Smallholder cassava planting material movement and grower behaviour in Zambia: implications for the management of cassava virus diseases

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ABSTRACT

Cassava is an important food crop across sub-Saharan Africa, where production is severely inhibited by two viral diseases; cassava mosaic disease (CMD) and cassava brown streak disease (CBSD), both propagated by a whitefly vector and via human-mediated movement of infected cassava stems. There is limited information on growers' behaviour related to movement of planting material, as well as growers' perception and awareness of cassava diseases, despite the importance of these factors for disease control. This study surveyed a total of 96 cassava subsistence growers and their fields across five provinces in Zambia between 2015 and 2017, to address these knowledge gaps. CMD symptoms were observed in 81.6% of the fields, with an average incidence of 52% across the infected fields. No CBSD symptoms were observed. Most growers used planting materials from their own (94%) or nearby (<10 km) fields of family and friends, although several large transactions over longer distances (10-350 km) occurred with friends (15 transactions), markets (1), middlemen (5), and NGOs (6). Information related to cassava diseases and certified clean (disease-free) seed reached only 48% of growers. The most frequent sources of information related to cassava diseases included nearby friends, family and neighbours, whilst extension workers were the most highly preferred source of information. These data provide a benchmark on which to plan management approaches to controlling CMD and CBSD, which should include clean propagation material, increasing growers' awareness of the diseases and increasing information provided to farmers (specifically disease symptom recognition and disease management options).

Keywords: cassava, farmer behaviour, clean seed system, cassava mosaic disease, planting material movement, Zambia

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a perennial shrub of the Euphorbiaceae (spurge) family, native to South America (Allem 2002; Olsen and Schaal 2001) and cultivated as a tuberous crop in tropical and subtropical regions worldwide. It can be propagated by either stem cuttings or seed, where the former is by far the most common (Alves 2002). In Zambia, cassava is one of the most important food crops after maize, and the primary staple in northern parts of the country (Chitundu et al. 2009; Szyniszewska 2020). It is the mainstay for an estimated 30% of the country's population (Simwambana 2005), consumed throughout the year in Western, North Western, Luapula and Northern provinces.

Cassava use in Zambia ranges from subsistence production, marketed fresh or processed for human consumption, to livestock feed and industrial use (Cadoni 2010). Demand is increasing for both human and industrial consumption in urban and industrial centres due to a surge in industrial applications including bio-ethanol, starch, stock-feed and brewing (Breuninger et al. 2009; Nuwamanya et al. 2012; Taiwo 2006; Tonukari 2004). Notably, production and consumption of cassava is now expanding to southern parts of the country, where the Zambian Government and NGOs have been promoting cassava in response to an increasing occurrence of drought and heat stresses that have led to the failure of maize crops (Phiri 2011). The production of cassava has also recently expanded in the Eastern Province (Alene et al. 2013; Barratt et al. 2006). Cassava is propagated using cuttings – pieces of harvested cassava stem. Upon harvest, these stems can be stored for up to 3-4 weeks in a cool, dry space before replanting. Cassava planting in Zambia is typically between November and January, while harvesting is highly flexible and relatively late compared with other countries. Harvesting takes place anytime between 16 months and 3 years after planting. Later harvesting is more common among growers planting landraces, while those that use improved varieties typically harvest sooner. Smallholder growers typically have more than one field as a safeguard, and planting will take place in areas with previously harvested crop.

Despite the importance of cassava, according to FAOSTAT data, Zambia suffers from low average yields of 5.8 tonnes per hectare (t/ha) (Chikoti et al. 2019; FAOSTAT 2018). This is considerably lower than the reported average yield of neighbouring countries, including Malawi (22 t/ha), Angola (10.9 t/ha) and the Democratic Republic of Congo (DRC, 8.1 t/ha) (FAOSTAT 2018). The low yield in Zambia is due to several biotic and abiotic constraints such as cold and drought. Among the biotic factors, one of the most important is the high prevalence in most cassava-growing areas of cassava mosaic disease (CMD), caused by cassava mosaic geminiviruses (CMGs, family *Geminiviridae*, genus *Begomovirus*) (Chikoti et al. 2013). Two variants of CMGs were confirmed to be present in Zambia: African cassava mosaic virus (ACMV) and East African cassava mosaic virus (EACMV) (Chikoti et al. 2013). Strains of the CMGs in Zambia (Chikoti et al. 2013; Mulenga et al. 2015), a reliance on cassava landraces (Alene et al. 2013; Rey and Vanderschuren 2017) and underdeveloped extension services magnify the impact of disease on crop yield. CMD was first reported in Africa in Tanzania, in 1894, and by the 1940's it had spread to all cassava-growing regions of the African continent (Fargette et al. 2006). CMD was confirmed in Zambia in 1995, but it is likely that it had been present there for much longer (Mkuyamba 1995). CMD symptoms include characteristic patches of yellow and green mosaic, leaf curling and deformation, narrowing, reduced plant height and tuber root size.

In 2017, cassava brown streak disease (CBSD, caused by potyviruses, family *Potyviridae*, genus *Ipomovirus*), was also confirmed in both Northern and Luapula provinces (Mulenga et al. 2018). CBSD was first documented in 1936 in northeast Tanzania, where in the early 1990s it was reported to be restricted to low-altitude areas below 1000 meters above sea level (masl) along coastal East Africa and lakeshore districts of Malawi (Legg et al. 2011). Since the mid-1990s there has been a re-emergence of CBSD around Lake Victoria and across other East and Central African countries (Alicai et al. 2019; Legg et al. 2011). CBSD is caused by two variants of single-stranded RNA viruses: cassava brown streak virus (CBSV) and Ugandan brown streak virus (UCBSV) belonging to *Ipomovirus* genus, family *Potyviridae* (Mbanzibwa et al. 2009; Winter et al. 2010). CBSD symptoms include root necrosis, radial root constrictions, feathery foliar chlorosis along secondary vein margins which eventually coalesce to form blotches, chlorotic mottling with no veinal association and, infrequently, brown streaks or lesions on stems (Nichols 1950).

These two viral diseases cause considerable losses, estimated at $1 billion per annum across sub-Saharan Africa (Tomlinson et al. 2017). CMD and CBSD have been estimated to cause yield loses of 15-24% (Thresh et al. 1997) and 18-25% (Gondwe et al. 2003) respectively, and consequently lead to the deterioration of the livelihoods of millions of growers (Abaca et al. 2012; Alvarez et al. 2012; Legg and Thresh 2003; Mbanzibwa et al. 2011; Patil et al. 2015; Winter et al. 2010). Viruses responsible for CMD and CBSD are both transmitted by an insect vector, *Bemisia tabaci* (whitefly), and human-mediated propagation of infected planting stems (Maruthi et al. 2017). Spread of cassava brown streak viruses (CBSVs) by *B. tabaci* is reported to occur semipersistently and over relatively short distances, usually of the order of tens of meters (Katono et al. 2015; Maruthi et al. 2017). CBSV has a faster acquisition rate in the vector (<1 hour) compared to CMV (up to 8 hours), but lower persistence (up to 48 hours) in the insect vector compared to CMV, which can be retained in the vector for up to 9 days (Thresh and Cooter 2005; Maruthi et al. 2017). Longer virus retention rates for CMV imply that spread is likely to be more efficient and over longer distances (Jacobson et al. 2018). Under experimental conditions, acquisition and transmission of CMV by viruliferous *B. tabaci* on exposed healthy cassava plants occurs primarily within the first 6 hours (44±16% disease incidence), whereas for CBSV it is was at 22±16% in the same time interval (Njoroge et al. 2017). Maruthi et al. (2017) reported the highest CBSV transmission rate achieved in their experiments at 60% over a period of 24 hours. Reported virus transmission rates differ between studies, likely due to different methodologies, laboratory conditions, cassava cultivars and viral strains. It is difficult to conclude how the rates of spread observed in laboratory conditions compare to the rates of virus spread in the field. The regional epidemiology of cassava virus spread, and existing evidence related to virus retention times, suggest that CMD in the field is spread by *B. tabaci* more efficiently than CBSD (Legg et al. 2011b).

Strategies for disease management include the removal of infected plants (roguing), the adoption of resistant cultivars, and the use of certified disease-free planting material (known as 'certified clean seed' or 'CCS') (Hillocks and Jennings 2003; Kanju et al. 2003; Legg 1999). Each method faces particular challenges that include difficulties in identifying infected plants, a paucity of resistant varieties (in particular those resistant to both viruses), and unacceptable increases in costs (Legg et al. 2011; Patil et al. 2015; Rwegasira and Rey 2012).

Recently, a number of surveys have assessed the impact and extent of CMD and CBSD in sub-Saharan Africa. Many of these have focused on disease incidence at the field scale or disease severity at the regional scale (Alicai et al. 2007; Chikoti et al. 2013; Gondwe et al. 2003; Hillocks et al. 2002, 1999; Mbewe et al. 2015; Mulenga et al. 2018; Rwegasira and Rey 2012). However, surveys are primarily based solely on field observations of disease, without consideration of (i) the growers' ability to identify CMD and CBSD, (ii) their practices related to sourcing and exchange of cassava planting material, or (iii) cassava disease control strategies implemented by growers. To understand which method of disease control is most likely to be successful, it is important to understand the decision-making processes of growers; what risks and costs are acceptable and under what circumstances. Recent work on CBSD, European corn borer and Western corn rootworm has shown that grower knowledge and management practices can have significant impacts on the long-term success of disease control, and may represent the difference between success and failure of control (Carrasco et al. 2012; Legg et al. 2017; McQuaid et al. 2017b; Milne et al. 2015).

