at farmer-level, because the production of sweetpotato seed at farmer-level has never been technology intensive. However, as more areas become high-virus-pressure zones due to increasing vector populations, it is important for seed producers to adopt technologies that will lower the rates of reinfection of cleaned-up virus-tested sweetpotato seed. Use of low-cost insect-proof net tunnels to multiply cleaned-up virus-tested sweetpotato seed at seed producer level has been piloted in seven countries in SSA (Ogero et al., 2017). This pilot showed that the technology can be successfully used by better resourced, trained farmers, to maintain and produce high quality basic seed for two to three years. However, for successful uptake seed producers also need to adopt appropriate management practices to maintain the quality and quantity of planting material in the net tunnels.

Keeping the plants in a healthy status enables several harvests i.e., the plants can be let to sprout again after harvesting. In sweetpotato, the first harvest of vines can be done 90 days after planting and thereafter harvesting can be done after every 40 – 60 days. This is called ratooning and it consists of harvesting the crop, with the next crop being the result of the first crop's regrowth (Riga, 2008). However, this technique can affect the quality and quantity of planting material produced because the root stocks age with time. We assessed how ratooning affects vine production for two sweetpotato varieties multiplied in net tunnels over 14 months. This was compared with another cropping technique that entailed uprooting old plants at harvest and replanting with apical portions of the same crop. Here, the plants are cut at about 10 cm aboveground, root stocks uprooted, and the bed prepared afresh. The harvested vines are then cut into 3-node (10-cm) cuttings and replanted.

2 Materials and methods

a) Location and varieties

The experiment was conducted at a satellite research farm operated by the Tanzania Agricultural Research Institute (TARI) − Ukiriguru at Nyakasanga village, Misungwi district, Mwanza, Tanzania, located at 2° 46' 14" S, 32° 56' 34" E, at an altitude of 1139 m asl. The site was about 300 m from the shore of Lake Victoria from where irrigation water was drawn. The location has two rain seasons in a year (March – mid-May and October – December) (Fig. 1).

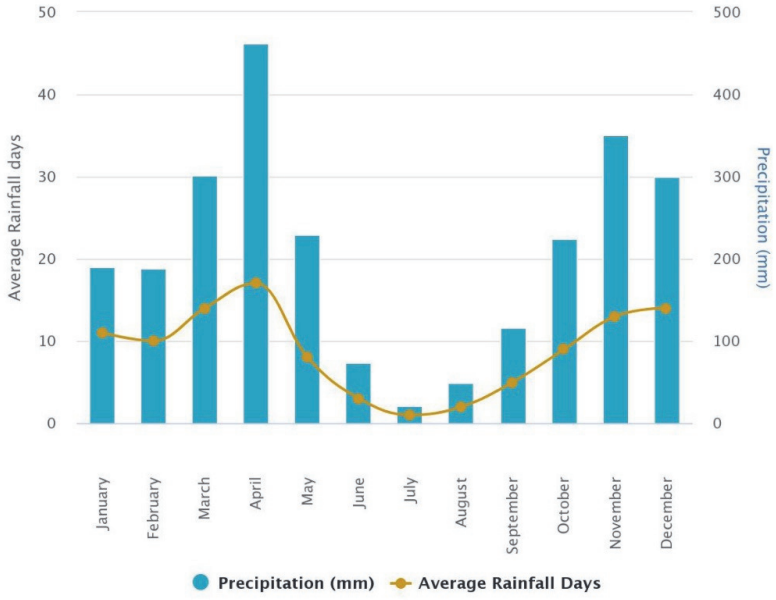

Figure 1. Average annual rainfall for Nyakasanga village, Mwanza, Tanzania.

Two varieties, Kabode and Mataya, were used in the experiment. Both varieties are orange-fleshed but have different growth characteristics. Kabode has a semi-erect canopy and is moderately resistant to sweetpotato viruses while Mataya is prostrate and susceptible to viruses.

b) Experimental set up

The trial was set up in a split-split plot design with the following factors:

a) Cover type (net tunnel vs open field) – main plot factor
b) Variety (cv. Kabode vs cv. Mataya) – sub plot factor
c) Cropping technique (ratooning vs replanting) – sub-sub plot factor

This led to eight treatments each replicated within each of the four main plots, four open field plots and four net tunnel-protected plots measuring 3 m × 1.7 m (Figure 1 and Photo 1).

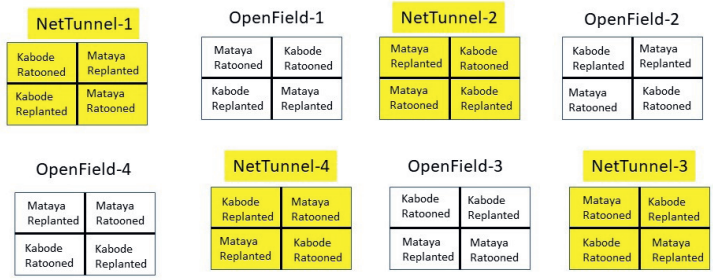

Figure 2. Schematic diagram showing the field layout.

Photo 1. Pictorial presentation of the field set up showing two net tunnels and two open field plots. Misungwi district, Mwanza, Tanzania. Photo credit: K. Ogero/CIP.

HOBO® Pro v2 Temperature/Relative Humidity data logger by Onset Computer Corporation (470 MacArthur Blvd, Bourne, MA 02532) was used to measure relative humidity and temperature in the net tunnels and open field plots.

c) Planting and crop management

At the beginning of the experiment each treatment was planted with forty 3-node, clean, virus-tested cuttings sourced from TARI – Ukiriguru. The first harvest was done 85 days after planting. Subsequent harvests were done after every 60 days. Vines of plants under the ratooning cropping technique were harvested at about 15 cm above the ground and left to sprout again. Therefore, the next crop was the result of the previous crop regrowth. This was repeated for the subsequent harvests. On the other hand, plants following the replanting cropping technique were uprooted during each harvest and 3-node vine cuttings obtained from the apical portions were replanted. The experiment was run for 14 months (September 21, 2019, to November 17, 2020) representing six generations of vine production. A mulch of rice husks was applied at establishment and after every harvest to suppress weeds. In addition, any weed that may have established during the growing period was uprooted during harvesting. During the dry season, irrigation was done three times per week. Three buckets of composite manure were applied per main plot during establishment. After every harvest, NPK (17:17:17) was applied at 200 g per main plot.

d) Data collection and analysis

The total number of vines produced per treatment was recorded at each harvest. At the same time, five vines were randomly selected from each treatment to determine the average number of nodes and vine length. Three-way analysis of variance (ANOVA) for split-split plot design was used to determine whether there were any statistically significant effects of the three factors: - cover type, variety, and cropping technique or their two-way or three-way interactions on the continuous dependent variables of number of vines, number of nodes and vine length. The analysis comprised of the second to the sixth generations representing the first to the fifth ratooning and replanting. Generation one was left out because it represented experimental establishment, i.e., initial planting of the crops that would be ratooned and those to be replanted and therefore no differences in treatments.

In recording virus incidences, the number of plants with visual virus symptoms was counted during harvest and virus incidence per treatment plot was calculated as follows:

$$Virus\ incidence\ (\%) = \frac{Number\ of\ infected\ plants}{Total\ number\ of\ plants\ per\ treatment\ i.e.\ 40} x100$$

3 Results

Temperature and relative humidity

The average temperature and relative humidity in net tunnels and open fields were similar. However, the highest temperature and relative humidity were recorded in the net tunnels (Table 1).

Table 1. Temperature and relative humidity in net tunnels and open fields.

	Temperature, °C			Relative Humidity, %		
	Average	Highest	Lowest	Average	Highest	Lowest
Net tunnels	23.8	32.0	19.1	80.4	100.0	51.9
Open fields	23.5	29.2	19.5	82.2	99.3	58.4

Number of vines per plot

There was a significant interaction between variety and cropping technique ($p = 0.04$), with larger differences between techniques for cv. Mataya than for cv. Kabode. The ratoon cropping technique produced more vines than the replanting technique ($p < 0.001$), irrespective of cover type (open field vs. net tunnel), as well as across the two varieties (Fig. 3). For cv. Kabode, the two cropping techniques did much better in open fields than in net tunnels. Open fields for ratooned crop consistently produced more vines than replanted crops across the generations ($p < 0.001$) and were significantly different at each generation. Cv. Mataya produced many more vines than cv. Kabode in both the net tunnels and in the open fields. In addition, the variety's ratooned plants produced significantly more vines than replanted plants in all but the sixth generation. This was observed both in net tunnels and open fields.

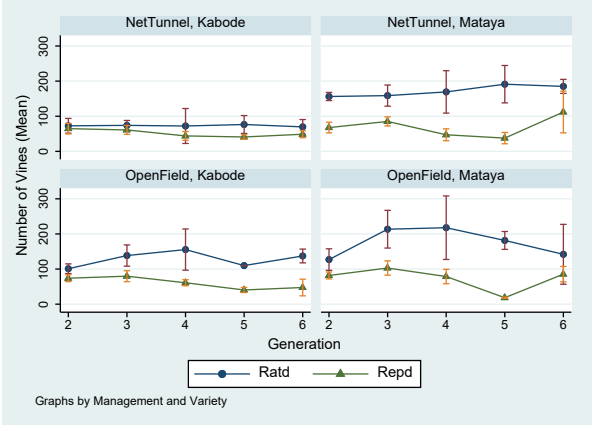

Figure 3. Trend in number of vines produced in net tunnels and open fields under ratoon cropping technique (Ratd) and replanting technique (Repd).

Vine length

Ratooned and replanted plants of both varieties planted in net tunnels produced longer vines than those planted in open fields ($p < 0.0001$; Fig. 4). The ratooned and replanted plants of the two varieties produced the longest vines in the third and sixth generations both in the net tunnels and open fields. For cv. Kabode, ratooned plants in open fields had longer vines compared to those replanted in all generations, with significant differences in the third and fifth generations ($p = 0.0006$). In net tunnels, the ratooned plants produced longer vines than replanted plants in all but the last generation. However, this was significant only in the fifth generation ($p = 0.004$). Plants of cv. Mataya grown in net tunnels under the ratoon cropping technique produced longer vines than replanted plants in all generations. This difference was significant only in the fifth generation ($p = 0.002$). In the open fields, the variety also produced longer vines under ratooning than after replanting in generations two to five, with significant difference observed in generation five ($p < 0.0001$). Plants of cv. Mataya replanted in open fields in generation six produced significantly longer vines than ratooned plants.

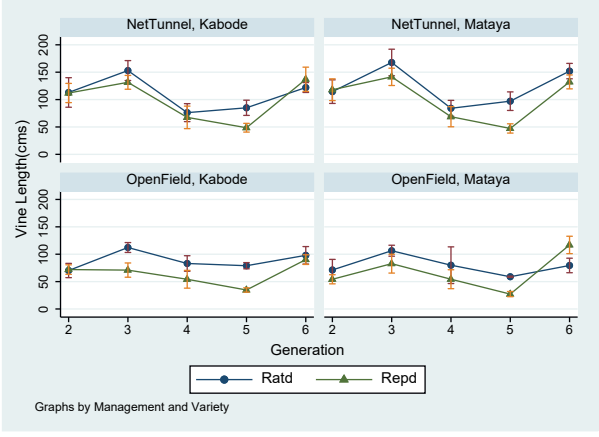

Figure 4. Trend in average vine length in net tunnels and open fields under ratoon cropping technique (Ratd) and replanting technique (Repd).

Number of nodes per vine

Vines from ratooned plants of cvs. Kabode and Mataya produced more nodes compared to replanted plants from the second to the fifth generation (Fig. 5). This was observed both in net tunnels and open fields. However, the difference between cultivars was not significant in all generations and cover types. For cv. Kabode, the difference between cropping techniques was significant only in open fields during the third and fifth generations. In the last generation, replanted plants of cv. Kabode grown in net tunnels produced more nodes per vine than the ratooned plants.

For cv. Mataya, ratooned crops produced significantly more nodes per vine than replanted crops both in net tunnels and open fields in the fifth generation.

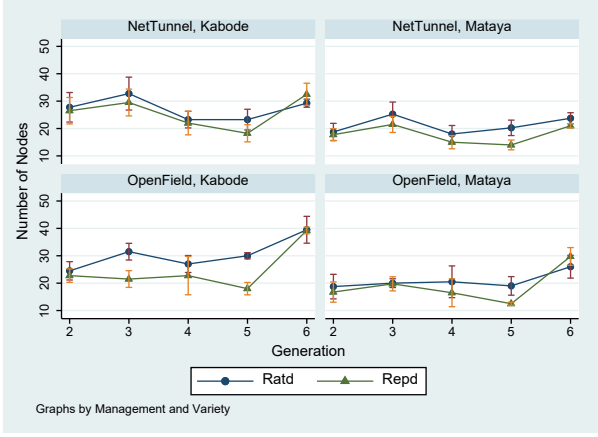

Figure 5. Trend in number of nodes per vine produced in net tunnels and open fields under ratoon cropping technique (Ratd) and replanting technique (Repd).

