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## EFFECTIVE ISOLATION DISTANCE FOR PREVENTION OF CASSAVA VIRUS INFECTIONS IN UGANDA

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### ABSTRACT

Cassava brown streak disease (CBSD) and cassava mosaic disease (CMD) are the major viral diseases of cassava in Uganda. Although isolation distance of “50 m” has been recommended by MAAIF in Uganda for prevention of virus infections in crops, the minimum isolation distance has not been verified for effectiveness in cassava. This study assessed the effective isolation distance for management of viral diseases in cassava. Virus-clean cassava cultivars (NASE 03, NASE 14 and NAROCASS 1) from farmers’ fields were used as field sourced (FS) planting materials. Tissue culture (TC) material of the same cultivars were sourced from the National Crops Resources Research Institute and Makerere University Agricultural Research Institute tissue culture laboratories. Both FS and TC materials were tested at isolation distances of 50, 100, 150 and 250 m for virus prevention. The experiment was laid out in a randomised complete block design and was run for 12 months after planting (MAP). Mean CBSD/CMD prevalence significantly varied ( $P < 0.05$ ) among isolation distances in both FS and TC plants, and the 250 m isolation distance was the most effective in reducing disease prevalence. Across cultivars and planting material category at 12 MAP, the 50 m isolation distance had the highest foliar incidence for CBSD (29.2%) and CMD (16.1%); while severity for CBSD was 1.4 and 1.2 for CMD. At 250 m, all FS and TC plants had CBSD/CMD severity of 1.0 and 0% incidence. These results show that 250 m isolation distance can provide an option to disseminate popular, but CBSD/CMD susceptible cassava cultivars thereby manage CBSD/CMD.

*Key Words:* Cassava brown streak disease, cassava mosaic disease

### RÉSUMÉ

La maladie des stries brunes du manioc (CBSD) et la maladie de la mosaïque du manioc (CMD) sont les principales maladies virales du manioc en Ouganda. Bien qu’une distance d’isolement de «50 m» ait été recommandée par le MAAIF en Ouganda pour la prévention des infections virales dans les cultures, l’efficacité minimale de la distance d’isolement n’a pas été vérifiée dans le manioc. Cette étude a évalué la distance d’isolement efficace pour la gestion des maladies virales dans le manioc.

Des cultivars de manioc sans virus (NASE 03, NASE 14 et NAROCASS 1) provenant des champs des agriculteurs ont été utilisés comme le source de matériel de plantation (FS). Le matériel de culture tissulaire (TC) des mêmes cultivars provenait des laboratoires National Crops Resources Research Institute and Makerere University Agricultural Research Institute tissue culture. Les matériaux FS et TC ont été testés à des distances d'isolement de 50, 100, 150 et 250 m pour la prévention des virus. L'expérience a été présentée dans un bloc complet randomisé et a été réalisée pendant 12 mois après la plantation (MAP). La prévalence moyenne de CBSD / CMD variait significativement ( $P < 0,05$ ) entre les distances d'isolement dans les plantes FS et TC, et la distance d'isolement de 250 m était la plus efficace pour réduire la prévalence de la maladie. À travers les cultivars et la catégorie de matériel de plantation à 12 MAP, la distance d'isolement de 50 m avait l'incidence foliaire la plus élevée pour le CBSD (29,2%) et le CMD (16,1%); tandis que la gravité pour CBSD était de 1,4 et 1,2 pour CMD. À 250 m, toutes les plantes FS et TC avaient une gravité CBSD / CMD de 1,0 et une incidence de 0%. Ces résultats montrent qu'une distance d'isolement de 250 m peut fournir une option pour disséminer des cultivars de manioc sensibles au CBSD / CMD, ce qui permet de gérer le CBSD / CMD.