Effective control of many diseases is based on a knowledge and understanding of how the pathogen spreads between fields as a function of distance. It is widely acknowledged that the incidence of CMD and CBSD can be amplified within an individual field by replanting infected material, i.e. cuttings left from the previous planting seasons (Samura et al. 2017), and on a larger scale by sharing planting material between fields (McQuaid et al. 2017a, b; Patil et al. 2015). However, more work is required to investigate and quantify the physical properties of human-mediated transmission, specifically the volume of (potentially infected) planting material that is exchanged and the distances over which this material is moved. Effective disease management is achieved based on an understanding of these dispersal characteristics.

The primary objective of the current study was to quantify and describe the movement of cassava planting material into and out of growers' fields (specifically the volume of cuttings moved over specified distances), and to identify the sources and recipients of that material. The secondary objective was to ascertain growers' knowledge (often referred to as awareness) of CMD and CBSD, including the symptoms associated with each disease and prevalence in the study area. Lastly, sources and preferences that growers had for obtaining information related to cassava pathogens, planting practices, CCS and disease management were explored. This information was obtained by a survey of 96 growers in five provinces of Zambia.

MATERIALS AND METHODS

Agro-ecological context of the study area

The study was conducted in five provinces of Zambia: Western, Luapula, Central, Northern, and Eastern (Fig. 1), which are among the major cassava growing areas and at the time of the survey were known to have CMD infections present, with CBSD infections confirmed in neighbouring Tanzania, Malawi, Mozambique, and the DRC (Gondwe et al. 2003; Hillocks et al. 2001; Mangana 2003; Mulimbi et al. 2012). These provinces encompass different agro-environmental conditions. Northern and Luapula provinces are located in Agro-Ecological Zone (AEZ) III, which comprises part of the Central African plateau with a monomodal rainfall pattern (Saasa 2003; The World Bank 2006). The rainy season occurs between November and April, and is followed by a dry spell lasting from May to October. Western, Central, and Eastern provinces are located in slightly drier AEZ II, (The World Bank 2006; Jain 2007). The rainy season occurs between December and April, followed by a similar dry spell to AEZ III.

Sample selection

Due to poor road infrastructure in Zambia, only fields located along the main motorable roads were selected for the study. A total of 96 smallholder cassava growers were selected in 10-15 km intervals along major motorable roads in the regions described above. We maximised the number of interviewees by restricting the survey to roadside fields, as reaching off-road fields was not feasible within the budgetary and time constraints for the survey. The survey was spread over a two-year period to accommodate staff constraints, while enabling us to maximise the number of respondents and obtain information from across five provinces of Zambia. Growers who were the field owners, or their family members, were informed of the scope and purpose of the survey and asked for permission and a signature confirming their consent to participate in the study, before the questionnaire and field sampling was conducted. A total of 24 growers were interviewed in 2015 in Eastern (9), Luapula (4) and Northern (11) provinces, and 72 growers were interviewed in 2017 in Central (15), Eastern (15), Luapula (15), Northern (14), and Western (13) provinces (see Fig. 1 and Table 1). The research team comprised a senior scientist and two research assistants, all conversant with the local languages and with experience in cassava production. The study was conducted between January and May in both years, alongside a survey to assess the prevalence of CMD and CBSD, following the protocol outlined by Sseruwagi et al. (2004). During the survey period most plants were assumed to be between three and nine months old, at which age cassava plants are regarded as ideal for the assessment of foliar and root symptoms, before shedding of their leaves.

Questionnaires

Structured interviews with a mix of closed- and open-ended questions were conducted with cassava growers who voluntarily agreed to participate. Local agriculture extension officers and, where available, village leaders were informed and asked for consent for the interviews to take place. A copy of the questionnaire template and results are available in an online repository (https://figshare.com/s/9c3331b503cc1c7401de, Szyniszewska et al. 2019). The names of the surveyed farmers and geographic coordinates of the locations were removed to ensure anonymity of respondents. The questionnaire was pre-tested on a small group of growers before the survey and adjustments were made to ensure that the questions were phrased clearly and understood correctly by the growers. The majority of critical questions were related to events that happened in the most recent year or harvest preceding the questionnaire, in order to mitigate the risk of bias due to respondents' recollection of events over a longer period of time. To encourage wider participation, the interviews and discussions were conducted in the local languages familiar to most growers; Bemba in Northern, Luapula and Central provinces, Lozi in Western Province and Nyanja in Eastern Province. Some of the questions were repeated and rephrased to enable growers to understand and respond fully, without changing the original meaning of the question.

In the first section of the questionnaire, general information on growers' field location, altitude and field size were recorded. Surveyors inspected the field for visual symptoms of CMD and CBSD, and visually assessed the number of varieties grown. Growers were asked open questions about planting and harvesting frequencies, and varietal preferences including the number of varieties in their fields. They were presented with a selection of reasons for choice of planting material and asked to order them according to their importance to the grower.

The second section of the questionnaire comprised questions related to the trade of planting material. Growers were asked how many bags (one bag of cuttings was defined as a bundle of 100 cuttings, each of 1 metre length) went to or were obtained from the following resources: their own fields, their stores (stored planting material), friends or family, markets, middlemen, NGOs, or research stations. Growers were also asked how far away the sources or recipients were located. Growers were presented with a selection of planting material sources and asked to order them according to their importance to the grower, as well as to identify how frequently they used each source (number of individual transactions).

The third section of the questionnaire comprised a set of open-ended questions to assess growers' awareness of CMD and CBSD in terms of symptom recognition, presence of the diseases in their fields and surrounding areas, and the mechanism of disease spread. After growers' knowledge related to CMD and CBSD was assessed, they all surmised it was a disease. Subsequently, they were asked whether they controlled for disease and, if yes, how they did so. Secondly, whether they are aware of CCS and, if so, where they would access it. Finally, what their sources of information were for advice on cassava planting material and methods.

The fourth and final section of the questionnaire was related to the sources and frequencies of obtaining information related to cassava diseases and CCS, and the ranking of pre-defined sources of information according to preference. These questions did not specify a timeframe, and the events could occur at any time in the past. Questions on the frequency of obtaining information were open-ended, and were classified by the researchers into five categories; often, sometimes, rarely, once and never. Unless explicitly stated by the grower, we classified 'often' as once a month or more frequently, 'sometimes' as quarterly or several times a year, and 'rarely' as once a year.

Growers were also asked open-ended questions about the factors that influenced their decisions related to disease control, including disease pressure, their concern about the disease, and market prices that they would be willing to pay for CCS at the time of the survey.

Disease incidence and severity

Plants at the fields visited were assessed for the presence and severity of CMD and CBSD foliar symptoms as part of a larger nation-wide survey monitoring cassava disease presence in Zambia. In each field, a total of 30 plants were inspected; 15 plants on each diagonal line across the field, following methodology outlined by Sseruwagi et al. (2004). Per field disease incidence was calculated using the number of plants with visual foliar symptoms present in the field divided by the total number of sampled plants. Foliar symptom severity for CMD was recorded on each plant using a five point ordinal rating scale outlined fully in Hahn et al. (1980), where 1 indicated no disease symptoms, 2 indicated mild disease symptoms (mild chlorotic pattern), 3 indicated moderate mosaic pattern throughout the leaf, 4 indicated severe mosaic, distortion of the leaflets and general reduction in size, and 5 indicated severe mosaic and/or distortion of the entire leaf and plant stunting. Similarly, the presence or absence of CBSD symptoms on the leaves and stems was recorded for each plant using an ordinal scale of 1 to 5, fully described by Gondwe et al. (2003) where 1 indicated no apparent symptoms, 2 indicated mild disease symptoms (slight leaf feathery chlorosis with no stem lesions), 3 indicated pronounced leaf feathery chlorosis, mild stem lesions, 4 indicated severe leaf feathery chlorosis, severe stem lesions, and 5 indicated defoliation, severe stem lesions and dieback.