Virus incidences

Ratooned and replanted plants grown in net tunnels had no virus symptoms during the entire experiment. Replanted plants grown in open fields also had no virus symptoms in all the generations. On the other hand, ratooned plants grown in open fields started showing virus symptoms in the fourth generation (Fig. 6). The symptoms increased in the fifth and sixth generations with cv. Mataya being more affected than cv. Kabode.

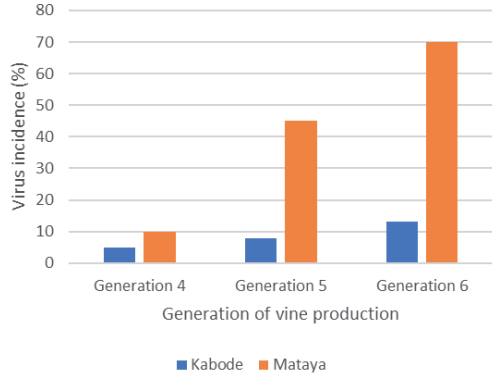

Figure 6. Percentage virus incidences on ratooned plants grown in open fields for cv. Kabode and cv. Mataya.

4 Discussion

Effect of ratooning on vine production

The ratoon cropping technique, also known as the 'reaping and regrowth system of production', has been reported to increase yields in plants that can regenerate leaves and/or shoots after harvesting leaves or shoots and is maximized in vegetable production (Fu, 2008; Riga, 2008). Sweetpotato behaves similarly producing new shoots from vines that have been harvested ten centimetres above ground thereby enabling seed producers to maximize seed production from a single planting. In this study, it was demonstrated that ratooning maintains vine production in net tunnels better than replanting after each harvest (Fig. 3). This may be explained by more frequent branching in ratooned crops by removing apical dominance (Wang et al., 2020). In addition, old stems and roots tend to have more carbohydrate reserves that give the ratooned crop a head start compared to the replanted crop (de Booysen & Nelson, 1975). This has been observed in other crops as well. For instance, sugarcane plants with the entire root system intact have been reported to produce photo-assimilates earlier, expediting regrowth and leading to more accumulation of biomass than in plants with 50% of the root system (Pissolato et al., 2021). The replanted crop requires more time to develop roots and leaves to photosynthesize. The newly planted crop does not use light and thermal sources efficiently at this stage of root development (Xu et al., 2021).

The recommended harvest time of seed vines from newly planted sweetpotato vine cuttings is 90 days whereas a ratooned crop can be harvested after 40 – 60 days (Stathers et al., 2018). This means that the replanted crop needs more time to fully develop. This may also explain why the ratooned plants had longer vine length than the replanted plants (Fig. 4).

The superior effect of the ratoon cropping technique was also noted in the number of nodes produced (Fig. 5). The number of vines and the number of nodes per vine are important parameters in sweetpotato seed production in sub-Saharan Africa. Therefore, by producing more vines and more nodes per vine, ratooning increases the number of seed cuttings produced. Each node has a meristem and seed is usually multiplied by planting three-node cuttings, with two nodes being buried in the soil to produce roots while one node remains above ground to produce a shoot. The more nodes per vine the higher the number of seed cuttings than can be realized.

Effect of genotype on vine production

Ratooned and replanted vine production in net tunnels and open fields was influenced by variety. Cv. Kabode produced more vines in open fields than in net tunnels regardless of the cropping technique (Fig. 3).

On the other hand, cv. Mataya produced a similar number of vines in net tunnels and open fields for both ratooned and replanted crops. Genotypic variation influences sweetpotato vine production and this has also been reported in plants produced in screen houses using a sandponics system (Makokha et al., 2020). The morphology of cv. Kabode might have contributed to the production of more vines in open fields than in net tunnels. The variety is semi-erect and tends to grow upwards when grown in areas with limited space as was the case in the net tunnels.

Open fields provided enough space for the variety to spread and branch. This might have increased the number of leaves and therefore increasing the leaf area. By spreading, the plant increases the area exposed to light therefore increasing plant photosynthesis. Plant type plays a key role in the interception and utilization of solar energy which is important in increasing canopy photosynthesis (Brodersen & Vogelmann, 2010; Feng et al., 2016). Yin and Struik (2017) reported that the daily canopy photosynthesis increases with increasing leaf area index because of a higher interception of photosynthetically active radiation.

Ratooning and virus incidences

The ratoon cropping technique produced more vines in both the net tunnels and in the open fields, but it also added to a progressive increase in virus incidences on plants grown in the open (Fig. 6). Ratooning has been reported to increase disease incidences and build-up of pests (Plucknett et al., 1970). Previous studies have demonstrated this in rice (Santos et al., 2003) and sugarcane (Young, 2018). Sweetpotato viruses are systemic infecting all parts of the plant, and ratooning may lead to a gradual increase in overall virus titres in plants. Previous reports indicated persistence of viruses in sweetpotato plants over several cropping cycles (Gibson & Kreuze, 2015). The plants grown in net tunnels did not show any virus symptoms, supporting previous findings by Ogero et al. (2019) that the insect-proof nets protect clean seed from reinfection. Open field production exposes clean seed to virus vectors that can transmit diseases from nearby fields. The extent of infection and symptom expression is influenced by virus tolerance levels of a particular cultivar as shown by differences in virus incidences between cv. Kabode and cv. Mataya in this study. The virus incidences in this study were based on visual symptoms. Testing of plant samples using a sensitive molecular-based diagnostic method could have given a better understanding of the incidences even for symptomless plants.

Conclusions

This long-term experiment has shown that ratooning has an advantage on sweetpotato vine production, not only in net tunnels but also in open fields. The technique can make management of vines grown in net tunnels easier. The net tunnels are small measuring 3.0 m × 1.8 m × 1.4 m which makes it tedious for an adult to enter for regular management.

Replanting means spending more time inside which can discourage someone from adopting the technology. With ratoon cropping technique the vine production cycle is reduced from 90 days (as is for newly planted cuttings) to 40 – 60 days thereby reducing labour costs per cycle. In addition, the ratoon cropping technique reduces costs of production because replanting is not required after every harvest. Based on these findings we recommend the technique for sweetpotato vine production in both net tunnels and open fields. The technique can contribute towards easier adoption of insect-proof net tunnels to protect clean sweetpotato seed from virus reinfection. If used in open field production, it should be accompanied with a suite of virus management practices to limit reinfection and increase the number of generations for seed production. This may include rogueing of infected plants, isolating seed production plots and using the technique for more resistant varieties that can withstand open field multiplication. This is the first detailed study investigating the effect of ratooning in sweetpotato seed production. The study focused mostly on parameters associated with the number of seed vines produced, i.e., number of vines, number of nodes per vine and vine length, and touched only partly on virus infection in open fields. Further studies are needed to investigate the effect of various environmental and management factors on regrowth of ratooned vines. These may include effect of temperature, water stress and carbohydrate reserves in root stocks. It is also important to regularly test ratooned plants for viruses to avoid keeping plants that are already infected.

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Female farmers cutting sweetpotato vines into 30 cm long cuttings. A single sweetpotato vine can provide 3 - 7 cuttings each measuring about 30 cm. The 30 cm long cutting is the one planted as seed for root production. Geita district, Tanzania. Credit: K. Ogero.

Farmers transporting sweetpotato seed on a donkey-drawn cart. Bukombe district, Tanzania. Credit: K. Ogero.

Chapter 5:

Managing Sweetpotato Seed Degeneration at the Lake Zone Tanzania: Farmers' Experiences, Actions and Seed Sourcing Strategies

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Abstract

Seed degeneration caused by virus infection is a key limitation to sweetpotato production in Tanzania as it leads to progressive decline in root yields. The use of clean virus-tested planting material is promoted as one way of reducing the effect of viruses on yields. However, adoption of this practice remains low with most farmers recycling planting material from season to season. We assessed farmers' perceptions and responses towards degeneration with an aim of gaining insights that can inform establishment of sustainable clean seed programs. A 'small-N' survey was conducted with 37 respondents in the Lake Zone Tanzania. We found that women farmers experienced degeneration more than men. The main action to address degeneration was to acquire a new variety, mostly from close sources in their social networks. Planting material was mostly acquired and shared as gifts. The findings of this study show that farmers' networks for sharing sweetpotato planting material, in combination with recycling degenerated seed, play an important role in degeneration. Reducing degeneration requires a strategy of introducing virus resistant varieties while involving women more in training in agronomic practices and as key nodes for dissemination of information on control of degeneration.

Key words: Virus infection, recycling, clean virus-tested seed, networks, seed vines

1 Introduction

Tanzania is the third largest producer of sweetpotato globally, but the yields are very low due to high virus pressure and on-farm recycling of planting material. Recycling of planting material in sweetpotato and other vegetatively propagated crops leads to pathogen accumulation in the starting material (so-called degeneration) and in the crop produced from it, thereby reducing crop yields (Adikini et al., 2016; Andrade et al., 2009; Jacobsen et al., 2019; McEwan et al., 2021; Shirima et al., 2019; Thomas-Sharma et al., 2016). Sweetpotato degeneration in Tanzania is largely attributed to synergistic interactions between sweet potato feathery mottle virus (SPFMV), belonging to the genus *Potyvirus*, and sweet potato chlorotic stunt virus (SPCSV), belonging to the genus *Crinivirus* (Gibson and Kreuze, 2015). Highest virus incidence and diversity have been reported in the regions around the Lake Victoria Basin which is also leading the country in sweetpotato production (Tairo et al., 2004). The synergistic interaction between SPFMV and SPCSV causes a disease complex known as sweet potato virus disease (SPVD) which can cause over 90% yield loss in susceptible varieties (Adikini et al., 2016). Sweetpotato-infecting viruses from the genus *Begomovirus,* known as sweepoviruses are also becoming increasingly important. These sweepoviruses can cause 10-80% yield loss depending on cultivar susceptibility (Kim et al., 2015; Wanjala et al., 2020). This yield loss happens even without clear symptoms in the above-ground parts of the infected plants.

The nature of the dominant sweetpotato seed system in Tanzania makes it difficult to control degeneration. More than 95% of seed flows are within informal, farmer-based seed systems which involve farmer-to-farmer exchange of planting material that is often sourced from the previous crop (Namanda et al., 2011). Farmers may also re-use vines that they have conserved from the previous season.

Several multi-stakeholder initiatives have been implemented in the Lake Zone of Tanzania with the aim of promoting an alternative system that can guarantee the quality of seed and therefore reduce degeneration. This includes distribution of clean virus-tested planting material whereby farmer-preferred landraces and improved varieties are cleaned of viruses and disseminated to farmers through centralized or decentralized approaches (Adam et al., 2018; McEwan et al., 2017). Decentralized strategies are the most popular approaches advocated by seed system experts and involve identifying and training farmers who can become specialized seed producers (Adam et al., 2018; Low and Thiele, 2020; McEwan et al., 2017). These decentralized seed producers (also known as decentralized vine multipliers or DVMs) are empowered with agronomic and business skills that are key in running a seed enterprise. The seed producers live within the communities they serve, making it easier for farmers to access quality planting material. The aim is to reduce distances travelled to acquire planting material and therefore discourage recycling.

However, sometimes such initiatives may overlook existing farmer-based seed systems (Almekinders et al., 2019). Complementarity of decentralized approaches often implemented by institutions such as the national agricultural research institutes and farmer-based seed systems can enhance farmer access to quality planting material but depends on farmers' motivations to use a particular source (Lukonge et al., 2015).

Uptake of clean virus-tested planting material also depends on farmer awareness on the benefits and sources of such seed. However, not all farmers can access such information because the government extension services, the primary channel for dissemination of agricultural information, are not effective due to low ratio of extension agents to farmers. The Tanzania national agriculture policy identified poor linkages between farmers, extension services and researchers as one of the challenges towards technology adoption in agriculture (The United Republic of Tanzania, 2013). Apart from the weakness of the extension system, interactions between farmers and other stakeholders, including scientists, are also minimal or missing (McEwan et al., 2020). This threatens not only adoption of quality seed but also other important productivity-enhancing technologies. Effective knowledge development and information sharing has been shown to be crucial in dissemination of agricultural technologies (Gildemacher et al., 2009). Other methods for information sharing include signposts, village meetings, champion farmers, mass media and mobile phones. Mobile phones are now widely acknowledged as a major channel for information sharing within the agricultural sector (Obong et al., 2018). Damtew et al. (2018) noted the role of mobile phones in catalyzing collaboration and information sharing among various stakeholders in the control of bacterial wilt and late blight in potato in Ethiopia. Here, extension officers combined mobile phones and farmer networks (groups) to disseminate information, including management strategies for the two diseases. Understanding how farmers get and share information can help identify key nodes for technology transfer and information sharing on management of degeneration.