*Mots Clés:* Maladie des stries brunes du manioc, maladie de la mosaïque du manioc

## INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a staple food crop, cultivated in many parts of Africa. In Uganda cassava is grown in all parts of the country, with eastern and northern regions being the major producer (FAOSTAT, 2017; UBOS, 2017). However, cassava production worldwide is constrained by a number of biotic factors. Of the biotic factors, cassava brown streak disease (CBSD) caused by *cassava brown streak viruses* and cassava mosaic disease (CMD) caused by different species of *cassava mosaic geminiviruses* (CMGs) are the major viral diseases leading to severe yield losses of up to 100% (Legg *et al.*, 2011; Legg *et al.*, 2014). CBSVs and CMGs are transmitted by whitefly, *Bemisia tabaci* Gennadius, and can also be spread in cuttings, which is exacerbated by the virtue that cassava is predominantly vegetatively propagated (Legg *et al.*, 2015).

CBSD and CMD are prevalent in all agroecological zones in Uganda and most cassava fields usually have both diseases on the same cassava plants at the same time (Kawuki *et al.*, 2017). Therefore, management strategies require that both diseases are simultaneously controlled. CBSD and CMD prevention is done using several ways; but

breeding for resistance is the most sustainable strategy, especially for the resource poor farmers. However, cassava varieties such as NASE 14, bred for resistance to CMV, succumb to CBSV (Kawuki *et al.*, 2017; Mukibi *et al.*, 2019). Genetic engineering has been tried to introduce CBSD resistance into CMD resistant varieties, such as TME 204; but after successful transformation, such varieties lost resistance to CMD (Beyene *et al.*, 2016; Beyene *et al.*, 2017). Besides, most times, resistant varieties are not the most preferred by farmers as they are deficient in some farmer preferred attributes (Nakabonge *et al.*, 2017). Therefore, cassava farmers in Uganda are largely left with only the option of using clean planting material of their preferred cultivars, when establishing new fields. Such clean planting materials are obtained from tissue culture laboratories and distributed to seed multipliers in 'isolated' fields (Legg *et al.*, 2011; Nakabonge *et al.*, 2017). The seed multipliers can multiply seed for several generations from the same tissue culture stock material, and such material ideally becomes field sourced material after exposure for several generations.

Several isolation distances have been recommended and adopted by seed multipliers and inspectors for the multiplication of clean

cassava planting materials. Isolation distance of “50 m” has been recommended by MAAIF in Uganda (MAAIF, 2010). According to Delimini (2012), cassava fields having breeder seed, should at least have 200 meters separating breeder seed from neighbouring cassava fields, and 100 meters separating basic seed from other fields. However, there has been no research done to empirically establish these isolation distances and compare CBSV and CMV infection rates between tissue culture derived and field sourced cassava planting materials. While shorter distances may not be effective, longer isolation distance would pose a constraint to small-scale and land limited farmers during certified clean seed multiplication, thus a need for an optimum isolation distance. This study was, therefore, conducted to establish the effective isolation distance for simultaneous prevention of CBSV and CMV infections in tissue culture derived and field sourced planting materials.

## MATERIALS AND METHODS

**Study site.** The study was carried out in a field at the Makerere University Agriculture Research Institute, Kabanyolo (MUARIK) in Uganda, in 2018/2019. MUARIK is located at a latitude of 0°28'22" N of the equator, longitude of 32°37'22" E and at 1200 meters above sea level. MUARIK is located near to Namulonge (longitude of 32°37'22" E, 0°32'22" S); a known hotspot for whiteflies and cassava viral diseases (Abaca *et al.*, 2012; Kaweesi *et al.*, 2014); hence buildup of inoculum and its spread was presumed.

**Plant materials.** A total of 288 plants of three cassava cultivars (NASE 03, NASE 14 and NAROCASS 1) were used in this study. NASE 03 and NASE 14 are the most preferred by farmers, at least in eastern Uganda; yet susceptible to CBSV and CMV (unpublished). NAROCASS 1 was included as a check because of its high levels of resistance to both viruses (Mukiibi *et al.*, 2019).