Collection and extraction of virus isolates

For cassava mosaic disease (CMD), a total of 208 leaf samples with CMD symptoms were collected from 96 fields during the survey. In each field, 3-4 leaf samples were collected; some with mild and the others with severe mosaic symptoms wherever they occurred, using brown envelopes to avoid contamination. The samples were transported to the Plant Virology Laboratory at the Zambia Agriculture Research Institute (ZARI) Mt. Makulu Central Research Station in Chilanga. The leaf samples were stored at -20˚C until use. Total nucleic acid (TNA) was extracted from 50 mg of each cassava leaf sample using the cetyltrimethylammonium bromide (CTAB) protocol (Lodhi et al. 1994). The extraction buffer contained 2% CTAB, 1.4 M NaCl, 100 mM Tris-HCl, 25 mM EDTA, 2% polyvinylpyrrolidone (PVP), and 2M NaCl. 2% mercaptoethanol was added to the extraction buffer just before use. The leaf samples were individually ground in 1000 µL extraction buffer using a mortar and pestle. Extracts of 800 µL were transferred into 2 mL microcentrifuge tubes and incubated at 65 °C for 15 minutes with regular shaking at intervals of 5 minutes, then cooled at room temperature. An equal volume of chloroform: isoamyl alcohol (24:1) was added to the cooled extract, vortexed for a minute and centrifuged at 12000 rpm for 15 min. The supernatant (500 µL) was transferred into new microcentrifuge tubes to which an equal volume (500 µL) of cold isopropanol was added followed by incubation at -20 °C for 30 min. The contents were centrifuged at 13000 rpm for 25 min and the supernatant discarded. The TNA pellet was washed once in 1000 µL of 70% ethanol and air dried at room temperature. The dried TNA pellet was resuspended in 50 µL Nuclease-free water. Partial fragments of 774 bp (DNA-A AV1/CP) and 556 bp (DNA-B) were amplified for both 2015 and 2017 CMD-symptomatic leaf samples using the specific primers JSP001/2 and EAB555F/R (Fondong et al. 1998) for the detection of the two cassava mosaic virus variants: African cassava mosaic virus (ACMV) and East African cassava mosaic virus (EACMV), respectively (Table 2, Supplementary Fig. S1). Polymerase chain reaction (PCR) was performed using a thermocycler (Technen 500) following the conditions as published in Chikoti et al. (2013).

To detect CBSD virus using RT-PCR, a two-step reverse transcriptase polymerase chain reaction (RT-PCR) protocol was used for virus detection. Complementary DNA (cDNA) was synthesized from 3 µg total

RNA in a 20 µL reaction mixture using M-MuLV reverse transcriptase primed with random hexamer according to the manufacturer's protocol and used in PCR with primers CBSDDF2 and CBSDDR (Table 2) (Mbanzibwa et al. 2011). PCR reaction and cycling conditions followed were as published in Munganyinka et al. (2018).

Electrophoresis was performed to detect the PCR products in a 1% agarose gel, stained in phenol blue, at 100 V for 60 min in gels buffered with 1X TAE using a Bio-rad gel apparatus. The gels were visualized using the gel documentation system (Gel Doc XR, Bio-rad).

Data analysis

Descriptive statistics including means, standard errors and cross tabulations were calculated to summarise the growers' responses and disease incidence. Results were expressed as percentages or absolute frequencies of responses obtained from growers, excluding records where data were not available (therefore the total may differ in each question). The answers were analysed using the R language for statistical computing (R Core Team 2016) and plotted with the *ggplot2* package (Wickham 2016). The relationship between growers' disease awareness as an independent binary response and disease incidence as a dependent variable was investigated with a logistic regression using the 'glm' function in the *lme4* package and a *chisq.test* function (Bates et al. 2015). Growers were classified as being aware or not aware of CMD based on their responses to the question "what do you know about CMD?". We compared responses of two groups of growers (ones informed about cassava diseases in the past, and those who never had information about cassava diseases) about their concern about cassava diseases on 10-point scale to see if there were significant differences in the responses of two groups using a non-parametric Mann-Whitney U test in the *wilcox.test* function of R.

RESULTS

Field properties, disease status and varieties preferences

Most growers' fields were small (mean = 0.59 ha, standard error SE = 0.12) and planted annually (92.9% of participants) (Table 1). Harvesting was based on need for own daily consumption or for sale (40% of participants). All survey sites in the Western Province were infected with CMD, based on visual symptoms assessment, with mean conditional incidence of 65.9%, where conditional incidence refers to mean incidence across infected fields only (Table 2). Approximately 90% of survey sites in Central, Luapula and Northern provinces had plants with CMD foliar symptoms apparent, with mean conditional incidence of 39.5 - 53.5%. Less than half (47.8%) of survey sites in Eastern Province were infected with CMD, with mean conditional incidence of 54.5%. In the infected fields, the highest ratio of plants with high severity scores (4 and 5) were observed in Eastern and Western provinces of the country, with the percent of plants with severity score 4 approximately 38% and those with severity score 5 approximately 5%. In contrast, plants with severity score of 4 ranged from 4.5-6.15%, and plants with severity score 5 ranged from 0-0.30%, in Central, Luapula and Northern provinces. No CBSD was observed in any of the study fields. Growers typically planted more than one variety of cassava in their fields (66.5% of growers) with a range of 1-7 varieties. Good taste and associated sweetness (31 growers), for a grower's own consumption and food security (22 growers), and with a high yield and large tubers (21 growers) were the most commonly cited traits determining varietal choice (Fig. 2). Early maturing and bulking (19 growers), and the availability of planting material (15 growers) were also cited as a priority determining choice. Among six preference criteria influencing choice of planting material presented to the respondents, varietal preference was the highest ranked, while availability-related answers were ranked second and third (Fig. 3).

Planting material movement and trade

Most planting material was recycled from the previous crop (83 growers) or stores (planting material stored previously, as opposed to material cut and immediately replanted - 11 growers), while a large proportion of growers (52/96) reported that they discarded some planting material. While sharing did occur with family and friends (55 and 39 growers respectively) this was generally within the same or nearby villages, with 94% of recipients located within a radius of 1-10 km (Fig. 4). However, some movement of planting material did occur over a greater distance, including a small number of large transactions with markets (100 bags over an average of 7.43 km), middlemen (9.5 bags over an average of 55 km), or NGOs (15 bags over an average of 28.5 km). Given the paucity of data on movement of cassava planting material, we provide some additional detail on selected individual transactions to illustrate the range of behaviours evident in a relatively small cohort. One transaction involved moving a large amount of planting material (100 bags) from a single grower with a large field of 4 ha to a market

40km away. Three further transactions with markets occurred, including 10 bags sold at a market a reported 0.05 km from the 1.5 ha field, and two smaller transactions of 7 and 1 bags over longer distances (3 km and 8 km, respectively) from very small fields (field size up to 0.25 ha). Growers who obtained their planting material from middle-men (intermediate suppliers) indicated material was moved over distances of 50 to 60 km, while six growers exchanged their planting material with an NGO or another organization over distances between 0 and 350 km.

CMD and CBSD awareness

Most of the growers surveyed (81%) responded that they did not know what CMD was when explicitly asked "what do you know about cassava mosaic disease?". After growers surmised it was a disease, most (60.5%) were unable to recognise it by its symptoms, or specify its mechanism of dispersal (75.6%), or likely effect on yield (39%). In a logistic regression model (Table 4), higher CMD incidence in a field was a significant predictor of growers' CMD knowledge. Nearly half of growers (44%) did not know whether the disease had an impact in their area, while 44% had observed an impact on the crop. Of those that had observed an impact of the disease, 25.9% identified yield losses.

Overall, when asked how concerned they were about CMD on a scale from 1 (not worried) to 10 (very worried), 53% of growers responded they were not at all or only slightly worried (1-3), 17% of growers were moderately worried (4-6) and 28% were very worried (7-10). When the respondents were grouped by whether they had heard about CMD at some point in the past ('informed' growers), or never heard about the disease ('not informed'), growers who had heard about CMD were more concerned compared with those who had not (Mann-Whitney U test $p = 0.0002$, W = 1235) (Fig. 5).

None of the growers had an awareness of CBSD, and no disease symptoms were detected in the surveyed fields.

Disease control and management

Disease management for CMD was rare among growers. Three quarters of growers (74.7%) declared that they did not practice any control measures (n = 83). In contrast, of the few growers that applied control measures, five used clean planting material while two, who were seeking help from agricultural extension workers, rogued the diseased plants and sprayed for insects. The majority of growers who used control measures were in Eastern Province (8 out of 12), which had the lowest mean disease prevalence and absolute incidence among surveyed farms. Most growers who implemented disease management cited their own experience as a source of disease control knowledge (7) while two cited agricultural extension workers, one cited a parent, and one a cooperative group.

Certified clean seed sourcing and awareness

Nearly half of the growers were aware of CCS (47.7%, n = 88), where 33.3% would seek it from agricultural extension workers if there was a need for it and 10.8% had used it in the past. At the same time, of those who were unaware of CCS (48.9%), after an explanation the majority (58%) responded that they would be happy to use it if it were available, while no growers indicated that they would not be happy to use CCS if it were provided to them. The remaining 3.4% of respondents stated that they were either aware of CCS for other crops or that CCS was not relevant to them. Northern and Western provinces had the highest awareness of CCS with 20 out of 24 and 9 out of 13 respondents declaring they knew about CCS respectively. In Central Province only one out of 12 respondents knew about CCS and in Luapula only 4 out of 19. In Eastern Province about half of the respondents (9 out of 17) declared they were aware of CCS.

Information sources

Among the surveyed growers, 30% relied on information passed on from their parents or grandparents as their source of cassava planting knowledge, slightly over a quarter (27.4%) relied on their own experience and 21.4% relied on information obtained from agriculture extension workers (n = 84). Other sources included friends (11.9%), other relatives (3.6%), other growers (1.2%), the radio (9.5%), researchers (3.6%), neighbours (2.4%) or NGOs (2.4%).

Information on cassava diseases and CCS had reached half of growers on at least one occasion in the past (50.6% and 51.8% respectively), although no single source of information reached the majority of individuals. The most frequent sources of information included nearby friends, family and neighbours, and the radio (Fig. 6a).