While the impact of degeneration on sweetpotato productivity in Tanzania has generated interest from researchers it is not clear how farmers experience it and what they do to address it or cope with it. In most cases, farmers recognize virus symptoms especially on highly susceptible varieties, but it is not clear if they associate these with seed degeneration. The assumption that yield loss will stimulate farmers to increase investments in quality seed does not seem to be valid. It is not clear if all sweetpotato farmers recognize seed degeneration. Another ambiguous issue is how those experiencing seed degeneration cope with it. In addition, it is important to understand how farmers exchange information about varieties, markets, and other agricultural technologies. This can provide important information on the best entry point for clean and virus resistant varieties to improve management of degeneration.

Herein, we first describe farmers' perceptions and actions towards seed degeneration in sweetpotato. Second, we identify popular sources of seed and seed sharing relationships. We finish by identifying farmers' most important sources of information on sweetpotato production. The results will contribute towards building sustainable channels for dissemination of clean seed and virus management strategies in sweetpotato.

2 Methodology

a) Study area

This research was conducted in Geita, Mara and Mwanza regions in the Lake Zone Tanzania (areas bordering Lake Victoria). The Lake Zone leads in sweetpotato production in Tanzania. Four districts that had previously benefited from a sweetpotato seed systems intervention (Marando Bora "Quality Vines") were covered (Fig.1).

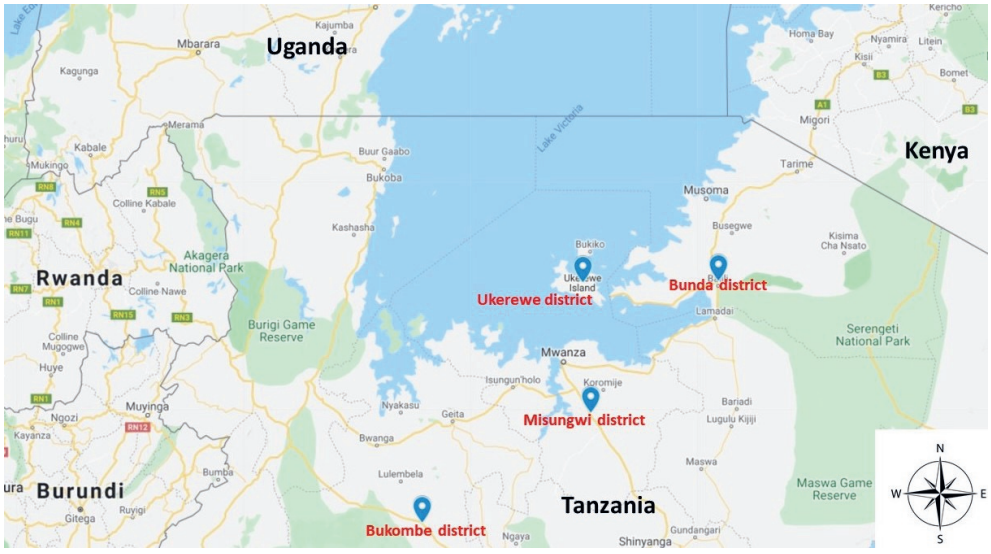

Figure 1. Map indicating Bukombe, Bunda, Misungwi and Ukerewe districts where the study was conducted.

The districts were purposively selected to cover differences in sweetpotato production practices (Table 1).

Table 1. Characteristics of study districts in terms of sweetpotato production.

District	Description
Bukombe	Major commercial production of sweetpotato roots; large scale farmers produce seed for own use but sell the surplus; farmers have embraced the habit of seed buying; one cropping season.
Bunda Rural	Semi-commercial production of sweetpotato; buying of seed is not common; one cropping season.
Misungwi	Mostly subsistence production; the district experiences a long dry period between May and September with most farmers losing all the vines; some seed buying emerging; two cropping seasons.
Ukerewe	Mostly subsistence production; buying of seed is not common; two cropping seasons.

b) Study design and data collection

The study piggybacked on another study that aimed at assessing the status of decentralized vine multipliers (DVMs) established under the "Marando Bora (Quality Vines)" component of the Sweetpotato Action for Security and Health in Africa (SASHA) Phase 1 project which was implemented in the Lake Zone, Tanzania from 2009 – 2012. This enabled us to also know if active linkages still existed between farmers and DVMs. The DVMs were farmers who had been trained in good agronomic practices and business skills for sweetpotato seed production. They were usually located in areas where there was extensive sweetpotato production but linked to upstream sources of early generation seed. Capacity building for DVMs was mostly through projects. Sometimes DVMs sold seed at subsidized prices due to the support they got from projects therefore leading to low costs of production.

A 'small-N' exploratory case study method was used to collect data from 37 respondents (Almekinders and Bentley, 2021). This method uses a survey that is exploratory in nature and comprises a small sample size of 35 – 50 respondents. It uses both qualitative and quantitative data and is more oriented towards a broad description than towards statistically significant differences and correlations. Thirty-seven interviews were conducted to understand various aspects associated with seed degeneration including farmers' experiences with degeneration, acquisition of new varieties, replacement of planting material of existing varieties and influence of social relationships on seed acquisition among others.

Respondents were purposively selected with the help of village extension officers (VEOs) to arrive at a gender-balanced group of farmers who were actively involved in sweetpotato farming as individuals. Twenty female and 17 male farmers were interviewed. The interviews were conducted in Kiswahili and technical terms were phrased in ways that farmers could understand them.

For instance, to ensure that we captured farmers' real experiences regarding degeneration and avoid researcher bias we asked if the respondents had experienced varietal tiredness. The question was phrased as follows: "Some people talk about a variety that has become 'tired'. Have you experienced that?" If they said yes, they were further asked to describe the signs they saw when a variety became tired, why they thought a variety became tired and what they did upon seeing signs of varietal tiredness. 'Tiredness (*kuchoka* in Kiswahili)' was therefore used as a synonym for degeneration. Another local term farmers used to describe this upon further probing was "*kuzara*". For consistency, "degeneration" will be used in the next sections. Qualitative and quantitative data were collected using a semi-structured questionnaire. Additional information and field observations were captured in notebooks. Each interview took approximately 1.5 hours.

c) Data analysis

Data from the interviews were entered and coded in Excel®. Analysis was done based on the frequencies of answers to identify differences between female and male interviewees for the various parameters. Numerical presentations were analyzed using descriptive statistics. The study was exploratory in character and did not warrant further statistical analysis apart from the scoring of common sources of planting material in terms of access, trust, availability, affordability and quality. Here, one-way analysis of variance (ANOVA) was used to understand whether the rating of sources of vines based on the different parameters differed by gender. For those variables that were tested statistically different, the Tukey test was used for mean separation.

3 Results

a) Farmers' experiences and actions towards degeneration in sweetpotato

Most farmers interviewed had experienced varietal degeneration. The number was higher for female respondents with 80% of them saying they had experienced degeneration while only 41% of the male respondents said they had experienced degeneration. The most common signs of degeneration referred to by both female and male farmers were related to yield reduction (Table 2). This included production of fewer and smaller roots over time, failure of plants to produce storage roots even when looking healthy, and prolonged maturity time. A female farmer noted: *"Roots do not bulk; sometimes the vines look healthy but there are no storage (bulked) roots. The vines can also become very small."* Yellowing of leaves, stunting and leaf curling, symptoms generally indicative of virus infection, were the second most common indicator for degeneration.

Almost half of both female and male respondents said that degeneration is caused by "*kuilima kwa miaka mingi*", i.e., recycling the same crop for a very long time. One female farmer linked this to aging in human beings, saying, "*The variety has become old. It is the same as human beings, a person is active when young but becomes less and less active as he/she grows old.*"

Table 2. Signs and reasons associated with sweetpotato varietal degeneration (n=23).

Signs and reasons for varietal degeneration	Female (n=16)*	Male (n=7)*
Signs of varietal degeneration:		
Does not produce any storage root even though the vines look healthy; prolonged maturity period; produces fewer and smaller roots; no root bulking	15	6
Yellow leaves; curled leaves; stunted growth	8	2
White hairs on the leaves; roots start to rot and are infested by white worms; vines get cut at the base	1	2
Leaves turn brown	0	1
Reasons for varietal degeneration:		
Recycling the same crop for a long time	7	3
Infection by diseases	4	4
Affected by drought	2	0
Infested by pests	1	3
Poor soil fertility	1	1
I do not know	6	1

*Farmers could mention more than one sign or reason.

Most female and male farmers said that they would get a different variety from other farmers if what they planted was degenerated (Fig. 2). Getting new planting material of the same variety was not a common strategy and removal of infected plants was only mentioned by one female farmer. Sixty-six percent (66%) of female respondents and 78% of the male respondents did not have specific timelines for acquiring new planting material of varieties they already had.

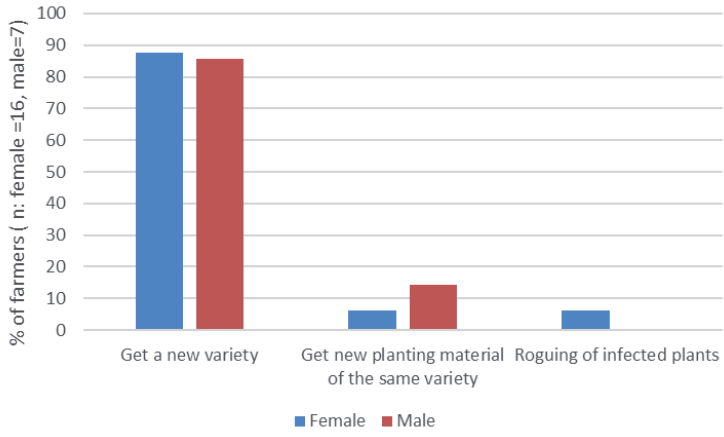

Figure 2. Percentages of female and male farmers (n=16 and n=7, respectively) taking actions in response to experiencing degeneration.

b) Sourcing of vines/planting material

Varieties being grown

Forty-three varieties were mentioned indicating high varietal diversity. Table 3 captures the top eleven varieties. On average a farmer was growing three varieties.

Table 3. Main sweetpotato varieties grown by the respondents.

Variety	Percentage of female farmers planting (n=20)*	Percentage of male farmers planting (n=17)*
Improved:		
Kabode	55	35
Polista	40	35
New Dimbuka	15	24
Mazao	20	12
Kakamega	15	12
Local:		
Umeme	25	18
Winnie	20	6
Chupi ya Mbole	15	12
Mwanatatata	15	6
Kilihona	20	0
Ukimwi	5	18

*Farmers could mention more than one variety

The own farm was the primary source for planting material (Table 4). Farmers sourced from DVMs because they considered them to supply quality seed and new improved varieties and when planting material was scarce. More women acquired seed through DVMs and projects than men.

Table 4. Number of female and male farmers mentioning use of different sources of planting material (n=37).

Source	Female (n=20)*	Male (n=17)*	Reasons for using source
Own farm	18	14	Able to conserve own vines; trusts own seed; reduce costs.
DVM	16	7	Scarcity of planting material; source of quality seed; source of new varieties.
Neighbor	12	11	Mutual dependence when in need to complement; affordable.
Relative	6	6	Mutual dependence when in need to complement; new variety.
Projects	6	2	Free seed; new variety.
Total	58	40	

*Farmers could mention more than one source.

Gender did not matter for the type of acquisition of vines for local varieties: both male and female farmers predominantly acquired their planting material from their own farm and through gifts (Fig. 3). For the acquisition of vines for improved variety, male farmers more often acquired them from a source with a cash-transaction.

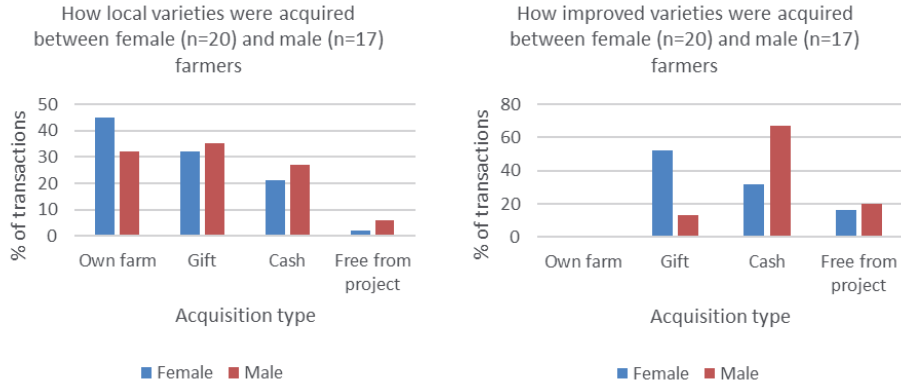

Figure 3. How female and male farmers acquired local and improved varieties planted in the previous season.