For field sourced materials, cultivars were sourced from farmers' fields in eastern Uganda (Bukedea and Kumi districts). Plants were checked for healthy status by extracting total nucleic acids from the leaves using a modified cetyltrimethyl ammonium bromide (CTAB) method (Maruthi *et al.*, 2002). The extracted nucleic acids were run in PCR/RT-PCR reaction protocols using CMV and CBSV degenerate primers.

For the tissue culture-derived materials, similar cultivars (NASE 03, NASE 14 and NAROCASS 1) were sourced from National Crops Resources Research Institute (NaCRRI) and MUARIK tissue culture laboratories and included in the field experiment.

### **Virus inoculation and management.**

Elephant grass (*Pennisetum purpureum*) was planted around the whole perimeter and longitudinally between different treatment blocks, 4 months before planting the cassava experimental treatment blocks. This was done to allow elephant grass to establish as a barrier crop so as to block whiteflies from transmitting CBSV and CMV from other unintended sources, and to separate the different treatment blocks (Maruthi *et al.*, 2017). The infected spreader rows of highly CBSV-susceptible TME 204 and CMV-susceptible Bao cassava cultivars (Abaca *et al.*, 2012; Kawuki *et al.*, 2016), were planted at one extreme end of the experimental block at spacing of 1 m x 1 m, two months before planting the cassava treatment blocks.

**Experimental design and management.** For the treatment blocks, the experiment was laid out in a randomised complete block design. The isolation distances used in this study were 50, 100, 150 and 250 m. For each distance, two planting material categories, field sourced (FS) and tissue culture-derived (TC), were used. Two blocks were used for each source of planting materials per isolation distance; and each block had three cassava cultivars (NASE 03, NASE 14 and NAROCASS 1). Six

replications/plants of each of the cultivars were planted at spacing of 1 m x 1 m.

The field was left to natural whitefly infestation, similar to farmers practice; without spraying throughout the study period. Experimental field was kept weed free by manual weeding and no fertiliser was applied. Occasional irrigation of the infectors and experimental blocks was done during excessive dry conditions, especially when plants were still young. Irrigation was done using a watering can in the evenings after every three days, for up to two weeks, giving three litres of tap water per plant per irrigation.

**Data collection and analysis.** The CBSD and CMD incidence and severity were assessed at 4, 8 and 12 months after planting (MAP). For CBSD severity, the assessment was done using foliar and stem symptoms, and was based on a scale of 1 - 5 (Gondwe *et al.*, 2003). Where 1 = no apparent symptoms; 2 = slight foliar feathery chlorosis and no stem lesions; 3 = pronounced foliar feathery chlorosis, mild stem lesions, and no dieback; 4 = severe foliar feathery chlorosis, severe stem lesions, and no dieback; and 5 = defoliation, severe stem lesions and dieback.

CMD severity was assessed using a 1 -5 severity scale (IITA,1990). Where 1 = no symptoms observed (shoot healthy); 2 = mild chlorotic pattern on most leaves, mild distortions at the bases of most leaves, with the remaining parts of the leaves and leaflets appearing green and healthy; 3 = a pronounced mosaic pattern on most leaves, with narrowing and distortion of the lower one-third of most leaves; 4 = severe mosaic distortion of two-thirds of most leaves, with general reduction in leaf size and some stunting of shoots; and 5 = very severe mosaic symptoms on all leaves, with distortion, twisting, and severe reduction in leaf size in most leaves, accompanied by severe stunting of plants. The CBSD and CMD incidences data were obtained from the number of plants showing foliar disease symptoms, expressed as a percentage of the total number of plants assessed.

For CBSD root severity at harvest (12 MAP), plants in each plot were uprooted and all roots cut transversally and assessed for CBSD root severity, using the 1-5 scale. Where, 1 = no necrosis, 2 = mild necrotic lesions (1-10%), 3 = pronounced necrotic lesion (11-25%), 4 = severe necrotic lesion (26-50%) with mild root constriction and 5 = very severe necrotic lesion (>50%) with severe root constriction (Kaweesi *et al.*, 2014). Disease root incidence of CBSD in harvested roots was quantified as a ratio of the number of roots showing roots symptoms to the total number of roots harvested per plant per genotype (Kaweesi *et al.*, 2014).