In terms of preferences for information, growers preferred to hear from extension workers, TV and radio, and people within the village (Fig. 6b), while village leaders and friends or relatives located in a different village were less preferred. Nearly 90% of growers who were aware of CMD had access to frequent information about it, whilst the majority of growers who were unaware of the disease had no access to information (Fig. 7). The most informed growers were located within the Northern and Eastern provinces, where over half of growers had often heard about CMD from various sources. The least informed growers were located in Luapula and Western provinces, where over two thirds of growers reported never receiving information about CMD.

Making decisions

High yield, low cost, and absence of disease were the most frequently reported factors (27.4%, 25% and 22.6%, respectively) influencing growers' decisions on whether or not to use CCS. The majority of growers indicated they would consider adoption of CCS to control for CMD if two to four neighbours were affected by the disease. Similarly, they would consider using CCS if two to four neighbours were using it too (Supplementary Fig. S2).

Growers were classified according to their answer to the question on CMD knowledge. Depending on their response they were classified as "having knowledge" for those who were aware of CMD, those who had "some knowledge", and finally those who "did not know" about the disease. In those three categories 40%, 18% and 8% of growers respectively controlled for the disease. However, differences between these groups were not statistically significant (χ^2 test $P = 0.19$, df = 2). When growers were classified into two groups (with or without knowledge), the differences were still not significant (χ^2 test $P = 0.16$, df = 1). The intention to buy CCS decreased with increasing price (Supplementary Fig. S3), where 20 KWZ per bag of 100 cuttings represented a key decision point for many growers (prices as presented to respondents and not inflation-adjusted for publication).

DISCUSSION

Cassava virus diseases constitute a major constraint to the production of cassava in sub-Saharan Africa, yet there have been few studies looking into some of the key aspects of human-mediated disease spread and control. These include awareness of the diseases, and the practices and decision-making of cassava growers themselves (Delaquis et al. 2018). Our study provides a valuable insight into the movement of planting material in Zambia, where we show that cassava planting material trade is largely informal with a limited number of commercial growers involved in the production and sale of planting materials. We found that growers mostly recycled materials from their own fields, attributing this to varietal preference as well as the fact that the material was readily available. This tendency to recycle material is consistent with previous studies, which have shown that a majority of planting material is recycled within the same field, while a considerable portion is also exchanged with close friends or family (Chikoti et al. 2016; Gnonlonfin et al. 2011; Houngue et al. 2018; Ntawuruhunga et al. 2007; Teeken et al. 2018). Although markets, NGOs or research organisations and intermediate suppliers (aka middle-men) were rarely involved in the movement of planting material for respondents in this study, the large scale of the distances and quantities of material moved in those transactions does indicate that these agents could transmit pathogens across large distances. This could lead to the establishment of new disease foci, which previous work has demonstrated could be severely detrimental to disease control (Delaquis et al. 2018; Legg et al. 2014; McQuaid et al. 2017a, b). Increasing the distance and quantity of movement of infected planting material increases the importance of the material over the whitefly vector in the dispersal of pathogens (McQuaid et al. 2017a).

In general, most growers in our study indicated that markets were more than 7 km from their homesteads. It has been shown in a previous study that the closer a household is to a market, the higher the probability it will adopt improved varieties, due to greater market accessibility (Salasya et al. 2007). Growers further away from markets are at a disadvantage, due to an increased difficulty in selling their own planting material and a reduced opportunity for information exchange, and are thus more inclined to subsistence production. Growers are also sensitive to the price of planting material, and an increase in the price of CCS relative to the local variety reduces adoption rates (Langyintuo and Mekuria 2008). However, while it seems likely that a lack of awareness of cassava diseases and control methods will affect cropping practices, our findings regarding this did not prove to be statistically significant.

There are inevitably sources of error and bias in the conduct of surveys that need to be borne in mind. Our survey was conducted over two years, but in each case critical questions were related to experience from the previous (i.e. most recent) year or harvest, in an effort to enhance comparability. Due to the poor road infrastructure in Zambia, participating growers were also located along the main motorable roads. Our inferences about movement distances therefore relate strictly to growers based along motorable routes. The Agricultural Extension System under the Ministry of Agriculture in Zambia spearheads activities that facilitate access of grower, their groups, organizations and other market actors to information and technologies. It groups all growers into camps that are irrespective of their proximity to either motorable or non-motorable locations. All the camps and growers therein are therefore provided with the same agricultural amenities, technical information and services, ensuring that all growers are at a par. Our inferences about access to information are therefore likely to hold for growers in motorable or non-motorable locations since both classes are targeted by communication from the Agricultural Extension Service in Zambia. The implications of small sample size and bias in the location of participating growers mean that additional work is required to confirm our findings. Particularly, it may be that the participants in our survey were more likely to have access to information than growers located further from motorable roads. It is important when considering issues of equity that these growers are not neglected, and future studies should attempt to identify whether our findings are consistent for these growers. Additionally, the sample size of our survey makes it more susceptible to stochastic differences amongst growers, so our findings should be viewed as exploratory, requiring further collection of evidence to support them. Sampling over multiple years may also have affected both the disease incidence and awareness we might expect to see, with both presumed to increase over time. Participant gender was not recorded, which raises a further limitation to the results. While the majority of smallholder growers are expected to be female, we might expect to see important behavioural and awareness differences between growers of different gender, as well as differences in obtaining access to information.

Our work supports previous studies that have shown that culinary properties and varietal taste are key factors in planting material selection, followed by economic traits such as yield, while the presence of disease makes little to no difference to choice (Houngue et al. 2018; Kombo et al. 2012; Njukwe et al. 2013; Ntawuruhunga et al. 2007). With this in mind, efforts to use CCS to control disease epidemics need to address growers' varietal preferences and needs (Evenson and Gollin 2003; Kiros-Meles and Abang 2008), something that also applies to the use of disease-resistant or tolerant varieties. If new varieties are not suited to local tastes the level of adoption is likely to be low, a factor to be considered by both cassava breeders and CCS producers alike. At the same time, the importance of yield to varietal choice presents an opportunity to educate and reassure growers about the economic advantages of CCS and the adoption of improved varieties.

Our study findings show a striking lack of awareness of cassava diseases amongst growers. While this is unsurprising for CBSD, the result for CMD was unexpected. CMD was widespread in growers' fields as evidenced from detected cassava mosaic virus variants in this study, and has been present across the country for more than two decades, with estimated yield losses of 50 – 70% (Muimba-Kankolongo et al. 1997). This lack of disease awareness is likely to be a reflection of the scarcity of information about diseases available to growers; only half of growers received any information on disease or its control at some point, and few received information frequently or on a regular basis. Access to information is critical to decision-making, and this lack of information increases concerns about the disease. Our results indicate that a reduced awareness as well as reduced receipt of information about disease can significantly affect growers' concerns and perceptions of the diseases, as well as their willingness to apply control measures. In particular, a lack of awareness of the risk and impact of disease on yield could lead to the failure of disease control measures implemented at a wider level, where it is necessary for a large proportion of growers to engage in disease management in order for effective, sustainable control to work (McQuaid, et al. 2017b). It is certainly highly likely that the lack of awareness, combined with high incidence, contributes significantly to the spread of the disease. The high rate of reuse of planting materials by growers within the same field, due often to a lack of alternative sources, could also result in a low genetic potential with an increase in susceptibility of the material to pests and diseases, as observed in Malawi (Chipeta et al. 2016). While replanting material resistant to disease could potentially protect growers from the arrival of infected cuttings from their own or other fields, no cassava variety is currently fully resistant to both CMD and CBSD (Kawuki et al. 2016; Mukiibi et al. 2019; Tomlinson et al. 2017). Ultimately, therefore, uninformed growers who do not practice management strategies will still be vulnerable to disease acquired from whitefly infections and, as a consequence of high rates of recycling of material, a rapid build-up of disease over seasons. Nonetheless, although there are improved cassava varieties bred by the Zambia Agriculture Research Institute that are tolerant to CMD, early bulking and high yielding, most of the farmers grow local varieties that are susceptible to CMD in Zambia (Alene et al. 2013; Chikoti et al. 2013). Persuading farmers to use CMD-resistant varieties is a challenge because of farmers' preferences for particular cassava traits other than disease resistance.

Lastly, our results underscore the important role of two key sources in providing information to growers; radio (as well as the less widely available TV) and extension workers. While our study demonstrated that extension workers were a highly trusted source of information, only a small proportion of growers were reached by these workers. Growers were more likely to share information in their network of neighbours, friends and relatives. This does suggest, however, that information received by a grower from an extension worker or the media could percolate (albeit with reduced trust in the source) through the grower's networks to reach a larger number of growers.

The combination of low levels of knowledge and information seen in our results suggests that there is a need for grower education, through extension workers and media, to improve awareness that is vital to controlling cassava disease. Reducing the presence of cassava virus diseases, and increasing the yields of small-holder growers across Zambia and cassava growing regions in Africa as a whole, will not happen without well-informed growers acting at an individual level to implement disease control.

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TABLES

Table 1. Summary of the number and per-province distribution of interviewed growers, average field size, number of varieties planted in the field and planting frequency.