Female respondents scored material sourced from own farm the highest in terms of accessibility, availability, and trust (Table 5). Men put more trust in planting material from DVMs followed by that from their own farm. This was opposite to women who put more trust in planting material from their own farm than that from DVMs. Both female and male respondents said planting material from projects was the most affordable. However, it was more accessible for female farmers than male farmers. Decentralized vine multipliers got the highest score in terms of quality among both the male and female respondents.

Table 5. Scoring of common sources of sweetpotato seed by interviewed female and male farmers in terms of access, trust, availability, affordability, and quality.

		Parameter and average scores (1=Very poor; 5=Very good)				
Gender	**Source**	**Accessibility**	**Availability**	**Affordability**	**Quality**	**Trust**
Female	DVM	3.69 (1.25)	3.56 (1.09)	3.81 (1.05)	4.75 (0.58)	4.50 (0.73)
	Neighbor	2.93 (1.22)	3.53 (1.06)	3.40 (1.24)	3.47 (0.92)	3.47 (0.92)
	Own farm	4.17 (1.20)	4.06 (1.06)	4.33 (0.84)	4.50 (0.86)	4.61 (0.78)
	Project	3.50 (1.64)	2.50 (1.52)	4.67 (0.82)	4.50 (0.84)	4.33 (0.82)
	Relative	3.75 (0.96)	3.25 (0.96)	3.75 (0.96)	3.75 (0.50)	4.00 (0.63)
	P value	0.1	0.07	0.05	0.0003	0.001
Male	DVM	4.00 (1.00)	4.00 (1.15)	4.57 (0.79)	4.86 (0.38)	4.86 (0.38)
	Neighbor	3.60 (0.99)	3.60 (1.30)	3.47 (1.36)	4.13 (0.92)	4.20 (0.86)
	Own farm	4.29 (0.99)	3.86 (1.35)	4.29 (1.07)	4.50 (0.76)	4.64 (0.63)
	Project	1.00 (0.00)	2.00 (0.00)	5.00 (0.00)	4.50 (0.71)	4.00 (0.00)
	Relative	3.33 (1.03)	3.33 (1.03)	4.17 (0.98)	4.50 (0.84)	4.50 (0.84)
	P value	0.002	0.3	0.1	0.4	0.2

Standard deviation in brackets.

c) Seed sharing relationships

Sweetpotato planting material was shared as a gift, sold or exchanged with another item (Table 6). Gifting was the most common mode of transaction and was partly influenced by the quantity of planting material provided by the supplier. Farmers looking for a lot of planting material would be told to pay whereas those seeking smaller quantities could usually get them as gifts. The amount of planting material shared was dependent on the type of variety. It was common for local varieties to be shared and exchanged for cash in large quantities compared with improved varieties. The amount of planting material shared of improved varieties was higher when the transaction was cash-based compared to gifts.

Table 6. The type of transactions and average quantity in number of vines associated with sharing of sweetpotato planting material by the respondents.

	Improved varieties		Local varieties		
Transaction type	**No. of transactions***	**Av. Qty**	**No. of transactions***	**Av. Qty**	**Reasons for type of transaction**
Gift	21	374	50	2402	Close relatives; Mutual dependence when in need to complement; fear of being cursed; neighbors; had more than enough; almost root harvesting time hence did not need the vines; gave with a condition that they conserve; in the Wasukuma culture if someone requests for vines you give for free; needed a few to multiply further; so that they could assist in marketing
Cash	5	950	18	2185	They wanted to multiply further; I do not know them; scarcity of vines; customer came with money; from distant village; had also bought; had invested money to produce; urgently needed money
Exchange with another item (barter trade)	0	0	2	600	Recover some costs of production
Total	26		70		

*Some respondents had multiple transactions.

Selling and gifting were also influenced by the social closeness and gender of the beneficiary and the seed producer. Eighty-four percent of transactions within close social networks (relatives, friends and neighbors) were as gifts with only 13% being cash transactions (data not presented). On the other hand, 68% of planting material shared with unknown people was through cash transactions. Women shared predominantly with women and men mostly with men. A female respondent said, *"Sharing depends on relationship, but it is mostly between women".* In addition, female farmers shared more often than male farmers.

The maximum number of vine cuttings (30-cm long) shared for improved varieties was 1000. On the other hand, farmers shared between 1000 to 6000 cuttings for local varieties. Some farmers did not bother to quantify how much of the local varieties they gave rather letting the beneficiary to go to the farm and harvest without being monitored. However, it was considered important to show someone how to harvest.

A female respondent narrated how she lost her vines due to bad harvesting techniques: *"One day people from Simiyu region came to get vines and I directed them to the farm without showing them how to cut and they cut at the soil level. The vines did not sprout, and I lost everything."* Gifting was common at the end of the season when most people had a lot of 'unwanted' vines. The peak season for selling was December to allow time for multiplication of enough material between September and November. Also crop prioritization meant that most people would be planting maize in November. A male farmer in Bukombe district, said *"Most sales are in December because I do not like selling directly from the multiplication plot to avoid having a shortage".* A female respondent from Bunda district reiterated *"I start selling in November, but the peak is December because people are still planting maize in November.* Stress conditions can influence decisions to sell. A male respondent said the following regarding his decision to start selling, *"It is not the tradition to sell vines. However, we started selling/buying when drought became frequent."*

d) Information sources

Neighbors and other farmers were the main source of information in relation to growing a sweet potato crop, both in frequency and the breadth of the information (Table 7). Decentralized vine multipliers and extension staff followed, whereas signboards and mass media were the least used sources.

Table 7. Sources of different types of information.

Source	n*	Type of information	Reasons for using source	Info shared with
Neighbors and other farmers	24	New varieties; sources of seed; markets; production trends	Farmers are always on the lookout for better varieties; we interact regularly; they also grow sweetpotato; they live nearby	Neighbors; other farmers in my village; customers; relatives; friends
Decentralized vine multipliers	15	New varieties; production practices; benefits of orange-fleshed sweetpotato (OFSP) varieties and marketing	Trained; has links with TARI	Neighbors; friends; relatives
Government extension staff	13	Good agronomic practices including preparation of seed beds, proper planting, management of pests and diseases; new varieties; marketing techniques	Reliable; information has been vetted by the government; professionally trained; it is their mandate	Relatives; friends; farmers who source seed from me
NGOs	6	Benefits, uses and processing of OFSP; sources of seed and production	They brought us a project; their staff visit us often	Neighbors; farmers in my group; other farmers in my village
Customers (of both roots and vines)	4	Preferred varieties; markets	They understand demand; they know what is preferred in the market	Neighbors; farmers in my village
Mass media (radio, TV)	3	New varieties; benefits of OFSP; pests and diseases	They are also channels that are used to share agricultural information	Neighbors and other farmers in my village
Signboards	2	New varieties	Not answered	No one
Total	67			

*Farmers could mention more than one source.

4 Discussion

Experiences and actions on degeneration

This study indicated that more women experienced sweetpotato seed degeneration compared with men at the Lake Zone Tanzania. In Tanzania, sweetpotato is mostly grown by women for household consumption and has long been considered a 'female crop' (Echodu et al., 2019; Kapinga et al., 1995). The differences in cultivation, including purpose, scale of production and time dedicated to the crop, between men and women means that women have more experience with and better understanding of sweetpotato agronomy compared to men.

This is supported by Adam (2015) who reported that the number of sweetpotato plots controlled by a woman had an influence on knowledge and management of sweetpotato diseases, with this knowledge increasing as the number of plots increased. These differences between men and women in experience with sweetpotato cultivation may have implications on both management of seed degeneration and adoption of new varieties.

It is noteworthy that most of the farmers recycled seed, yet recycling was mentioned as the main cause of degeneration. Recycling of planting material leads to accumulation of viruses within the plant which causes progressive yield reduction (Adikini et al., 2015; Gibson et al., 2014; Ssamula et al., 2019). The progressive loss of productivity from season to season and symptoms of virus infection were mentioned as the main signs of seed degeneration indicating that most farmers in the study area have substantive knowledge on the phenomena. This adds to the need to probe further why farmers recycle seed even when they know the negative impacts. It is also important to understand why rogueing out virus-infected plants did not feature highly as a degeneration management strategy.

Sustainable models for seed systems are mostly anchored in farmers' willingness to purchase high-quality planting material regularly (Legg et al., 2022). However, in the case for sweetpotato and other vegetatively-propagated crops (VPCs) (Almekinders et al., 2019; McEwan, 2016) this is difficult to achieve in reality. The respondents in this study did not look for high quality planting material of the degenerated varieties rather opting to replace them with different varieties. This strategy may support a high varietal diversity within a small region as indicated by the high number of varieties (43) mentioned by the respondents. This is good for variety turnover and capitalizing on it can support the adoption of more and new improved varieties.

Sourcing and sharing of seed

The first 'go to place' for seed was the own farm although it was not mentioned as the most affordable. Friends and relatives were the dominant external sources of new seed and varieties within the study area indicating that sweetpotato seed is shared within very close social networks. Therefore, training those farmers in these networks, in particular women, who are usually relied upon for seed, can increase availability and access to quality seed. Farmers in the study area indicated that DVMs were the key source for high-quality seed. These DVMs had previously received training in good practices for quality seed production through past projects including Marando Bora (McEwan et al., 2017; Shikuku et al., 2019). Trainings aimed at improving the existing system can also lead to a spillover in terms of information sharing. New information can be targeted at farmers that are already sharing. We found that friends and other farmers were the key sources of information indicating that knowledge shared with a few farmers can reach many more. These can also act as key entry points for clean, virus-tested seed and new improved varieties.

Whereas the respondents acquired more improved varieties through cash-based transactions compared to local varieties, they shared both mostly through gifting. The improved varieties were mainly sold when the recipient needed high quantities. Achieving a more commercial system requires change of mind-sets to embrace more cash-transactions when acquiring and sharing seed. However, this is also influenced by other socio-cultural factors that lead to gifting as previously indicated by Tadesse et al. (2017) in Irish potato and Kilwinger et al. (2020) in banana.

Conclusions

Strategies seeking to reduce seed degeneration need to use experienced female farmers as champions for knowledge sharing. Taking advantage of farmers' practice of addressing degeneration by getting different varieties can contribute towards increased adoption of improved varieties as envisioned in the CGIAR 2030 strategy (CGIAR, 2021). However, the lack of systematic plans for replacing planting material of the same varieties and reliance of own farm as a primary source of cuttings may negatively affect profitability of quality seed production. Farmer-farmer networks play an important role in sweetpotato seed sourcing but can lead to spread of viruses, especially those that are symptomless. Given that these systems will continue to be crucial in sweetpotato seed production for a long time it is important to implement strategies that improve the capacity of key seed producers to detect and manage sweetpotato viruses. Using experienced women farmers to mentor others and identifying mechanisms whereby new knowledge and skills are passed between spouses may lead to better management of seed degeneration. Such efforts can create an interface between the 'formal' and 'informal' seed systems. It is also important to understand why farmers recycle seed even when it has degenerated. Understanding the other sociocultural factors that determine retention of degenerated planting material and varieties can provide crucial information towards building sustainable seed delivery models.

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The author (L) and the District Crops Officer, Mtatiro Nyandisi (second left) joining farmers in showcasing high yields from clean seed of improved varieties. Bukombe district, Tanzania. Credit: Z. Machunde.

Chapter 6:

General Discussion

1 Introduction

How can cleaned-up, virus-tested seed be incorporated into smallholder farming systems to reduce degeneration in sweetpotato in the Lake Zone Tanzania? This is the overarching question that led to my research on sweetpotato seed degeneration at the Lake Zone Tanzania and the role of clean virus-tested seed as a control strategy. Sweetpotato seed degeneration is defined as yield loss caused by virus infection carried in vine cuttings (Gibson & Kreuze, 2015). Thomas-Sharma et al. (2016) also defined seed degeneration in vegetatively propagated crops as the build-up of diseases and pests in/on the seed over consecutive seasons of on-farm propagation therefore reducing seed health and progeny yield. The main cause of seed degeneration in sweetpotato is viruses and these are systemic with titers increasing progressively within the plant with time and across generations. Use of infected plants for propagation leads to decline in yields as the virus titers increase from season to season and the virus spreads through the crop.

So far, the main viruses causing seed degeneration in sweetpotato are the aphid-transmitted sweet potato feathery mottle virus (SPFMV) and the whitefly-transmitted sweet potato chlorotic stunt virus (SPCSV). Sweet potato feathery mottle virus is a mild virus and can cause negligible to 40% yield losses depending on the environment and cultivar susceptibility while SPCSV can cause more than 50% yield losses (Adikini et al., 2016; Loebenstein, 2012). When co-infecting, the two viruses cause a disease complex known as the sweet potato virus disease (SPVD) which can lead to 100% yield loss in susceptible varieties (Ateka et al., 2004; Kreuze et al., 2021; Mukasa et al., 2006; Ndunguru et al., 2009). Sweetpotato infecting members of the genus *Begomovirus*, known as sweepoviruses, are also becoming of interest as they have been shown to cause considerable yield losses even without above-ground symptoms (Mulabisana et al., 2019; Wanjala et al., 2020).