Data on disease severity and incidence for cultivars, planting materials and isolation distance were subjected to analysis of variance using GenStat 14<sup>th</sup> edition (Payne *et al.*, 2011). Comparisons of means were made using Fisher's protected LSD at  $P < 0.05$ .

## RESULTS AND DISCUSSION

This study assessed a number of 288 cassava plants; 144 plants from TC material and 144 plants from FS material of NASE 03, NASE 14 and NAROCASS 1 cassava cultivars. The aim was to assess for CBSV and CMV infection at varying isolation distances of 50, 100, 150 and 250 m in cassava plants from TC and FS material. The study was carried out at the Makerere University Agricultural Research Institute, Kabanyolo (MUARIK), a known hotspot for whitefly population and viral cassava diseases hence buildup of inoculum and its spread was assured (Abaca *et al.*, 2012; Kaweesi *et al.*, 2014).

Results indicated that foliar CBSD and CMD incidences (Tables 1 and 2) and severity (Tables 3 and 4) varied significantly among plants from both tissue-culture derived and field sourced cassava planting materials. Similarly, CBSD root incidence and severity also varied significantly among plants from both TC and FS material (Table 5). These results indicate a differential response of the different cassava planting material category

TABLE 1. Cassava brown streak disease foliar incidence (%) for tissue culture and field sourced planting materials per isolation distance at different months after planting at the Makerere University Agriculture Research Institute, Kabanyolo (MUARIK) in Uganda

Planting materials	Isolation distance (m)											
	50			100			150			250		
	4 MAP	8 MAP	12 MAP	4 MAP	8 MAP	12 MAP	4 MAP	8 MAP	12 MAP	4 MAP	8 MAP	12 MAP
NAROCASS 1 FS	0	0	8.3	0	0	0	0	0	0	0	0	0
NAROCASS 1 TC	0	0	0	0	0	0	0	0	0	0	0	0
NASE03 FS	33.33	58.33	75	25	41.67	58.3	0	0	16.67	0	0	0
NASE03 TC	25	41.67	58.3	8.33	25	41.7	0	0	8.3	0	0	0
NASE 14 FS	0	16.67	25	0	0	8.3	0	0	0	0	0	0
NASE 14 TC	0	0	8.3	0	0	0	0	0	0	0	0	0
Mean	9.7	19.5	29.2	5.6	11.1	18.1	0	0	4.2	0	0	0
LSD(0.05)	4.37	4.98	7.46	4.37	4.98	7.46	4.37	4.98	7.46	4.37	4.98	7.46
CV(%)	110.7	63.0	56.2	110.7	63.0	56.2	110.7	63.0	56.2	110.7	63.0	56.2

LSD = the least significant difference at P=0.05, CV = the coefficient of variation, MAP = months after planting, TC = tissue culture-derived material, FS = field-sourced planting material

Isolation distance for prevention of cassava virus infections

TABLE 2. Cassava mosaic disease foliar incidence (%) for tissue culture and field sourced planting materials per isolation distance at different months after planting at the Makerere University Agriculture Research Institute, Kabanyolo (MUARIK) in Uganda <sup>6</sup>

Planting materials	Isolation distance (m)											
	50			100			150			250		
	4 MAP	8 MAP	12 MAP	4 MAP	8 MAP	12 MAP	4 MAP	8 MAP	12 MAP	4 MAP	8 MAP	12 MAP
NAROCASS 1 FS	0	0	0	0	0	0	0	0	0	0	0	0
NAROCASS 1 TC	0	0	0	0	0	0	0	0	0	0	0	0
NASE03 FS	25	41.67	58.33	16.67	33.33	41.67	0	8.33	25	0	0	0
NASE03 TC	16.67	33.33	50	0	16.67	33.33	0	0	16.67	0	0	0
NASE 14 FS	0	0	0	0	0	0	0	0	0	0	0	0
NASE 14 TC	0	0	0	0	0	0	0	0	0	0	0	0
Mean	7.0	12.5	18.1	2.8	8.3	12.5	0	1.4	7.0	0	0	0
LSD(0.05)	2.49	3.44	4.12	2.49	3.44	4.12	2.49	3.44	4.12	2.49	3.44	4.12
CV(%)	99.0	59.9	42.5	99.0	59.9	42.5	99.0	59.9	42.5	99.0	59.9	42.5