Province	Number of growers		Field size [ha]		Median number of in-field varieties	Planting frequency [number of respondents]		
	2015	2017	Mean	SE[a]		Biennial	Yearly	Twice a year
Central	-	15	0.26	0.06	2	0	2	2
Eastern	9	15	0.82	0.43	1	0	22	2
Luapula	4	15	0.29	0.06	3	1	18	0
Northern	11	14	0.45	0.09	2	0	23	0
Western	-	13	1.25	0.29	3	0	12	1

[a] SE = standard error

Table 2. Primers used to detect variants of cassava mosaic viruses using PCR in cassava leaf samples collected. Cassava mosaic disease (CMD) was diagnosed using primers for African cassava mosaic virus (ACMV) and East African cassava mosaic virus (EACMV), and cassava brown streak disease (CBSD) was diagnosed using primers for cassava brown streak viruses (CBSV) and Ugandan cassava brown streak virus (UCBSV).

Primer	Sequences (5'–3')	Specificity	Product size
JSP001[a]	ATGTCGAAGCGACCAGGAGAT	ACMV	774
JSP002[a]	TGTTTATTAATTGCCAATACT	ACMV	
EAB555/F[a]	TACATCGGCCTTTGAGTCGCATGG	EACMV	556
EAB555/R[a]	CTTATTAACGCCTATATAAACACC	EACMV	
CBSDDF2[b]	GCTMGAAATGCYGGRTAYACAA	CBSV, UCBSV	344, 440
CBSDDR[b]	GGATATGGAGAAAGRKCTCC		

[a]Cassava mosaic begomovirus (CMB) specific primers used for the study as described by Fondong et al. (1998). [b]Cassava brown streak potyviruses specific primers described by Mbanzibwa et al. (2011).

Table 3. Summary of cassava mosaic disease (CMD) per-province presence in the fields of interviewed growers. Prevalence refers to the proportion of fields with any disease symptoms observed. Per field incidence was calculated based on visual foliar symptoms across 30 surveyed plants, where absolute incidence refers to the average percent of infected plants across all fields and conditional incidence refers to the average incidence across infected fields only. Disease symptoms severity score 1 indicates no

observed CMD symptoms, 2 indicated mild disease symptoms (mild chlorotic pattern), 3 indicated moderate mosaic pattern throughout the leaf, 4 indicated severe mosaic, distortion of the leaflets and general reduction in size, and 5 indicated severe mosaic and/or distortion of the entire leaf and plant stunting (Hahn et al. 1980).

		CMD								
		Absolute incidence [%][b]		Conditional incidence [%][c]		Mean per-field percent of plants classified in each disease severity category [%]				
Province	Prevalence [%]	Mean	SE[a]	Mean	SE	1	2	3	4	5
Central	92.9	36.7	7.4	39.5	7.7	60.5	12.1	2.1	6.1	0.3
Eastern	47.8	26.1	7.2	54.5	9.6	41.5	5.1	10.0	38.5	4.9
Luapula	89.5	47.9	6.0	53.5	5.1	46.5	10.4	38.6	4.5	0
Northern	91.7	43.8	6.3	47.7	6.4	54.1	3.0	33.8	8.9	0.2
Western	100	65.9	6.3	65.9	6.3	34.1	1.3	20.8	38.2	5.6

[a] SE = standard error
[b] Absolute incidence = incidence among all fields (both infected and where disease was not reported)
[c] Conditional incidence = incidence among infected fields only.

Table 4. Logistic regression model of in-field cassava mosaic disease (CMD) incidence to predict growers' answer to the question "do you know what CMD is", where cases are represented by "no" answers and controls by "yes" answers (number of respondents = 84).

Model	β estimate	Std. error	Z value	Pr (>|z|)
Intercept	2.1223	0.1042	20.36	<0.001
CMD incidence	-1.8838	0.1674	-11.26	<.001

Figures

Figure 1. Locations of interviewed growers in five provinces of Zambia, showing field size and cassava mosaic disease (CMD) incidence (proportion of infected plants within the field).

Figure 2. Different cassava traits dictating varietal choice cited by growers, where multiple answers were permitted. 'Resistant' refers to resistance to disease. Number of respondents = 96.

Figure 3. Planting material (A) reason for choice and (B) preferred source. Ranking 1 represents the most preferred, whilst rankings 6 (A) and 4 (B) represent the least preferred (number of respondents = 96).

Figure 4. Total number of (A) bags of planting material moved (received or given away/sold) and (B) individual transactions over a given distance. One bag of cuttings is defined as a bundle of 100 cuttings, each of 1 metre length. An organisation was defined as a non-profit entity involved in the movement of cuttings, such as an NGO or research station (number of respondents = 96).

Figure 5. Growers' response to the question: "How worried are you about cassava mosaic disease, on a scale of 1 to 10, where 1 is the least worried and 10 is the most worried?". Growers are categorised based on whether they reported hearing about cassava mosaic disease (CMD) in the past on at least one occasion (defined as 'informed') or never ('not informed'). Number of respondents = 87.

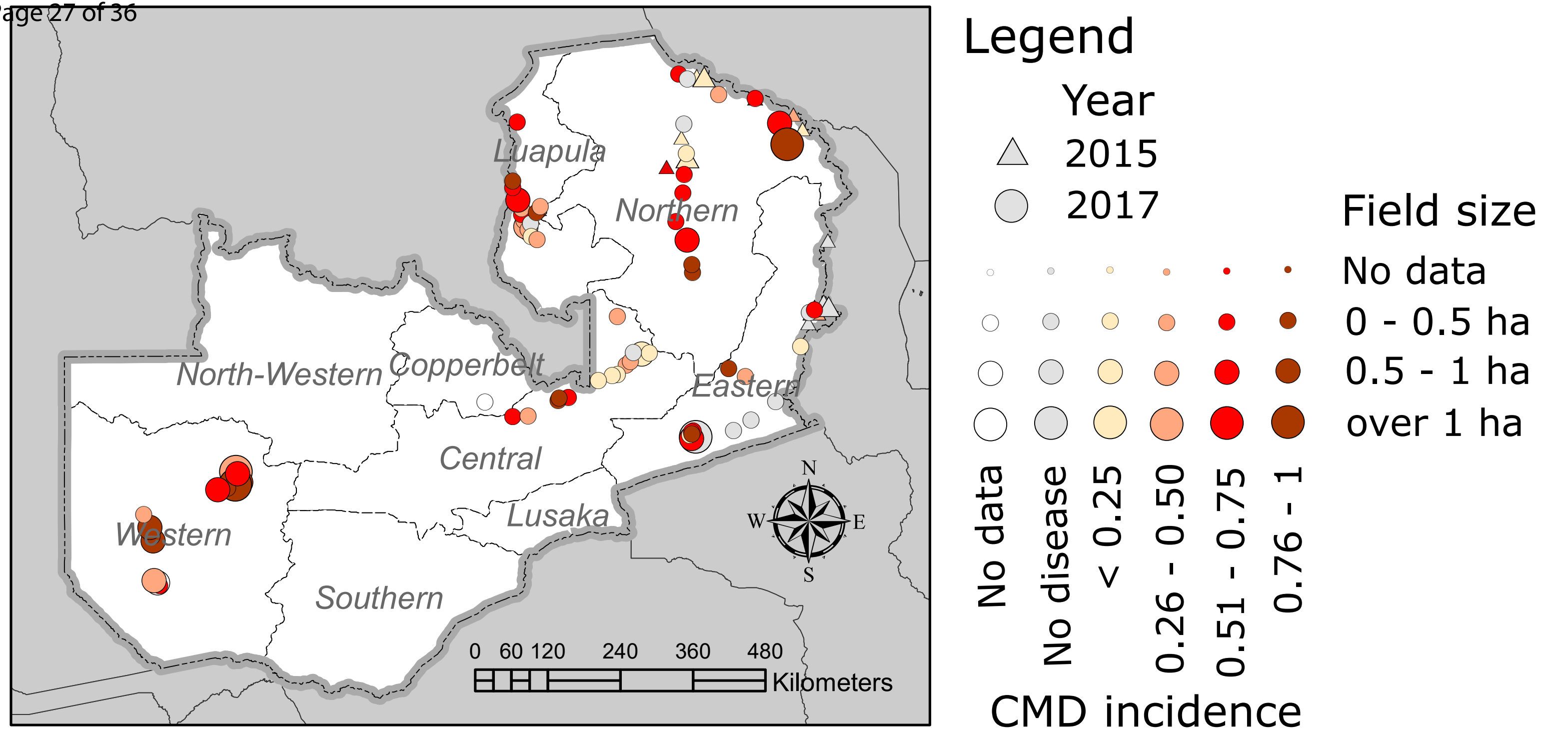
Figure 6. (a) Frequency of receiving information on cassava, and (b) ranking of source of information on cassava diseases from the most (1) to least preferred (7). Friends and relatives from a different village are classified as 'friends or relatives from far away' (number of respondents = 75).