The overarching research question was informed by the promotion of cleaned-up, virus-tested seed as one of the control strategies, the others being breeding for virus-resistance and on-farm management of infections (Loebenstein, 2012; Thomas-Sharma et al., 2016; Valverde et al., 2007). Use of cleaned-up, virus-tested seed being one of the key strategies in sub-Saharan Africa, Tanzania included (McEwan, 2016), it was important to understand how clean seed degenerates under farmer conditions in different environments, considering differences in cultivar resistance to viruses. Experiments were conducted in environments with a high or a low virus pressure to assess how seed sourced from clean virus-tested plants of varieties with different virus resistance levels degenerated following successive seasons of propagation by farmers (Chapter 2). Although this would contribute towards building evidence on the yield benefits of clean seed, it was important to explore other factors that would influence adoption of such seed.

Farmers' demand for quality seed has also been reported to be influenced by factors that are beyond the physical seed characteristics e.g., prices, sources and social relations (Kilwinger et al., 2020; McEwan et al., 2021). Also, it is not clear if all sweetpotato farmers recognize seed degeneration and what they do to manage it. Chapter 5 investigated farmers' seed sourcing strategies, their experiences with seed degeneration and actions to address degeneration.

It has been noted that clean seed can get infected once it gets out of the controlled screenhouse environment and farmers are encouraged to implement measures that can limit infection, e.g., by keeping vectors out of the seed beds (Dennien & Henderson, 2018; Milgram et al., 1996). Screenhouses play an important role in keeping virus vectors out but they are unaffordable to most farmers in Tanzania. We assessed the effectiveness of relatively low-cost insect-proof net tunnels as an alternative to the screenhouses and demonstrated that they were able to maintain the virus-free status of the clean plants for up to 20 months (Chapter 3) (Ogero et al., 2019). However, successful uptake of the technology also required inclusion of appropriate management practices to maintain the quality and quantity of planting material in the net tunnels. It was particularly important to understand how ratooning affected the quantity of planting material produced in the net tunnels overtime (Chapter 4).

In this chapter, I reflect upon the key findings from the various research questions in the context of a wider scientific debate in relation to sweetpotato seed systems. The findings are brought into perspective to draw insights that can contribute towards designing sustainable seed systems not only for sweetpotato but also other vegetatively propagated crops, in particular roots, tubers, and bananas.

2 Seed degeneration, does it matter?

Seed degeneration has been reported to cause significant yield losses in various vegetatively-propagated crops including banana, cassava, potato and sweetpotato (Gibson & Kreuze, 2015; Jacobsen et al., 2019; Navarrete, 2021; Shirima et al., 2019). It is important to consider how it translates into yield losses: is it a linear or a non-linear process? Chapter 2 of this thesis showed that the susceptible sweetpotato variety, cv. Ejumula, degenerated in a linear manner in the high-virus-pressure environment. This has also been observed in the USA on the variety Beauregard (Bryan et al., 2003). In addition, a progressive decline in yield has been reported in cassava plants infected with cassava brown streak disease (CBSD) in Tanzania (Shirima et al., 2019). While this hypothesis may hold true for some varieties and environments it is not always universally applicable.

For instance, Navarrete et al. (2021) did not find a progressive yield decline following several seasons of on-farm propagation of potato under farmers' conditions in Ecuador. Lewthwaite et al. (2011) also demonstrated that sweetpotato degenerated non-linearly in New Zealand with an initial rapid yield decline followed by a diminishing reduction with increasing iterations of field growing cycles. Chapter 2 also presented a non-linear pattern for yield loss for the susceptible variety at the low virus environment and for the more tolerant variety at both the high and low virus environments.

Some varieties have been reported to show signs of reversion whereby the host recovers from disease symptoms therefore making degeneration non-linear (Gibson & Kreuze, 2015; Jacobsen et al., 2019). Reversion is the ability of virus-infected plants to become mostly virus-free. In addition, other factors such as mineral nutrition may slow down the process or make it completely non-linear (Barker, 2009). Adding NPK fertilizer to the soil has been shown to increase the rate of reversion in sweetpotato (Ssamula et al., 2019). Chapter 2 showed higher yields in the high-virus-pressure site compared to the low-virus-pressure site despite the high virus incidences, differences that were directly related to soil characteristics. Hence, it is important to also consider soil fertility in seed degeneration models. Soil fertility is missing in the risk assessment framework for seed degeneration described by Thomas-Sharma et al. (2017). Consequently, the model's predictions on rates of degeneration may be inaccurate when a variety is grown in areas with different soil characteristics.

As we continue to study and develop better strategies to address degeneration, it is important to revisit the definition(s) of seed degeneration to understand where to focus on. Currently, the seed degeneration definitions can be divided into two types:

a) Progressive yield loss following season to season planting of infected planting material (Clark et al., 2002; Gibson & Kreuze, 2015).
b) Build-up of diseases and pests in/on the seed over consecutive seasons of on-farm propagation therefore reducing seed health and, consequently, progeny yield (Thomas-Sharma et al., 2016).

The first type of definition puts more emphasis on yield loss associated with poor seed health whereas the second type of definition stresses more the disease/pest incidences. Both definitions are valid but may influence what is seen as the impact of seed degeneration and subsequently farmer actions.

The first type of definition may resonate well with farmers because in the end the economic importance of degeneration is associated with yield losses and as shown in Chapter 5 farmers seek new varieties when they notice a decline in yield.

Farmers may not act to remove infected plants (known as rogueing) if that is not reflected in the root yields. This means that more tolerant varieties, which show less symptoms, may be recycled over many years even when infected. Unfortunately, this exposes susceptible varieties grown nearby to infections and risk of being wiped out. In addition, it may lead to an overall high disease pressure in production areas.

On the other hand, the second type of definition may lead to strategies that focus more on reducing disease/pest incidences. Such strategies can lead to reducing disease pressure in farming systems especially for varieties that are susceptible to viruses and for symptomatic viruses but may be challenged if the infected varieties are farmer-preferred. This might be circumvented by disseminating cleaned-up, virus-tested seed of the farmer-preferred varieties. However, adoption may be low if yield benefits are not clearly demonstrated when comparing clean seed and farmer-sourced seed. As presented in Chapter 2, even though clean seed of resistant varieties may yield more than farmer-sourced seed of the same varieties, the differences may not be significant enough to convince a farmer to invest in replacing seed. This may also be influenced by market dynamics and the commercial orientation of the farmer

3 How can clean seed be successfully incorporated in smallholder systems to address degeneration?

Chapter 2 showed that use of clean seed by farmers can reduce yield losses associated with degeneration, especially for susceptible varieties in high-virus-pressure environments. This means that the effective incorporation of clean virus-tested seed into smallholder farming systems needs to consider cultivar resistance and the prevailing agroecological conditions. Seed replacement rates for more resistant cultivars may be lower than for susceptible varieties.

Identifying a functional nexus between formal and informal seed systems is needed for a successful incorporation of clean seed in smallholder farming systems. More than 95% of sweetpotato seed is shared within close social networks as shown in Chapter 5. Therefore, it is important to consider the characteristics of these important networks to identify entry points for clean seed. The informal seed systems are highly flexible and able to supply a diverse range of varieties for different uses (Almekinders et al., 1994). Understanding this flexibility and the need for varietal diversity can contribute towards creating strong linkages with formal sources of clean seed and improved varieties. Usually, the formal system tends to focus on a few varieties therefore contradicting the informal system where diversity is embraced.

Understanding and incorporating market-preferred traits in breeding pipelines can lead to developing varieties that can be easily adopted within the informal system.

In addition to advising farmers to start with clean planting material it is important to consider the practices that have enabled them cope with degeneration and keep most of their landraces symptomless. The adoption of new varieties to replace degenerated ones (Chapter 5) is an indication that farmers are continuously selecting varieties and may end up with more resistant varieties. However, it is also important to probe why removal of infected plants is not a popular strategy.

It is important to understand the demand of different types of farmers. Farmer demand for seed is usually complex comprising of other characteristics beyond genetic and sanitary quality e.g., prices, delivery mechanisms and volumes (McEwan et al., 2021). Dissemination of clean seed should consider the channels through which farmers are currently getting seed. This is because some sources may be more trustworthy and reliable than others. Kilwinger et al. (2020) reported that sources of seed also matter because farmers have different views regarding attractiveness of various sources. Chapter 5 showed that men and women farmers trust different sources. It was also shown that social relations are important in sweetpotato seed acquisition and sharing. The main off-farm sources of planting material were friends, neighbours and relatives. Social relations are important not just for seed but also in information sharing and they are based on trust that has been built over time. These networks are very important in acquisition of seed of roots, tubers and bananas, whose seed systems are largely informal. Nduwimana et al. (2022) supports this in banana whereby banana seed in Burundi was shared within and between villages based on social and family linkages. This has also been reported in potato and cassava (Kilwinger et al., 2021; Tadesse et al., 2017). Characterizing the seed exchange networks can give a detailed description of the value embedded in them and how they can be tapped to disseminate clean seed. It can also help understand access of different types of farmers to different sources. For instance, in Burundi it was shown that male farmers had more access to clean seed from formal sources than female farmers (Nduwimana et al., 2022).

4 How can infection of clean seed be reduced?

Clean seed is not immune to viruses and can get rapidly infected once it gets into farmers' fields. It is therefore important to implement measures that can reduce the rate of infection. Chapter 3 showed that insect-proof net tunnels can successfully protect clean seed from attack by whiteflies and aphids thereby minimizing infection (Ogero et al., 2019).

Protected structures such as net tunnels and screenhouses can therefore be incorporated at seed producer level to maintain the integrity of clean seed. The technology is especially important in high-virus-pressure areas and may be better adopted by well-resourced seed producers. For successful adoption of net tunnels and screenhouses, it is important to look at the supply chain for the insect-proof net and commercial viability of using protected structures. A study in Uganda showed that using planting material sourced from net-protected structures led to higher root yields compared to planting material sourced from open fields (Namanda et al., 2019). The authors found out that using net tunnel (3.0 × 1.8 m) conserved material increased root yield by 23.6% over material sourced from open fields whereas using material conserved in larger mini-screenhouses (8 × 4 m) increased root yield by 52.6% over material sourced from open fields. This shows that larger net-protected structures may even be better than the net tunnels investigated in this thesis.

It is also important to consider other factors that may affect the physiology of plants and vine production in net tunnels including ratooning. Protection of the plants from insect vectors means that it is possible to harvest several times. Chapter 4 showed that ratooning leads to higher vine production. The ratooned plants have a head start in growth compared with newly planted plants because of carbohydrate reserves in the root stocks (de Booysen & Nelson, 1975). Ratooning is already recommended in sweetpotato seed production in open beds whereby a new harvest of vines is possible after every 40 – 60 days (Stathers et al., 2018). The ratooned crops tend to produce multiple branches leading to more vines. Chapter 4 also showed that vines produced by ratooned crops had more nodes compared with the replanted crops. Therefore, ratooning increases the quantity of seed by producing more vines and nodes per vine. This technique can also reduce labour costs because regular replanting is avoided. However, ratooning in open fields should be accompanied with regular virus management including rogueing out symptomatic plants. This is because the ratooned plants grown in open beds tend to accumulate viruses (Chapter 4). Ratooning has also been reported to increase disease incidences and build-up of pests in rice (Santos et al., 2003) and sugarcane (Young, 2018). For successful application of the ratooning technique, it is also important to investigate the effect of factors such as temperature, water stress, and carbohydrate reserves in root stocks, on regrowth of ratooned vines.

In addition to net-protected structures, there are other on-farm management practices that can be implemented to reduce infection of clean seed at seed producer level. These include regular monitoring of seed production plots and rogueing out plants showing virus symptoms. In addition, it is necessary to locate seed production fields away from other sweetpotato farms. An isolation distance of 15 m between sweetpotato seed plots and other sweetpotato farms has been reported to limit the spread of SPVD (Gibson et al., 2004).

However, this may not be feasible in Tanzania and other intensively farmed areas in Africa. Growing barrier crops such as maize between the seed production plots and other farms may help circumvent this limitation. Also, seed production plots of more resistant varieties can be alternated with those of susceptible varieties to limit rapid spread of viruses.

Lastly, it is important to map locations in terms of virus pressure to facilitate successful dissemination of clean seed. This can be two-prong by conducting surveys to assess the level of virus inoculum in various areas and establishing a surveillance system to monitor aphid and whitefly populations.