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LSD = the least significant difference at P=0.05, CV = the coefficient of variation, MAP = months after planting, TC = tissue culture-derived material, FS = field-sourced planting material

TABLE 3. Cassava brown streak disease foliar severity for tissue culture and sourced field planting materials per isolation distance at different months after planting at the Makerere University Agriculture Research Institute, Kabanyolo (MUARIK) in Uganda

Planting materials	Isolation distance (m)											
	50			100			150			250		
	4 MAP	8 MAP	12 MAP	4 MAP	8 MAP	12 MAP	4 MAP	8 MAP	12 MAP	4 MAP	8 MAP	12 MAP
NAROCASS 1 FS	1.0	1.0	1.083	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
NAROCASS 1 TC	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
NASE03 FS	1.33	1.83	2.25	1.25	1.5	1.83	1.0	1.0	1.17	1.0	1.0	1.0
NASE03 TC	1.25	1.5	1.75	1.08	1.25	1.5	1.0	1.0	1.08	1.0	1.0	1.0
NASE 14 FS	1.0	1.17	1.25	1.0	1.0	1.08	1.0	1.0	1.0	1.0	1.0	1.0
NASE 14 TC	1.0	1.0	1.17	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mean	1.1	1.3	1.4	1.1	1.1	1.2	1.0	1.0	1.0	1.0	1.0	1.0
LSD(0.05)	0.07	0.12	0.15	0.07	0.12	0.15	0.07	0.12	0.15	0.07	0.12	0.15
CV(%)	16.9	26.2	32.1	16.9	26.2	32.1	16.9	26.2	32.1	16.9	26.2	32.1

MAP = months after planting, TC = tissue culture-derived material, FS = field-sourced planting material

Isolation distance for prevention of cassava virus infections

TABLE 4. Cassava mosaic disease foliar severity for tissue culture and field sourced planting materials per isolation distance at different months after planting at the Makerere University Agriculture Research Institute, Kabanyolo (MUARIK) in Uganda <sup>8</sup>

Planting materials	Isolation distance (m)											
	50			100			150			250		
	4 MAP	8 MAP	12 MAP	4 MAP	8 MAP	12 MAP	4 MAP	8 MAP	12 MAP	4 MAP	8 MAP	12 MAP
NAROCASS 1 FS	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
NAROCASS 1 TC	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
NASE03 FS	1.25	1.42	1.83	1.17	1.33	1.5	1.0	1.08	1.25	1.0	1.0	1.0
NASE03 TC	1.17	1.33	1.58	1.0	1.17	1.33	1.0	1.0	1.17	1.0	1.0	1.0
NASE 14 FS	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
NASE 14 TC	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mean	1.1	1.1	1.2	1.0	1.1	1.1	1.0	1.0	1.1	1.0	1.0	1.0
LSD(0.05)	0.06	0.08	0.12	0.06	0.08	0.12	0.06	0.08	0.12	0.06	0.08	0.12
CV(%)	14.2	19.2	27.2	14.2	19.2	27.2	14.2	19.2	27.2	14.2	19.2	27.2

MAP = months after planting, TC = tissue culture-derived material, FS = field-sourced planting material

TABLE 5. Cassava brown streak disease root severity and incidence (%) for tissue culture and field sourced planting materials per isolation distance at 12 months after planting at the Makerere University Agriculture Research Institute, Kabanyolo (MUARIK) in Uganda