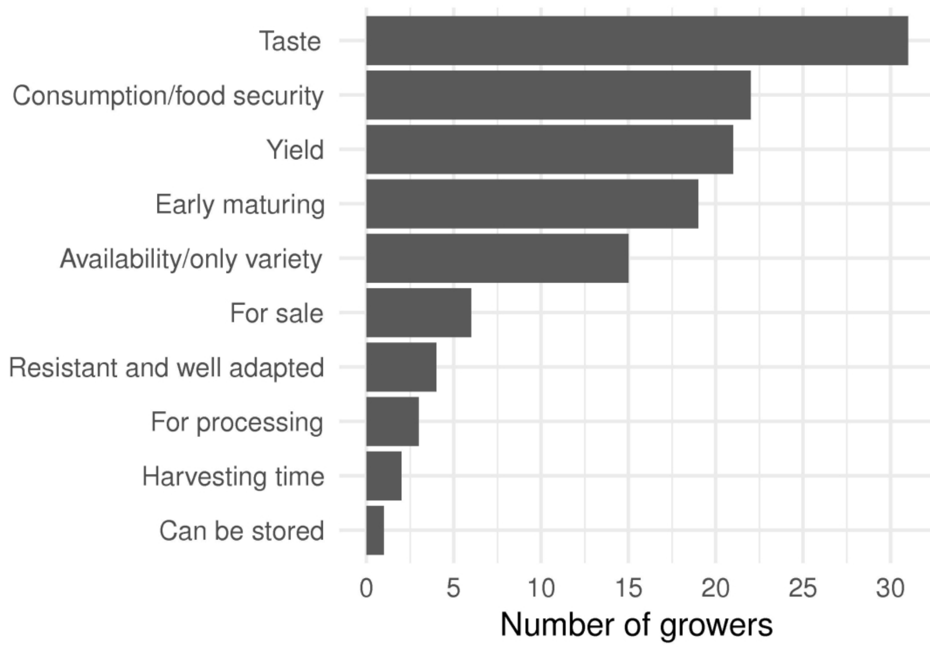
Figure 7. Response to the question (a) "What do you know about cassava mosaic disease?" classified into growers who knew about the disease, those who had some idea of the disease, and those who did not know about the disease (number of respondents = 85). (b) Frequency with which growers received information about cassava mosaic disease (CMD) by province (number of respondents = 86).

Supplementary Figure S1. Gel electrophoresis of DNA fragments of representative isolates of a) African cassava mosaic virus (ACMV) (774bp) using the specific primers JSP001/002 and b) East African cassava mosaic virus (EACMV) (556bp) using the specific primers EAB555F/R

Supplementary Figure S2. Response to the questions (a) "after how many of your neighbours had cassava mosaic disease (CMD) would you think about control?" (number of respondents = 86) and (b) "after how many of your neighbours used clean seed systems (CCS) would you think about control?" (number of respondents = 83).

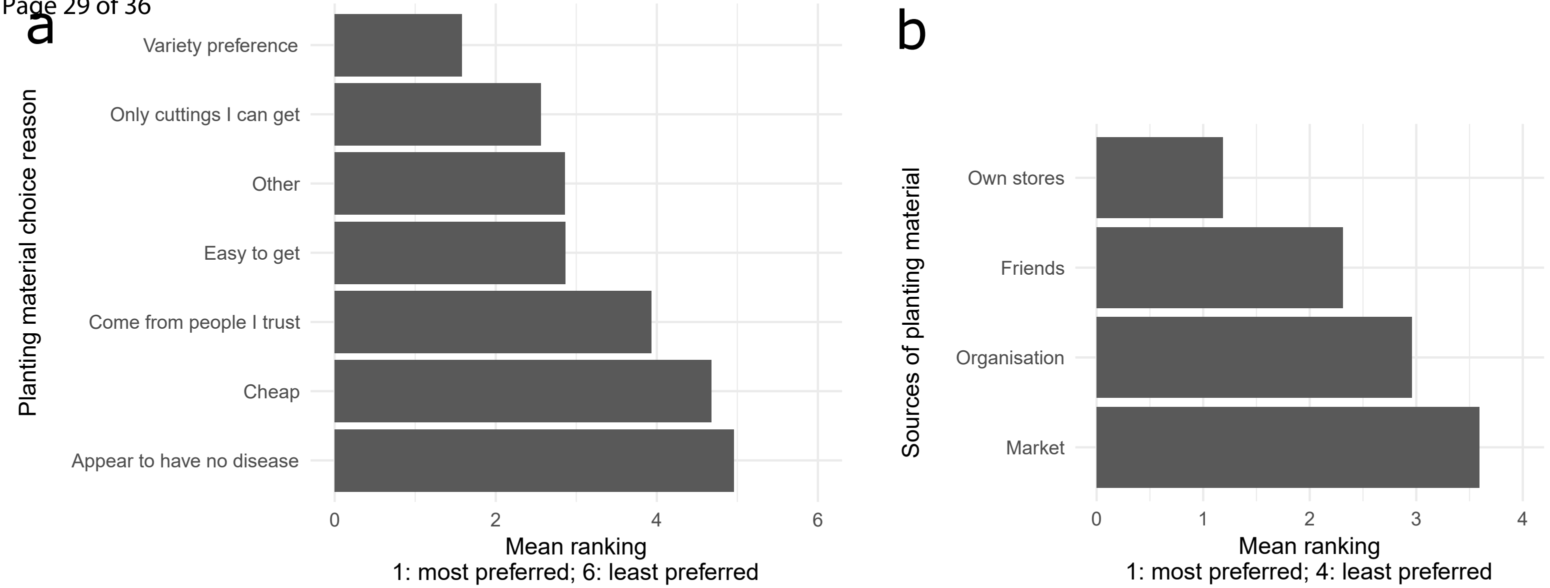
Supplementary Figure S3. Response to the question: "would you buy a bundle of 100 certified clean plant cuttings if the cost were: 10, 15, 20, 30 or 40 Zambian kwacha?" (number of respondents = 96).

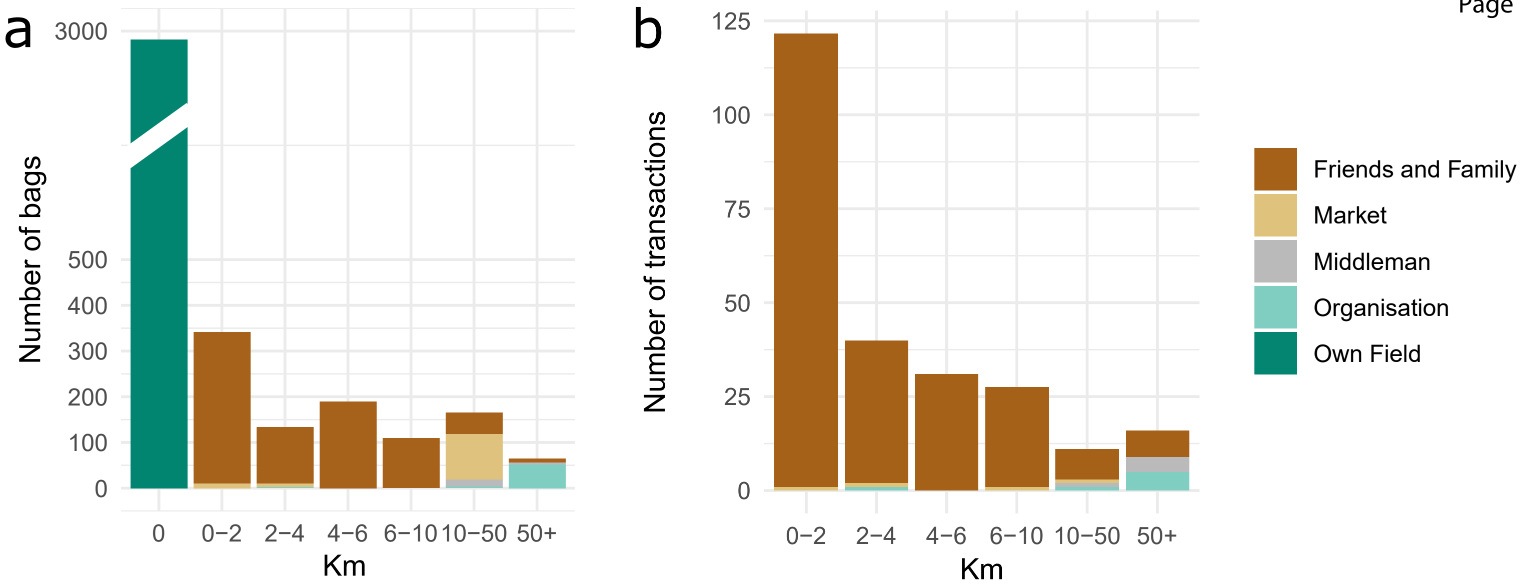

Luapula
Northern
North-Western
Copperbelt
Eastern
Central
Lusaka
Western
Southern
N
W
E
S
0 60 120 240 360 480
Kilometers
Legend
Year
2015
2017
Field size
No data
0 - 0.5 ha
0.5 - 1 ha
over 1 ha
No data
No disease
< 0.25
0.26 - 0.50
0.51 - 0.75
0.76 - 1
CMD incidence