5 How do farmers respond to degeneration?

Actions to address degeneration depend on how farmers perceive it and what they think causes it. Chapter 5 showed that farmers in the Lake Zone Tanzania associate sweetpotato seed degeneration with yield losses and are likely to seek a new variety as a response. Getting cleaner planting material of the degenerated variety was not a popular option raising the question whether farmers consider degeneration a seed or a variety issue. It should be noted that the new variety could also be infected with viruses. Also, removal of infected planting material did not feature as a major strategy to control degeneration. This shows that farmers still expect to get some yields from the infected plants. Indeed, Chapter 2 supported this in the case of more tolerant varieties, but it may not work for susceptible varieties as was the case for cv. Ejumula in the high-virus-pressure area where there was a progressive decline in yield following virus infection. It is important to emphasize and create awareness on the integrated seed health management approach: use of resistant varieties, clean seed and on-farm management practices (Thomas-Sharma et al., 2016). On-farm management practices are particularly important in the smallholder system where seed recycling is common. Practices such as rogueing, selecting healthy plants as sources of planting material and isolating seed plots can contribute towards reducing disease inoculum (Navarrete et al., 2022). In potato, positive selection has been shown to reduce degeneration and increase yields in East Africa (Gildemacher et al., 2011; Priegnitz et al., 2020; Schulte-Geldermann et al., 2012). It is important to create more awareness on such on-farm management practices especially among seed producers.

As indicated in Chapter 5, more female farmers reported degeneration compared to male farmers. This suggests that women can become better champions in addressing degeneration. Interventions seeking to address degeneration can tap into this and train more female farmers on measures to control degeneration. However, it might be better to take a household approach where men are also involved.

This is because men are key decision makers on production resources within a household. In banana, it has been reported that men are more likely to buy clean seed due to better access to production resources within the household (Kikulwe et al., 2018).

6 Implications for designing seed systems interventions

In answering my overarching research question, this research provides some lessons that can be adopted when designing seed systems to supply clean sweetpotato seed. First, my research demonstrated that clean, virus-tested seed can play an important role in managing seed degeneration. However, promotion of clean seed needs to consider varietal resistance, agroecology, and farmer practices. These factors influence the frequency at which farmers acquire new seed. Varieties that are more susceptible to viruses may succumb quickly in high-virus-pressure areas therefore requiring regular replacement. However, the rate of replacement can also be influenced by other, social characteristics such as farmer preferences, seed sources, prices and uses.

Second, the lack of significant yield differences between plants sourced from clean, virus-tested seed and those sourced from farmers' fields explains why farmers keep recycling planting material. This can be partly attributed to reversion whereby some varieties produce virus-free branches that can be used as healthy planting material. It is therefore important to explore further and consider the paradigm of reversion as it can contribute towards ensuring that farmers get access to quality seed within their localities. Embracing reversion can be complementary to using clean virus-tested seed.

Third, Chapter 5 showed that sweetpotato seed is still shared within close farmer-farmer networks, mostly based on social ties. These networks will continue to play an important role in sweetpotato seed flows and therefore it is important to devise innovative ways of linking them with the formal seed system for access to clean seed and improved varieties. One approach is to identify key seed producers within the informal sector, train them on quality seed production and link them with upstream sources of clean, virus-tested seed. They can also help in providing information on market-preferred varieties.

Lastly, the importance of net-protected structures in reducing sweetpotato seed degeneration at the seed producer level is shown. However, every added technology comes with a cost which can potentially make the price of seed to go up. Selling sweetpotato seed at high prices may discourage purchase especially since buying of sweetpotato seed is not common.

It is therefore crucial to strategically promote such technologies in high-virus-pressure environments and with well-resourced seed producers who can do medium or large-scale production therefore absorbing associated costs through the economies of scale.

7 Conclusion

The research captured in this thesis set out to investigate how clean, virus-tested seed can be incorporated in smallholder farming systems in the Lake Zone Tanzania to address degeneration. The findings show that clean seed can help address degeneration with the use of agronomic practices like the use of net tunnels, ratooning and rogueing among others. Net-protected structures are especially important in high-virus-pressure areas and can help reduce virus infection of clean seed by blocking out vectors. It is, however, necessary to consider other important, social factors that can influence the adoption of practices around the use and re-production of clean, virus-tested seed. These include farmer seed sourcing strategies and the risk of infection once planted in open fields. It was shown that seed recycling is common, with off-farm sources such as neighbours, relatives, and friends also playing a key role in seed acquisition. It is therefore important to consider these sources when designing seed system interventions. The use of these sources is anchored on trust that is built on social relations and non-monetary benefits that farmers get from close social networks. Tapping into the power of such networks can facilitate quicker adoption of clean virus-tested seed and provide a link between the formal and informal seed systems.

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Summary

A farmer harvesting clean sweetpotato vines grown in an insect-proof net tunnel. Misungwi district, Tanzania. Credit: K. Ogero.

Summary

Seed degeneration caused by viruses is a major constraint to sweetpotato production at the Lake Zone, Tanzania. Dual infection with sweet potato feathery mottle virus (SPFMV) and sweet potato chlorotic stunt virus (SPCSV) can cause up to 100% yield losses. Use of cleaned-up, virus-tested vines disseminated through a formal system is one of the strategies to address this constraint. However, clean seed can get infected once exposed to virus-spreading vectors in the field leading to degeneration and yield losses therefore requiring replacement. There is a need to understand the interactions between viruses, the crop and the environment better. This will enable farmers to know the pattern of degeneration of planting material in their localities, which control measures to use and when to purchase new planting material. Understanding variations in yields of clean planting material after several generations of field propagation defines how seed producers can sustain the supply of quality planting material. Several practices have been promoted to aid on-farm management of sweetpotato viruses during seed production. These include use of insect-proof net tunnels to protect clean seed from virus vectors. However, their effectiveness in reducing virus infection is not well understood. Moreover, successful uptake of clean seed requires an understanding of the current seed sourcing strategies among farmers in Tanzania. This includes understanding how they perceive and react to degeneration.

This thesis sought to assess how cleaned-up, virus-tested seed can be incorporated into smallholder farming systems to address degeneration in sweetpotato. The first chapter is a General Introduction on seed degeneration, sweetpotato production in Tanzania and the challenges posed by virus-related seed degeneration. Chapters 2 to 5 are research chapters that sought to answer the following questions:

a) How does sweetpotato seed sourced from clean virus-tested plants degenerate in different agroecologies following several seasons of on-farm propagation? (Chapter 2)
b) Can insect-proof net tunnels limit virus infection of clean sweetpotato planting material? (Chapter 3)
c) How does ratooning affect vine production in insect-proof net tunnels? (Chapter 4)
d) What are farmers' experiences with and actions towards degeneration including seed sourcing strategies? (Chapter 5).

Chapter 2 was based on experiments conducted over five seasons in a high- and low-virus-pressure environment. The experiments compared degeneration of seed sourced from clean virus-tested plants and farmer-sourced seed over the five seasons. The analysis in this chapter shows that clean virus-tested seed slows down degeneration especially for susceptible varieties grown in high-virus-pressure environments. It also shows yield stability for farmer-sourced material of resistant varieties. This provides more evidence on why farmers recycle planting material. This chapter shows that it is important to consider cultivar resistance and agroecology when promoting adoption of clean virus-tested seed.

Chapter 3 captures the effectiveness of insect-proof net tunnels in reducing seed degeneration. It presents findings from a 21-month experiment assessing virus infection on vines grown in net tunnels and open fields. In addition, a SeedHealth model (see https://tools4seedsystems.org/) was used to model percentages of yield loss over ten seasons in scenario analyses for a high-virus-pressure site. The chapter shows that insect-proof net tunnels can prevent infection in a high-virus-pressure area for up to 20 months. The SeedHealth modeling showed that plants sourced from net tunnels would still attain more than 60% of the expected yields after 10 generations while those sourced from open fields would not do so after only 4 generations. The findings form a basis for recommending adoption of net-protected structures such as screenhouses in high-virus-pressure environments to reduce rapid infection of clean seed. These can be adopted by well-resourced farmers producing at medium or large scale.

Following the successful demonstration by Chapter 3 that insect-proof net tunnels can reduce the rate of virus infection in clean seed it was necessary to assess how farmers can successfully manage the plants to avoid losses not related to virus infection. This included evaluating the effect of ratooning in vine production in the net tunnels. **Chapter 4** captures findings from a 14-month experiment comparing a ratoon cropping technique and a replanting technique in net tunnels and open fields. This chapter shows that ratooning increases vine production. However, the technique leads to higher virus incidences on plants grown in the open. Ratooning is recommended for vine production in net tunnels and screenhouses. If used in the open fields, it should be accompanied with virus control measures.

To understand farmers' experiences with and actions towards degeneration, a 'small N' survey was conducted with 37 farmers (20 female and 17 male). The findings captured in **Chapter 5** show that more women reported seed degeneration than men. Those experiencing degeneration said that the main signs were production of fewer and smaller roots. This is indicative of declining yields. The Chapter also shows that farmers associate degeneration with yellowing and stunting of plants – key signs of virus infection.

In addition, Chapter 5 shows the main action to address degeneration was to seek a new variety rather than getting cleaner planting material of the degenerated variety. This indicates the need to inform farmers that degenerated varieties can be cleaned-up and returned into the system especially if they are market-preferred. Chapter 5 also indicates that sweetpotato planting material is shared within very close networks (friends, neighbours and relatives) and that on-farm seed recycling still dominates.

Chapter 6 puts the results of Chapters 2 up to and including 5 in a broader context. It also draws some comparisons with seed degeneration in other root, tuber and banana crops. Chapter 6 notes the importance of getting more information on farmers' experiences with degeneration. Such data can be combined with data from biophysical experiments to come up with better management strategies.

The findings captured in this thesis show that using clean seed can help improve seed quality in sweetpotato across generations. However, it is important to consider other important factors that can influence its adoption. These include farmer seed sourcing strategies and risk of re-infection. Characterizing the farmer seed exchange networks can give a detailed description of and bring to the forefront the value embedded in them and how they can be tapped to disseminate clean seed. It can also help understand how different types of farmers have access to different types, sources and origins of planting material.

Samenvatting

Farmers harvesting sweetpotato roots. Bukombe district, Tanzania. Credit: K. Ogero.

Samenvatting

Kwaliteitsverlies van uitgangsmateriaal (in het Engels: seed degeneration) veroorzaakt door virussen is een belangrijke beperkende factor in de teelt van zoete aardappel in de Lake Zone, Tanzania. Menginfecties van sweet potato feathery mottle virus (SPFMV) en sweet potato chlorotic stunt virus (SPCSV) kunnen leiden tot wel 100% opbrengstverlies. Het gebruik van 'opgeschoond', op virus getest uitgangsmateriaal, verspreid via een formeel systeem, is één van de mogelijke strategieën om dit kwaliteitsverlies tegen te gaan. Virusvrij uitgangsmateriaal zal echter onvermijdelijk weer geïnfecteerd worden als het in het veld wordt blootgesteld aan virus-verspreidende vectoren. Dit resulteert in achteruitgang van het gewas en opbrengstverlies en maakt vervanging van het uitgangsmateriaal noodzakelijk.

Er is meer kennis nodig over de interacties tussen de virussen, het gewas en zijn omgeving zodat het voor boeren mogelijk wordt om patronen in het achteruitgaan van het gewas te leren herkennen, te weten welke maatregelen ze hiertegen kunnen nemen en wanneer nieuw uitgangsmateriaal te kopen.

Begrijpen welke variaties in opbrengst kunnen optreden na enkele generaties van vermeerdering op het veld helpt de producenten bij een constante aanvoer van uitgangsmateriaal. Er zijn verschillende manieren mogelijk om de virussen van zoete aardappel beter te beheersen. Eén daarvan is het gebruik van insecten-vrije gaaskooien om virus-vrij uitgangsmateriaal te beschermen tegen virusvectoren. Hoe efficiënt ze dat doen, wordt echter nog niet goed begrepen.
Bovendien vereist een succesvolle acceptatie van virusvrij uitgangsmateriaal een beter begrip van de verschillende manieren waarop de boeren in Tanzania aan hun uitgangsmateriaal komen, hoe ze de achteruitgang van hun materiaal ervaren en hoe ze hierop reageren.

Dit onderzoek beoogde te onderzoeken hoe gezond, virus-vrij uitgangsmateriaal van zoete aardappel het beste ingebracht kan worden in de kleinschalige landbouw om het probleem van kwaliteitsverlies van uitgangsmateriaal tegen te gaan.