Planting materials	Isolation distance (m)							
	50		100		150		250	
	Rs	Ri	Rs	Ri	Rs	Ri	Rs	Ri
NAROCASS 1 FS	1.17	1.11	1.0	0	1.0	0	1.0	0
NAROCASS 1 TC	1.0	0	1.0	0	1.0	0	1.0	0
NASE03 FS	2.5	41.13	2.25	28.7	1.58	9.09	1.0	0
NASE03 TC	2.08	38.24	1.5	11.16	1.0	0	1.0	0
NASE 14 FS	1.67	17.26	1.33	4.17	1.0	0	1.0	0
NASE 14 TC	1.33	9.09	1.25	2.3	1.0	0	1.0	0
Mean	1.6	17.8	1.4	7.7	1.1	1.5	1.0	0
LSD (0.05)	0.19	4.75	0.19	4.75	0.19	4.75	0.19	4.75
CV (%)	36.6	174.9	36.6	174.9	36.6	174.9	36.6	174.9

LSD = the least significant difference at P=0.05, CV= the coefficient of variation, Rs = root severity, Ri = root incidence (%), TC = tissue culture-derived material, FS = field-sourced planting material

(tissue culture versus field sourced) to CBSV and CMV infection. Results also revealed differential prevalence of CBSD and CMD for the different cultivars (Tables 1, 2, 3, 4 and 5), an observation consistent with earlier findings (Alicai *et al.*, 2007; Kawuki *et al.*, 2017; Mukiibi *et al.*, 2019).

Both FS and TC planting materials of NASE 14 and NAROCASS 1 cassava cultivars had CMD severity of 1.0 and CMD incidence of 0% (Tables 2 and 4). These findings are in agreement with observations by Mukiibi *et al.* (2019), who also observed no CMD symptoms on these cassava cultivars. Absence of CMD symptoms on these cassava cultivars demonstrates high levels of resistance or tolerance to the disease (Kawuki *et al.*, 2016). These cultivars, however, succumbed to CBSD (although with less prevalence compared to NASE 03). These observations indicate a big gap that still exists for combined resistance for the two diseases. Plants from TC and FS cassava planting materials of NASE 03 had

very high incidence (Tables 1, 2 and 5) and severity (Tables 3, 4 and 5) for both CBSD and CMD confirming that this cultivar is most susceptible to the two diseases (Kawuki *et al.*, 2017).

CBSD and CMD prevalence was generally higher among FS than TC planting materials (Tables 1, 2, 3, 4 and 5). This confirms the superiority of using tissue culture derived clean planting materials in the management of viral diseases in cassava compared to field sourced planting materials (Legg *et al.*, 2011; Legg *et al.*, 2015; Legg *et al.*, 2017; Tumwegamire *et al.*, 2018). These results provide an indication that phytosanitary measures, such as those involving the use of virus-free planting material, preferably TC derived, coupled with isolation from surrounding potential sources of infection, offer excellent potential for CBSD and CMD control (Maruthi *et al.*, 2017).

The less prevalence of CBSD and CMD in plants from TC planting material is probably because through tissue culture, challenges

such as diseases often associated with conventional practices can be overcome. Consequently, Nakabonge *et al.* (2017) suggested tissue culture as an important technology in setting up cassava seed systems; although many farmers in Uganda still use FS planting materials. Cyclic-propagation of FS planting materials in cassava leads to continued virus presence and build up in the environment (Maruthi *et al.*, 2005).

CBSD was generally more prevalent than CMD (Tables 1, 2, 3 and 4). This is probably because, the cultivars used in this study were more susceptible to CBSV than CMD (Kawuki *et al.*, 2016). CBSV and CMD prevalence was lowest in planting materials of recently released cassava cultivars NAROCASS 1 (released in 2015) and NASE 14 (released in 2011); while highest in NASE 03 (released in 1993). This shows virus evolution and progress done by the breeding program.