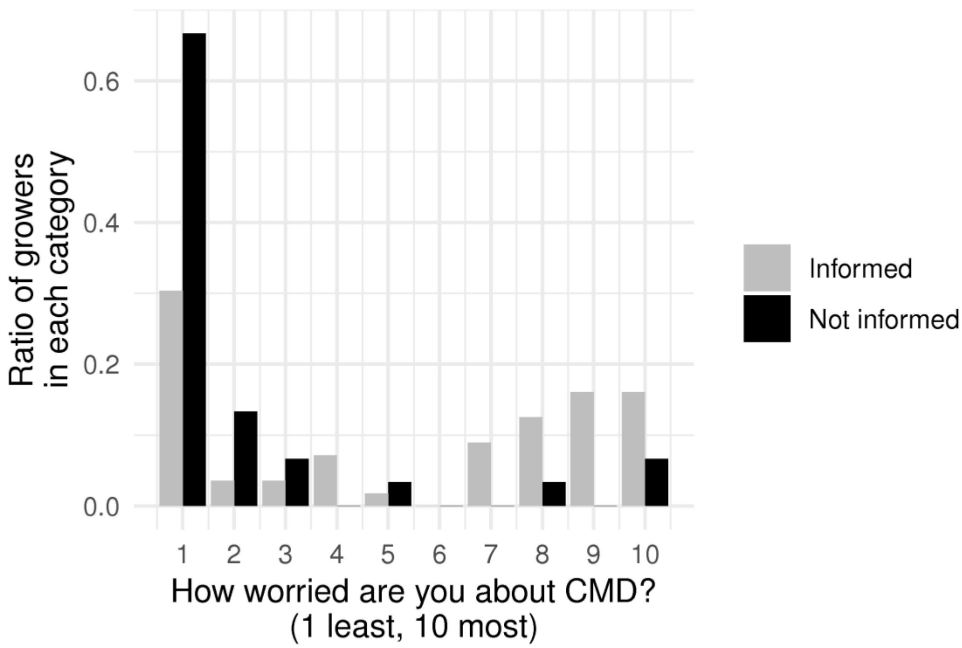


Different cassava traits dictating varietal choice cited by growers, where multiple answers were permitted. 'Resistant' refers to resistance to disease. Number of respondents = 96.

119x80mm (600 x 600 DPI)

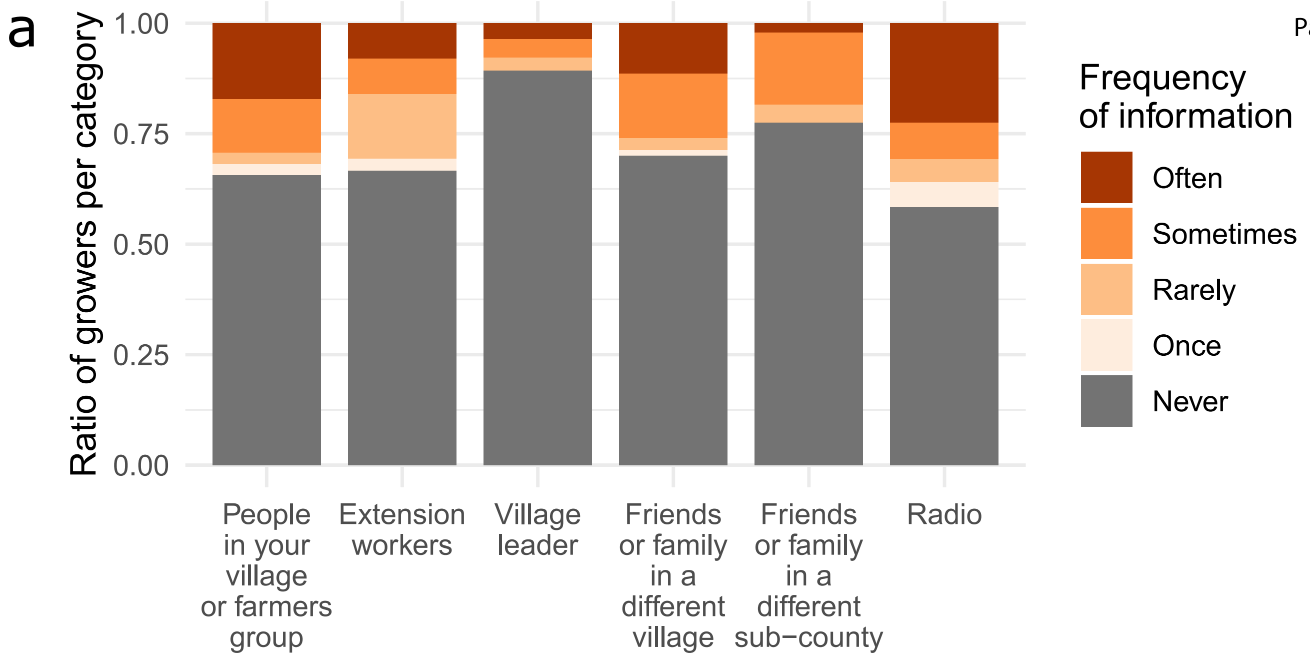

a
Variety preference
Only cuttings I can get
Other
Easy to get
Come from people I trust
Cheap
Appear to have no disease
Planting material choice reason
0
2
4
6
Mean ranking
1: most preferred; 6: least preferred
b
Own stores
Friends
Organisation
Market
Sources of planting material
0
1
2
3
4
Mean ranking
1: most preferred; 4: least preferred

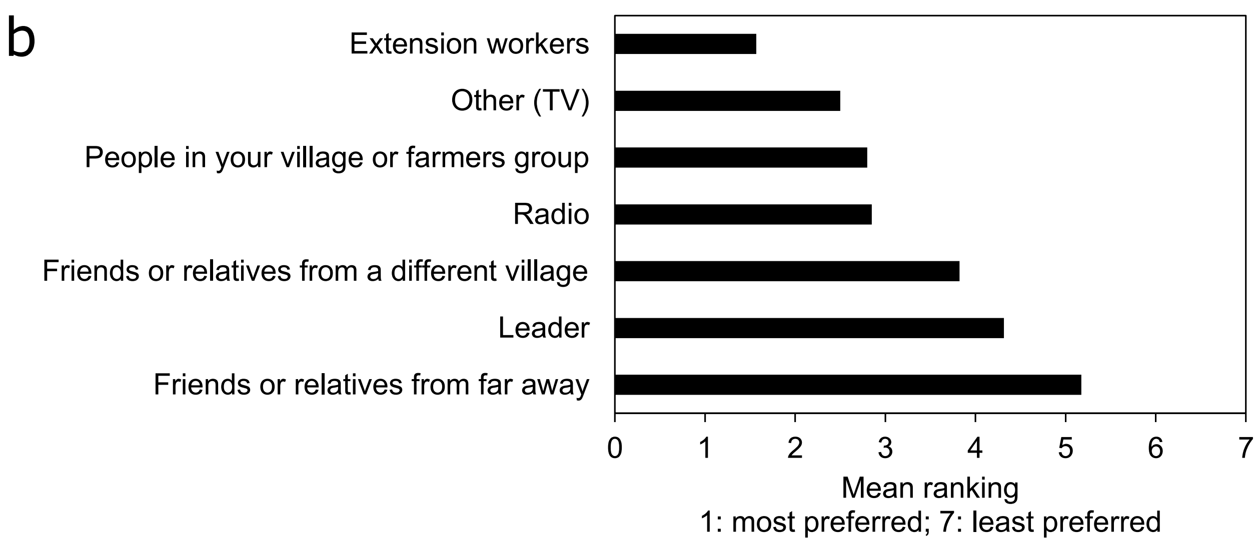

a
3000
500
400
300
200
100
0
Number of bags
0
0−2
2−4
4−6
6−10
10−50
50+
Km
b
125
100
75
50
25
0
Number of transactions
0−2
2−4
4−6
6−10
10−50
50+
Km
Friends and Family
Market
Middleman
Organisation
Own Field