Het eerste hoofdstuk van dit proefschrift is een algemene introductie over het verlies van kwaliteit (vooral gezondheid) van uitgangsmateriaal na meerdere vermeerderingen, de teelt van zoete aardappel in Tanzania en de uitdagingen die door de virussen van deze teelt worden veroorzaakt. Hoofdstukken 2 tot en met 5 beschrijven het onderzoek aan de hand van de volgende vragen:

a) Hoe snel gaat gezond en op virus getest uitgangsmateriaal van zoete aardappel achteruit in de loop van achtereenvolgende teeltseizoenen onder verschillende teeltomstandigheden? (Hoofdstuk 2)
b) Kunnen insecten-vrije gaaskooien bijdragen aan het terugdringen van her-infecties van gezond, virus-vrij plant materiaal? (Hoofdstuk 3)
c) Hoe beïnvloedt de teelt van een hergroeigewas (in het Engels: ratooning) de productie van stekken in insectenvrije gaastunnels? (Hoofdstuk 4)
d) Welke ervaringen hebben de boeren met kwaliteitsverlies van uitgangsmateriaal en welke acties ondernemen ze hiertegen, inclusief het verkrijgen van nieuw uitgangsmateriaal? (Hoofdstuk 5)

Hoofdstuk 2 is gebaseerd op experimenten die gedurende vijf teeltseizoenen in gebieden met hoge en lage virusdruk werden uitgevoerd. In deze experimenten werd kwaliteitsverlies van gezond, virusvrij uitgangsmateriaal vergeleken met dat van uitgangsmateriaal afkomstig van de boeren zelf. Gebruik van gezond, virusvrij uitgangsmateriaal remt de achteruitgang, met name bij vatbare cultivars in een omgeving met hoge virusdruk; daarentegen blijft boerenmateriaal van resistente cultivars stabiel in opbrengst. Dit hoofdstuk laat zien dat het inzetten van resistente cultivars en het rekening houden met teeltomstandigheden belangrijk zijn bij het promoten van het gebruik van gezond, virusvrij uitgangsmateriaal.

Hoofdstuk 3 beschrijft de effectiviteit van insectenvrije gaastunnels in het tegengaan van kwaliteitsverlies van uitgangsmateriaal. Gedurende 21 maanden werd uitgangsmateriaal geteeld in gaastunnels of geteeld in de volle grond vergeleken met betrekking tot de ontwikkeling van virusinfecties. Daarnaast werd het SeedHealth model (https://tools4seedsystems.org/) gebruikt in een scenarioanalyse om de te verwachten opbrengstdervingen te modelleren over een periode van 10 groeiseizoenen onder hoge virusdruk. Dit hoofdstuk laat zien dat insectenvrije gaastunnels gedurende 20 maanden virusinfecties kunnen tegenhouden. Het SeedHealth model liet zien dat planten afkomstig uit de gaastunnels na 10 generaties nog 60% van hun verwachte opbrengst zouden halen, terwijl de planten in de volle grond dat al niet meer halen na slecht vier generaties. Deze resultaten vormen de basis voor de aanbeveling gaastunnels toe te passen in gebieden met hoge virusdruk om snelle infectie van gezond uitgangsmateriaal te voorkomen. Zulke gaastunnels zouden vooral ingezet kunnen worden door boeren die op middelgrote tot grote schaal uitgangsmateriaal produceren.

Na de succesvolle demonstratie dat gaastunnels virusinfecties in gezond uitgangsmateriaal kunnen terugdringen was het nodig om na te gaan hoe boeren het beste hun uitgangsmateriaal kunnen vermeerderen om niet-virus-gerelateerde opbrengstverliezen te vermijden. Daartoe is onder meer onderzocht wat het effect is van het telen als hergroeigewas op de productie van uitgangsmateriaal. **Hoofdstuk 4** beschrijft de resultaten van een experiment waarin gedurende 14 maanden de teelt van een hergroeigewas werd vergeleken met het herplanten van stekken. Hieruit blijkt duidelijk dat met een hergroeigewas de opbrengst aan uitgangsmateriaal kan worden verhoogd maar dat dit ook gepaard gaat met meer virusinfecties bij teelten in de volle grond. Het telen van een hergroeigewas wordt aanbevolen voor de productie van uitgangsmateriaal in gaastunnels en schermkassen. Wanneer het toegepast wordt in de volle grond dan zijn aanvullende virusbeperkende maatregelen noodzakelijk.

Om te begrijpen hoe boeren kwaliteitsverlies begrijpen en ermee omgaan is een ‘kleine N’ enquête gehouden onder 37 boeren (20 vrouwen en 17 mannen). De resultaten hiervan, beschreven in **Hoofdstuk 5**, laten zien dat vrouwen vaker kwaliteitsverlies zien dan mannen. Zij die kwaliteitsverlies ervoeren, beschreven het vooral als minder en kleinere wortels. Dit is een duidelijk aanwijzing voor lagere opbrengsten. Dit hoofdstuk laat ook zien dat als belangrijkste reactie hierop men op zoek ging naar een nieuw ras in plaats van gezond uitgangsmateriaal van het bestaand ras. Dit toont aan dat het nodig is om de boeren erop te wijzen dat hun gedegenereerde rassen virusvrij gemaakt kunnen worden en toch geteeld kunnen worden, zeker als ze gewild zijn door de markt. Hoofdstuk 5 laat ook zien dat uitgangsmateriaal van zoete aardappel uitgewisseld wordt binnen nauw verbonden netwerken (vrienden, buren en familie) en dat hergebruik van uitgangsmateriaal binnen de eigen boerderij nog steeds het belangrijkst is.

Hoofdstuk 6 plaatst de resultaten van Hoofstukken 2 tot en met 5 in een bredere context. Het legt ook verbanden met kwaliteitsverlies van uitgangsmateriaal in andere wortel-, knol- en banaangewassen. Dit hoofdstuk concludeert ook dat het van belang is om meer informatie over de ervaringen van boeren met kwaliteitsverlies te verkrijgen. Zulke informatie kan, in combinatie met resultaten van biofysische experimenten, leiden tot betere gewas management strategieën.

De resultaten beschreven in dit proefschrift laten zien dat het gebruik van virusvrij materiaal kan bijdragen aan het verbeteren van de kwaliteit van uitgangsmateriaal over meerdere generaties. Het is echter belangrijk te beseffen dat meerdere factoren hierbij van belang zijn, zoals bijvoorbeeld hoe boeren aan hun uitgangsmateriaal komen en welke risico’s er zijn op nieuwe virusinfecties.

Het in kaart brengen van de netwerken waarlangs uitgangsmateriaal wordt uitgewisseld kan belangrijke informatie geven over deze netwerken, duidelijk maken welke waarden ze in zich dragen en hoe ze gebruikt kunnen worden om virusvrij uitgangsmateriaal te verspreiden. Het kan ook helpen te begrijpen hoe verschillende soorten boeren toegang hebben tot uitgangsmateriaal van verschillend herkomst.

Farmers packing sweetpotato seed on lesos/kangas for transportation to their farms. Geita district, Tanzania. Credit: K. Ogero.

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My PhD journey was initiated by my co-promotor Conny J.M. Almekinders who introduced me to two great scientists, Paul C. Struik and René van der Vlugt. Conny, you asked me if I was ready to go through the whole process and I said yes, lest did I know that it would not be a smooth ride. I am grateful that you stuck with me and provided guidance and valuable materials when I got stuck. I am greatly indebted to my promotors René van der Vlugt and Paul C. Struik for their guidance and positive criticisms during my research work. René, thank you for letting me know that 'it was never that serious' and it was okay to take time off and share a drink with friends. Paul, thank you for enabling me to pay attention to details and scientific language. I would also like to thank my co-promotor Jan Kreuze for not only being a great team leader but also a mentor. Thank you for sharing with me your wisdom and for being available whenever I needed you. I am greatly indebted to Margaret McEwan for convincing senior management at the International Potato Center (CIP) to support my PhD studies and ensuring that I did not lack research funds. Thank you too for making me think outside the box and to always look at the bigger picture of scientific research. Your constructive criticism has made me a better scientist.

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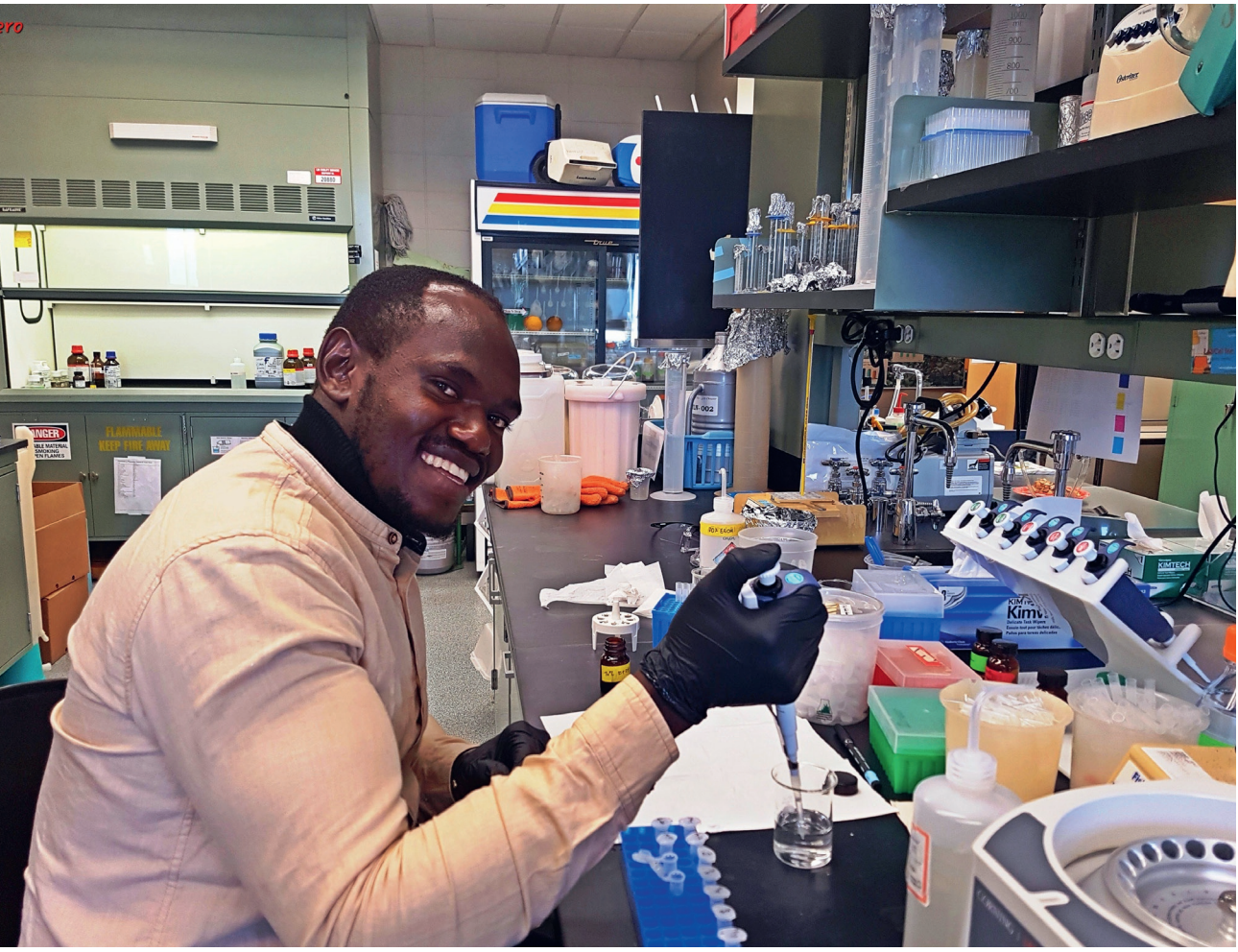
Doing a PhD is not an easy task and sometimes one may get stuck. I had several of such moments but was able to keep pushing, thanks to three people who were always a phone call away: my fellow PhD candidates, Erik and Israel, and my colleague Srini Rajendran. Thank you for your encouraging words.

I want to say thank you to my wife, Lucy, and daughter, Zuri, for the moral support you gave me during the whole PhD trajectory. Thank you for braving my absence from home. You are a great inspiration to my life.

Many people have given generously their time and expertise which assisted me during my research and while writing this thesis. My special thanks go to Bramwel Wanjala from the Kenya Agricultural and Livestock Research Organization (KALRO) for support and guidance on virus diagnostics. Thank you for taking time to visit BecA-ILRI laboratories and help whenever my assays did not work. I highly appreciate the support received from CIP colleagues in Nairobi. Thanks a lot Bernice, Eric, Dorca and Ethel. You were all amazing and made my laboratory visits bearable. I thank my former colleague at CIP, Haile Selassie Okuku, for taking me through the basics of data analysis. Many thanks to the scientists at the Tanzania Agricultural Research Institute (TARI) with whom I have done collaborative research on sweetpotato seed systems for the last eight years. A special recognition to Adventina, Deus, Hadija, Machunde and Sonda.

I have spent the better part of the last eight years, including the time spent conducting the research captured in this thesis, in Tanzania. There are several people who have made my stay a bit easier including my friends Daniel, Lotty, Robert and Sandra. Thanks a lot Lotty and Robert for the morning runs, gym sessions and for being a great company during the early days of the COVID 19 pandemic. Sandra, I appreciate our friendship which started in Mwanza and extended to the Netherlands. Thank you for the trips to Utrecht and Rotterdam.

Thanks to the many sweetpotato farmers I have interacted with, not just in Tanzania but across the world. The conversations with you kept me going and enabled me to always ‘think impact’.