CBSD and CMD severity and incidence for plants from TC and FS planting materials decreased with increase in isolation distances; the 50 m isolation distance had the highest prevalence and 250 m had the lowest prevalence (Tables 6 and 7). Maruthi *et al.* (2017) also reported a greater CBSV incidence in plots that were closer to CBSV spreader plants. However, our results were in contrast to earlier suggestion by Maruthi *et al.* (2017), who reported that an isolation distance of 100 m is sufficient to significantly minimise the spread of CBSVs between infected and disease-free plots. Short distances favour whitefly semi persistent transmission mechanism for CBSVs (Delimini, 2012; Maruthi *et al.*, 2017). Short isolation distance combined with high whitefly population in our study area (Abaca *et al.*, 2012; Kaweesi *et al.*, 2014), probably explain the high CBSV prevalence observed at the 50 and 100 m isolation distances. The 250 m isolation distance could have limited a big number of CBSV- and CMV-carrying whiteflies from reaching the healthy plants, thus the observed no CBSV and CMD prevalence at 250 m isolation distance although the study was set

TABLE 6. Cassava brown streak disease and cassava mosaic disease foliar severity and incidence for the varying isolation distances at different months after planting at the Makerere University Agriculture Research Institute, Kabanyolo (MUARIK) in Uganda

Isolation distance (m)	4 MAP						8 MAP						12 MAP					
	Severity		Incidence (%)		Severity		Incidence (%)		Severity		Incidence (%)		Severity		Incidence (%)			
	CBSD	CMD	CBSD	CMD														
250	1.0	1.0	0.0	0.0	1.0	1.0	0.0	0.0	1.0	1.0	0.0	0.0	1.0	1.0	0.0	0.0		
150	1.0	1.0	0.0	0.0	1.0	1.01	0.0	0.0	1.01	1.01	1.39	0.0	1.04	1.07	4.17	6.94		
100	1.06	1.03	5.55	2.78	1.13	1.08	11.11	8.33	1.24	1.24	11.11	8.33	1.24	1.14	18.05	12.5		
50	1.1	1.07	9.72	6.94	1.25	1.13	19.44	12.5	1.42	1.42	19.44	12.5	1.42	1.24	29.17	16.06		
Mean	1.0	1.0	3.8	2.4	1.1	1.1	7.6	5.6	1.2	1.2	7.6	5.6	1.2	1.1	12.8	8.9		
LSD (0.05)	0.06	0.05	3.57	2.03	0.09	0.07	4.06	2.81	0.12	0.12	4.06	2.81	0.12	0.1	6.09	3.36		
CV (%)	16.9	14.2	110.7	99	26.2	19.2	63	59.9	32.1	27.2	56.2	42.5	32.1	27.2	56.2	42.5		

LSD = the least significant difference at P=0.05, CV = the coefficient of variation, MAP = months after planting

TABLE 7. Cassava brown streak disease root severity and incidence (%) for the varying isolation distances at 12 months after planting at the Makerere University Agriculture Research Institute, Kabanyolo (MUARIK) in Uganda

Isolation distance (m)	CBSD root severity	CBSD root incidence (%)
250	1.0	0.0
150	1.1	1.52
100	1.4	7.7
50	1.6	17.8
Mean	1.3	6.8
LSD (0.05)	0.15	3.88
CV (%)	36.6	174.9

LSD = the least significant difference at P=0.05, CV = the coefficient of variation

up in an area that is a hotspot for CBSD and CMD and with a high whitefly infestation (Abaca *et al.*, 2012; Kaweesi *et al.*, 2014). Our findings, therefore, can be applied both in low and high disease pressure zones to effectively manage CBSD and CMD.

### CONCLUSION

This study has revealed that 250 m isolation distance can be instrumental in significantly preventing CBSV and CMV infections in cassava plants from tissue culture and field sourced cassava planting materials in Uganda. This distance can prevent CBSV and CMV infections in both cultivars that are susceptible and tolerant as seen by the cultivars used in this study. This provides hope for enhanced CBSD and CMD management in farmer preferred yet susceptible cultivars. Therefore, cassava seed multipliers and inspectors in Uganda can adopt this isolation distance for the production and dissemination of clean planting material. To effectively utilise findings for our study, we recommend strict regulations by inspectors to only allow cassava seed multiplication by multipliers with enough land for the 250 m isolation distance especially for the high disease pressure zones in Uganda.

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