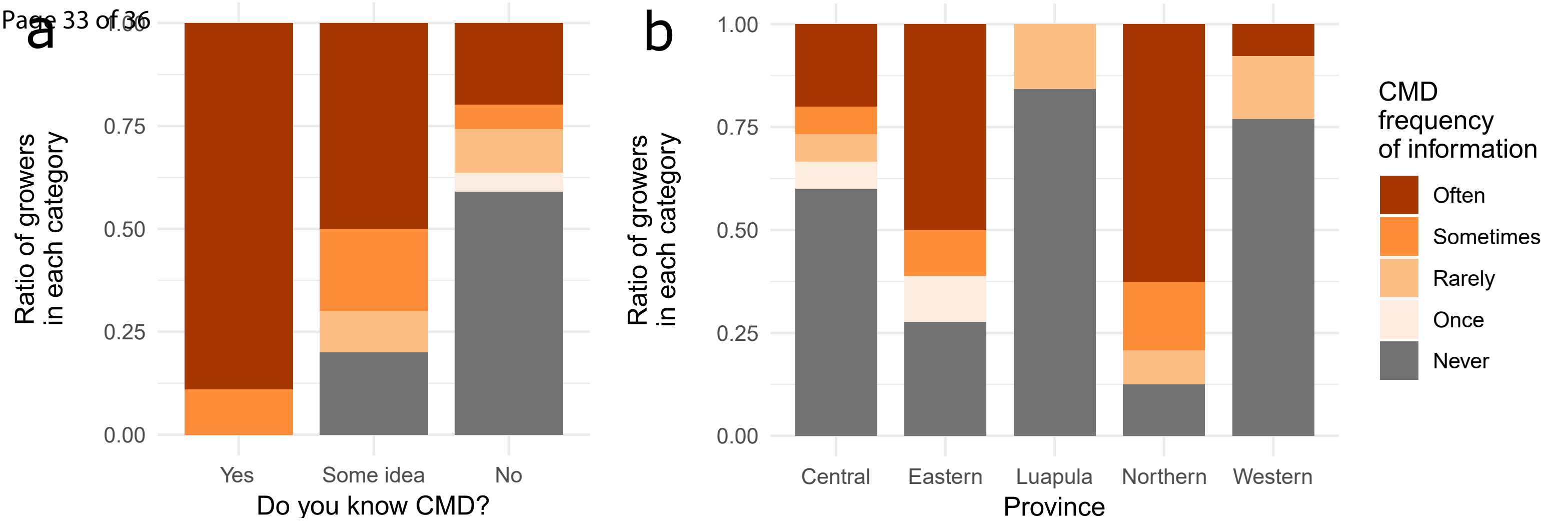


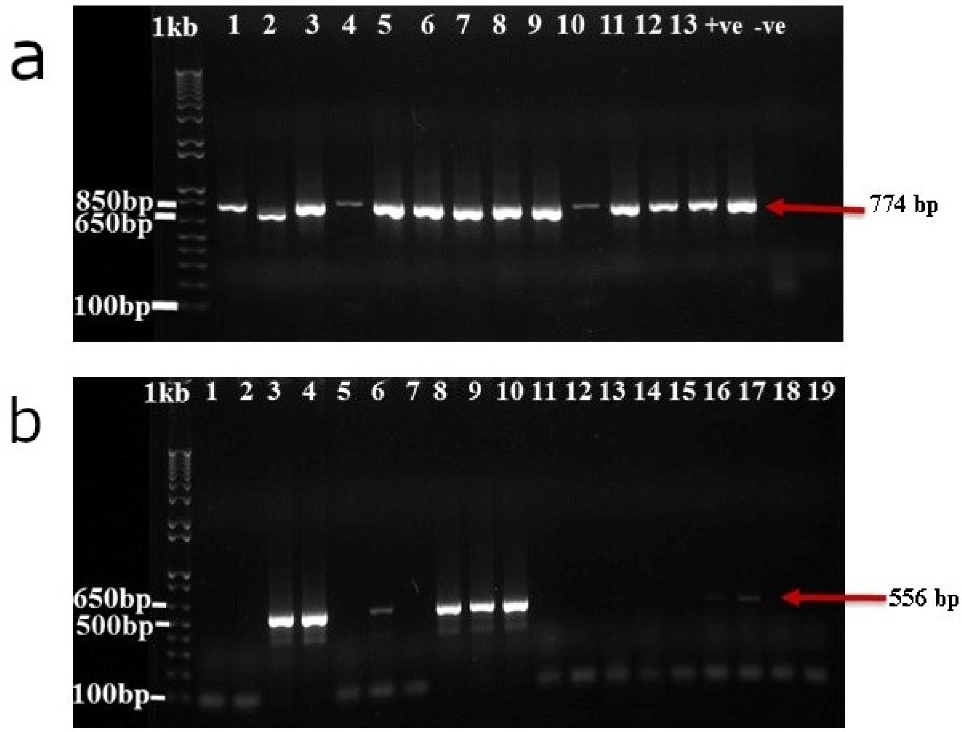
Growers' response to the question: "How worried are you about cassava mosaic disease, on a scale of 1 to 10, where 1 is the least worried and 10 is the most worried?". Growers are categorised based on whether they reported hearing about cassava mosaic disease (CMD) in the past on at least one occasion (defined as 'informed') or never ('not informed'). Number of respondents = 87.

119x80mm (600 x 600 DPI)


a
Ratio of growers per category
1.00
0.75
0.50
0.25
0.00
People in your village or farmers group
Extension workers
Village leader
Friends or family in a different village
Friends or family in a different sub−county
Radio
Frequency of information
Often
Sometimes
Rarely
Once
Never

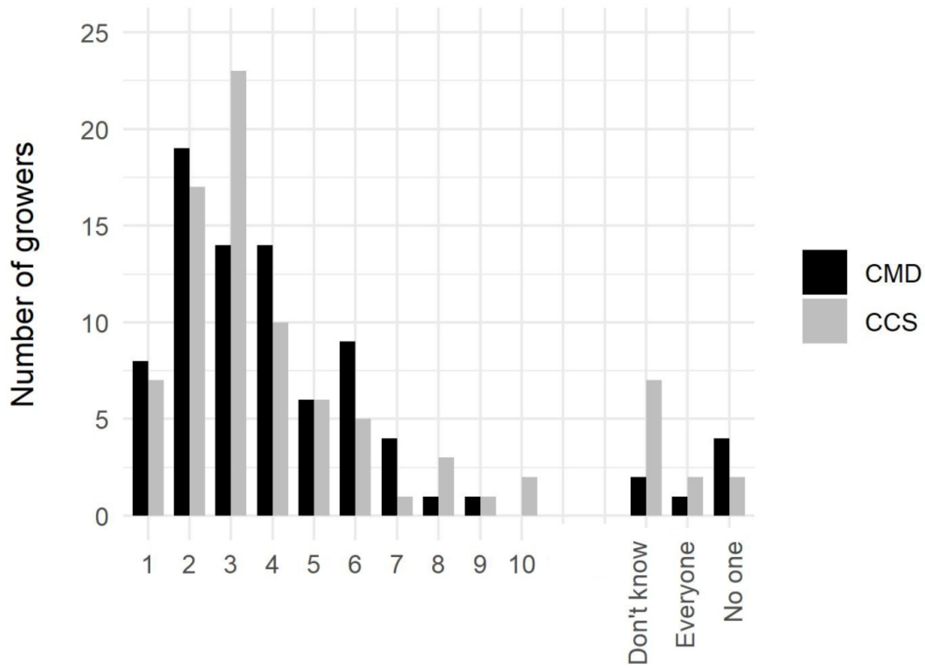

b
Extension workers
Other (TV)
People in your village or farmers group
Radio
Friends or relatives from a different village
Leader
Friends or relatives from far away
0
1
2
3
4
5
6
7
Mean ranking
1: most preferred; 7: least preferred


a
1.00
0.75
0.50
0.25
0.00
Ratio of growers in each category
Yes
Some idea
No
Do you know CMD?
b
1.00
0.75
0.50
0.25
0.00
Ratio of growers in each category
Central
Eastern
Luapula
Northern
Western
Province
CMD frequency of information
Often
Sometimes
Rarely
Once
Never



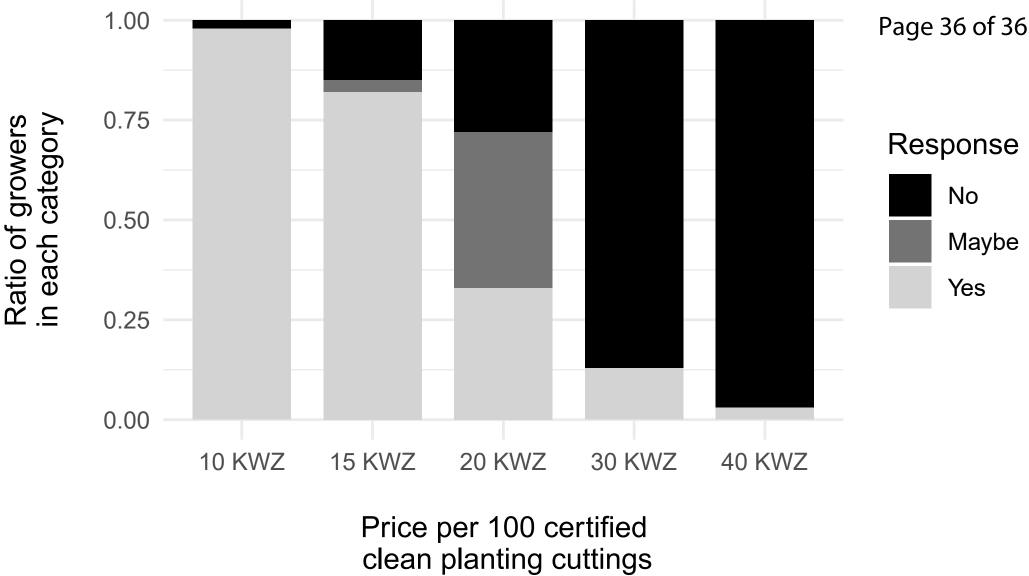
Gel electrophoresis of DNA fragments of representative isolates of a) African cassava mosaic virus (ACMV) (774bp) using the specific primers JSP001/002 and b) East African cassava mosaic virus (EACMV) (556bp) using the specific primers EAB555F/R

165x136mm (96 x 96 DPI)



Response to the questions (a) "after how many of your neighbours had cassava mosaic disease (CMD) would you think about control?" (number of respondents = 86) and (b) "after how many of your neighbours used clean seed systems (CCS) would you think about control?" (number of respondents = 83).

521x405mm (72 x 72 DPI)


1.00
0.75
0.50
0.25
0.00
Ratio of growers
in each category
10 KWZ
15 KWZ
20 KWZ
30 KWZ
40 KWZ
Price per 100 certified
clean planting cuttings
Response
No
Maybe
Yes