The author conducting DNA extraction from sweetpotato root samples for virus testing via PCR during a CGIAR-Norman Borlaug Fellowship at Louisiana State University. LSU AgCenter, Baton Rouge, Louisiana, USA. Credit: K. Speyrer.

About the author

About the author

Kwame Ogero was born on October 27, 1986, in Nyamira County, Kenya. He began his primary school education in 1992 at Motagara Primary School in Nyamira County. In December 1999 his family moved to Nairobi, Kenya's Capital, and he had to complete his last year of primary education in a different school. In Nairobi, he joined Hekima Primary School where he sat for his Kenya Certificate of Primary Education (KCPE) examination in 2000. Kwame exceled in his KCPE examination and joined the prestigious Lenana School in 2001. He completed his secondary school education in 2004 and was admitted to the School of Medicine of the University of Nairobi in 2006 to pursue a Bachelor of Science degree in Biochemistry (Molecular Biology and Biotechnology major). Kwame graduated from the University of Nairobi in 2009. While at the University of Nairobi Kwame was involved in student politics and was elected as the Chairman of the Nairobi University Biochemistry Students' Association (NUBSA) for the academic year 2008/2009. As a student leader Kwame was involved in organizing scientific outreach events including seminars and workshops on emerging issues. A good number of these events focused on raising awareness on the use of genetic engineering and other biotechnological applications in agriculture. These events provided opportunities to network with agricultural scientists from institutions such as the Africa Agricultural Technology Foundation (AATF), African Biotechnology Stakeholders Forum (ABSF), Africa Harvest, International Service for the Acquisition of Agri-biotech Applications (ISAAA), Kenya Agricultural and Livestock Research Organization (KALRO), Monsanto (now Bayer) and Syngenta among others. Slowly, Kwame's interest would shift from medicine to agriculture. After completing his BSc studies Kwame teamed with two of his classmates and came up with an idea of establishing a tissue culture company to produce clean planting material for banana. The three visited ISAAA, who had experience in tissue culture for banana, to get advice and information on how to set up the business. At ISAAA, they met the Senior Programs Officer, Faith Nguthi, who gave them useful information and linked them with Prof. Gitonga Nkanata who was the Dean of the School of Agriculture and Enterprise Development at Kenyatta University. Prof. Nkanata was doing research focusing on low-cost tissue culture technologies.

Kwame and one of his two colleagues secured an appointment with Prof. Nkanata. On the material day, the duo went to Prof. Nkanata's Cell and Tissue Culture Laboratory at Kenyatta University. Prof. Nkanata would join them a few minutes later and he was excited by the three young men's ambitions and offered two partial scholarships for MSc studies with thesis research on low-cost tissue culture. Kwame took up one of the scholarships and enrolled for a Master of Science in Agronomy studies at Kenyatta University in March of 2010. Kwame graduated with an MSc. Agronomy degree in 2012 with a thesis titled '*Low-cost Tissue Culture of selected Cassava (Manihot esculenta Crantz) and Sweet Potato (Ipomoea batatas (L) Lam.) Varieties'.* While studying for his MSc Kwame worked as a Research Assistant at the Cell and Tissue Culture Laboratory, Kenyatta University and Associate Lecturer at Mt. Kenya University. In September 2011 he joined the International Service for the Acquisition of Agri-biotech Applications (ISAAA) *Afri*Center as a Program Assistant and Liaison Officer for the Open Forum on Agricultural Biotechnology in Africa (OFAB), Kenya Chapter, gaining vast experience in science communication and policy outreach. He worked at ISAAA until May 2014 when he joined the International Potato Center (CIP) as a Regional Research Associate based in Mwanza, Tanzania. Kwame has been involved in several seed systems projects since joining CIP, including the Quality Seeds and Access to Improved Varieties component of the the CGIAR Research Program on Roots, Tubers, and Bananas (RTB). The collaborations with Wageningen University and Research (WUR) under the RTB project led to an opportunity to enroll for PhD studies in November 2017 at WUR with a thesis focusing on sweetpotato seed degeneration in Tanzania. Kwame continues to work at CIP on research geared towards developing sustainable seed systems for sweetpotato. His research is focusing on developing crop-specific models and decision support systems for understanding and managing seed degeneration in sweetpotato. This includes identifying scenarios where on-farm management, resistant varieties, and seed replacement management strategies would be most optimal. Kwame is also interested in understanding effects of climate change on epidemiology of sweetpotato viruses and socioeconomic factors that influence farmers' decisions to use different sources of seed. He serves on the Committee of the International Society for Tropical Root Crops - Africa Branch (ISTRC - AB) as the East Africa Representative. Kwame is married to Lucy Mosotah with whom they have a 3-year-old daughter, Zuri. He is a fitness enthusiast and spends most of his leisure time in the gym.

Multiplication of sweetpotato seed in net tunnels and open field nurseries. Buchosa district council, Tanzania. Credit: K. Ogero.

Publications

List of publications

Ogero, K. O., Kreuze, J. F., McEwan, M. A., Luambano, N. D., Bachwenkizi, H., Garrett, K. A., Andersen, K. F., Thomas-Sharma, S., & van der Vlugt, R. A. A. (2019). Efficiency of insect-proof net tunnels in reducing virus-related seed degeneration in sweet potato. Plant Pathology. ISSN: 0032-0862. 68: 8. pp. 1472–1480. https://doi.org/10.1111/ppa.13069.

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The author contributing to a discussion during the Emerging Technologies for Global Food Security Conference held on June 19 - 21, 2018. Saskatoon, SK, Canada. Credit: D. Stobbe.

Select conference presentations

Select conference presentations

Date	Conference/Workshop Name & Place	Presentation Title & Mode Of Presentation
June 27– July 1, 2022	12th Triennial Conference of the African Potato Association, Lilongwe, Malawi.	Understanding Sweetpotato Seed Flows in Tanzania for Better Linkages Between the Formal and Informal Seed Systems (Oral)
June 5 – 8, 2022	15th International Symposium of Plant Virus Epidemiology, Madrid, Spain.	Degeneration of Clean Virus-tested Sweetpotato Seed in High and Low Virus Pressure Areas at the Lake Zone, Tanzania (Poster)
Dec 10 - 11, 2020	The 2020 Carter Conference on Shifting Momentum in African Agriculture Through Research and Technologies. Online.	Developing Sustainable Seed Systems for Delivery of Orange-fleshed Sweetpotato in Africa (Oral)
August 25 – 29, 2019	11th Triennial Conference of the African Potato Association, Kigali, Rwanda.	Improving Irrigated Production of Sweetpotato Vines in Net Tunnels (Oral)
May 13 – 17, 2019	14th International Plant Virus Epidemiology Symposium, Seoul, South Korea.	Using Insect-proof Net Tunnels to Reduce Virus-related Seed Degeneration in Sweetpotato (Oral)
June 19 – 21, 2018	Emerging Technologies for Global Food Security Conference, Saskatoon, SK, Canada.	1. Addressing Virus-related Cultivar Decline in Sweetpotato: Clean Seed Systems and On-farm Management in Tanzania (Oral) (https://www.youtube.com/watch?v=t6mcbFISLa4) 2. Interactive Effects of Phosphorus and Virus Infection on Sweetpotato Vine and Root Production (Poster)
Mar 5 – 10, 2017	The 13th International Symposium of The International Society for Tropical Root Crops-Africa Branch (ISTRC-AB), Dar es Salaam, Tanzania.	Using net tunnels in on-farm management of sweetpotato viruses during basic seed production (Poster)
Oct 10 – 12, 2016	10th Triennial Conference of the Africa Potato Association, Addis Ababa, Ethiopia.	Factors affecting adoption of Insect-proof Net Tunnels for production of Quality Sweetpotato Vines among Farmer-multipliers in Tanzania (Poster)
Jun 14 – 16, 2016	Emerging Technologies for Global Food Security, Saskatoon, Saskatchewan, Canada.	Assessing Virus Degeneration of Clean Sweetpotato Planting Material maintained in Net Tunnels under farmer-management in the Lake Zone, Tanzania (Poster)
Jan 18 – 22, 2016	World Congress for Root and Tuber Crops, Nanning, Guangxi, China.	Towards Improved Sweetpotato Seed System Management: Use of Affordable Net Tunnels and Decentralized Inspection Schemes (Oral)

Oct 5 – 8, 2014	Agricultural Biosciences International Conference (ABIC), 2014, Saskatoon, Saskatchewan, Canada.	1. Enhancing access to disease-free sweetpotato planting materials through low-cost tissue culture for food security and poverty alleviation in Eastern Africa (Poster). 2. Why STI is key to food security in sub-Saharan Africa (Oral) – Presented in the Tomorrow's Leaders Forum held during the conference.
Aug 5 – 9, 2013	2nd Annual National Biosafety Conference, Nairobi, Kenya.	Global and Regional Trends in Commercialization of Biotech/GM Crops 1996-2012 (Oral)
Oct 10 – 13, 2011	10th African Crop Science Society Conference 2011, Maputo, Mozambique.	1. Evaluating the feasibility of using locally available fertilizers as low-cost nutrients in sweet potato (*Ipomoea batatas* (L.) Lam) tissue culture (Oral). 2. Response of two sweet potato (*Ipomoea batatas* (L) Lam.) varieties regenerated on low-cost tissue culture medium (Poster).

The author participating in a session on use of Choice Experiments in determining farmers' demand and preferences for quality seed and varieties during an RTB Seed Systems Community of Practice meeting. Lunteren, Netherlands. Credit: R. Kahiu.

PE&RC Training and Education Statement

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (6 ECTS)

- Managing virus degeneration in sweetpotato for a better seed system at the Lake Zone, Tanzania: what and how to prioritize in decentralized vine multiplication?

Post-graduate courses (7.8 ECTS)

- PhD Masterclass on science and seed systems; PE&RC & CGIAR (2017)
- Means-end chains and laddering course; WASS (2017)
- Small RNA library preparation from plant tissues and bio-bioinformatics sequence analysis for detection of viruses; International Potato Center (2018)
- Plant breeding and resilient seed systems; PE&RC, RTB, Dynaversity and Liveseed (2020)
- Basic statistics; PE&RC and SENSE (2018)
- Advanced statistics course & Design of Experiments; PE&RC (2018)

Deficiency, refresh, brush-up courses (7.5 ECTS)

- Epidemiology and data science; University of Florida (2020)
- Research methods in crop science; CSA (2021)

Laboratory training and working visits (6 ECTS)

- Role of clean seed systems in addressing virus-related cultivar decline in sweetpotato; Department of Plant Pathology and Crop Physiology, Louisiana State University (2018)

Competence strengthening / skills courses (2.2 ECTS)

- Team effectiveness workshop; International Potato Center (2019)
- Peer review course; Publons Academy (2020)
- The craft of writing effectively; University of Chicago (2021)
- Writing beyond the academy; University of Chicago (2021)

Scientific integrity/ethics in science activities (0.3 ECTS)

- Course on genetic resource policies for CGIAR scientists; CGIAR Genebank Platform (2021)

PE&RC Annual meetings, seminars and the PE&RC weekend (0.9 ECTS)

- PE&RC First year weekend (2017)

Discussion groups / local seminars or scientific meetings (21 ECTS)

- Sweetpotato seed systems and crop management community of practice (2017-2021)
- RTB Seed systems community of practice (2017-2021)
- CIP Global science seminars (2019-2022)

International symposia, workshops and conferences (20.7 ECTS)

- 9th Sweetpotato seed systems & crop management community of practice consultation; poster and oral presentations; Kigali, Rwanda (2018)
- Emerging technologies for global food security conference; poster and oral presentations; Saskatoon, Canada (2018)
- 11th Triennial conference of the African potato association; poster and oral presentations; Kigali, Rwanda (2018)
- 14th International plant virus epidemiology symposium; oral presentation; Seoul, South Korea (2019)
- Sweetpotato commercial seed production screenhouse practices; oral presentation; International Potato Center; online (2020)
- The carter conference; oral presentation; University of Florida; online (2020)
- 15th International symposium of plant virus epidemiology; poster presentation; Madrid, Spain (2022)
- 12th Triennial conference of the African potato association; poster presentation; Lilongwe, Malawi (2022)

Societally relevant exposure (6 ECTS)

- Understanding cultivar decline in sweetpotato for better seed systems interventions (2020)
- Innovation at the last mile, reaching farmers with quality seeds (2021)
- LAMP testing paves the way for better and rapid virus diagnostics in cassava and sweetpotato seed in Tanzania (2021)
- RTB toolbox for working with root, tuber and banana seed systems (2021)
- Change agents and better seeds lead to sweetpotato success in Tanzania (2022)

Committee work (2 ECTS)

- International society for tropical root crops; ISTRC (2014-2017)

Dr. Graham Thiele, Director of the CGIAR Research Program on Roots, Tubers and Bananas (RTB), launching the program at Wageningen University in May 2017. RTB was the main sponsor of the research captured in this thesis. Wageningen, Netherlands. Credit: R. Kahiu.

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