Review Article

ROLE OF PREVALENT WEEDS AND CULTIVATED CROPS IN THE EPIDEMIOLOGY OF MAIZE LETHAL NECROSIS DISEASE IN MAJOR MAIZE GROWING AGROECOLOGICAL ZONES OF UGANDA

ABSTRACT

In Uganda, the severe Maize lethal necrosis (MLN) disease, which threatens subsistence maize production is elicited by co-infection of maize plants with *Maize chlorotic mottle virus* (MCMV) and *Sugarcane mosaic virus* (SCMV). However, there is no information about natural hosts of MLN causing viruses and role in epidemiology of MLN in Uganda. The aim of this study was to determine existence of natural alternative weed and cultivated crop hosts of MLN causing viruses. Three seasonal surveys between 2014 and 2015 were carried out in five major maize growing agroecological zones of Uganda. Weeds and cultivated crops growing in proximity to maize were observed for virus symptoms and tested for MLN causing viruses using Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay and Reverse Transcriptase Polymerase Chain Reaction. Data was collected on frequency of occurrence of weeds and cultivated crop species and MLN virus disease incidence. *Digitaria abyssinica, Bidens pilosa* and *Commelina benghalensis* were the most common weed species while *Phaseolus vulgaris, Manihot esculenta*, *Arachis hypogaea*), *Musa sp*, *Glycine max* and *Ipomoea batatas* were the most common cultivated crops. *Pennisetum purpureum, Digitaria abyssinica, Cyperus rotundus, Amaranthus spinosus, Commelina benghalensis* and *Eleusine indica* weeds species are natural hosts of *Maize chlorotic mottle virus*. **While** Among cultivated crops, *Phaseolus vulgaris*, *Manihot esculenta* and *Sesamum indicum* are natural hosts of MCMV **among cultivated crops**. Only Sorghum (*Sorghum bicolor*) and sweet potato (*Ipomoea batatas*) tested positive for SCMV. MCMV incidence in weeds ranged from 5.26% to 100% and 4.76% to 100% in cultivated crops. MLN causing viruses were prevalent in weeds and cultivated crops located in known hotspots for MLN in Uganda. The study has revealed that alternative hosts of MLN-causing viruses are present in major maize growing agroecological zones of Uganda and act as sources of inoculum to sustain MLN epidemics.

Keywords: Alternate weed hosts, epidemiology, Maize lethal necrosis, Uganda

1. INTRODUCTION

Maize lethal necrosis (MLN) disease has been reported in different countries of East and Central African , **region** including Rwanda [1], Tanzania [2], Kenya [3], Uganda [4, 5] and Democratic Republic of Congo [6] and is now considered to be the most widespread and serious virus disease on maize in sub-Saharan Africa. MLN is not indigenous to the African continent and Uganda **it** and is not yet widespread and abundant. The disease is caused when maize plants are co-infected with *Maize chlorotic mottle virus* and other cereal viruses in the potyvirus group such as *Maize dwarf mosaic virus* (MDMV) and *Sugarcane mosaic virus* (SCMV). However apart from maize, there is scanty information about its natural alternative hosts and ecology in Uganda. Maize was the only naturally occurring host of MCMV known [7], until recently when the virus was detected in sugarcane [8] and finger millet [9]. These new findings point to the possibility of other new natural hosts of MLN viruses that could be present in Uganda. In addition, MCMV has been reported to have a broad experimental host range including no less than 19 grass species making it difficult to manage the virus [10]. The wide host range has implications on the epidemiology of virus diseases and should be considered in development of an integrated disease management strategy.

Several studies on the significance of weeds as reservoirs of MLN causing viruses notably MCMV [8, 11, 7, 10] and MDMV and SCMV [12, 13, 14] have been conducted in maize production agro-ecologies in temperate regions of North America. Little or no related studies have been conducted in tropical regions [9, 15, 16]. Differences in agroecosystems and agro-ecologies result in differences in the biology and occurrence of different weed species, which may cause variation in their relation to MLN causing viruses and their vectors. Most of these alternative weed hosts are found growing in association with maize agroecosystems in these countries where **first** studies on MLN were first conducted may have acted as alternative hosts for vector feeding or reproduction of virus vectors, reservoirs of the MLN causing viruses or both. However, it is not known whether similar grass weed species that are hosts of MLN are present in Uganda and if they could have had a role in enhancing the spread of MLN. The alternative host status of related plant species and common intercrops grown with maize in Uganda for the Ugandan strain(s) of MLN -causing viruses and their role in the spread of MLN in Uganda is unknown. Some maize viruses have been known to have different reactions on the same alternative host due to variation in the strains. There have been no studies conducted on MLN- causing viruses and their natural or artificial hosts in Uganda. Some non-chemical methods of managing MLN such as crop rotation and fallowing, focus on the removal of the maize host from the field for a defined period. It is not known whether crop rotation is a feasible MLN disease control measure. The success of such cultural methods depends on the duration of survival of MLN causing viruses without a maize host or alternate hosts. In most cases, no attention is given to weeds which could be alternative hosts and sources of inoculum for MLN viruses. In order to develop an MLN management strategy that is effective, information should be generated through studies conducted to establish the host range of Ugandan strain(s) of MLN causing viruses. The aim of this study was to establish the weeds and cultivated crop species occurring in maize agroecological zones of Uganda and to determine the existence of natural alternative weed and cultivated crop hosts of MLN causing viruses. It was hypothesized that potential alternative hosts of Maize Lethal Necrosis-causing viruses are present in major maize growing agroecological zones of Uganda and act as natural sources of inoculum to maize fields.

2. MATERIALS AND METHODS

2.1 Description of the Study Area

The study was conducted over three seasons from 2014A, 2014B and 2015A and covered 14 major maize growing districts from five agroecological zones (AEZ) of Uganda (Fig. 1). The study agroecological zones were classified according to the National Agricultural Research Organization [17] based on distinct vegetation type, elevation and climatic conditions. The Eastern Agro-ecological Zone (EAZ) covered the districts of Tororo and Bukedea and is characterized by an annual average rainfall of 1197 mm with temperature ranges from 15-32.5 ˚C. The Lake Albert Crescent Agro-ecological Zone (LACZ) covered the districts of Hoima, Masindi, Kibaale and Kiryandongo and is characterized by an annual average rainfall of 1259mm with temperature ranging from 17.5-32.5˚C. The South Eastern Agro-ecological Zone (SEAZ) covered the districts of Iganga, Busia and Bugiri with average annual rainfall ranging from 1215-1328mm and temperature ranging from 15 to 32.5˚C. The Eastern Highlands Agro-ecological Zone (EHAZ) covered the districts of Mbale, Bulambuli, Sironko, Kween and Kapchorwa with average annual rainfall more than 1,400 mm and temperature ranging from 7.5 to 27.5˚C. The Western Highlands Agro-ecological Zone (WHAZ) covered the districts of Kasese and Kabarole with average annual rainfall of 1,270 mm and temperature ranging from 15 to 30 ˚C.

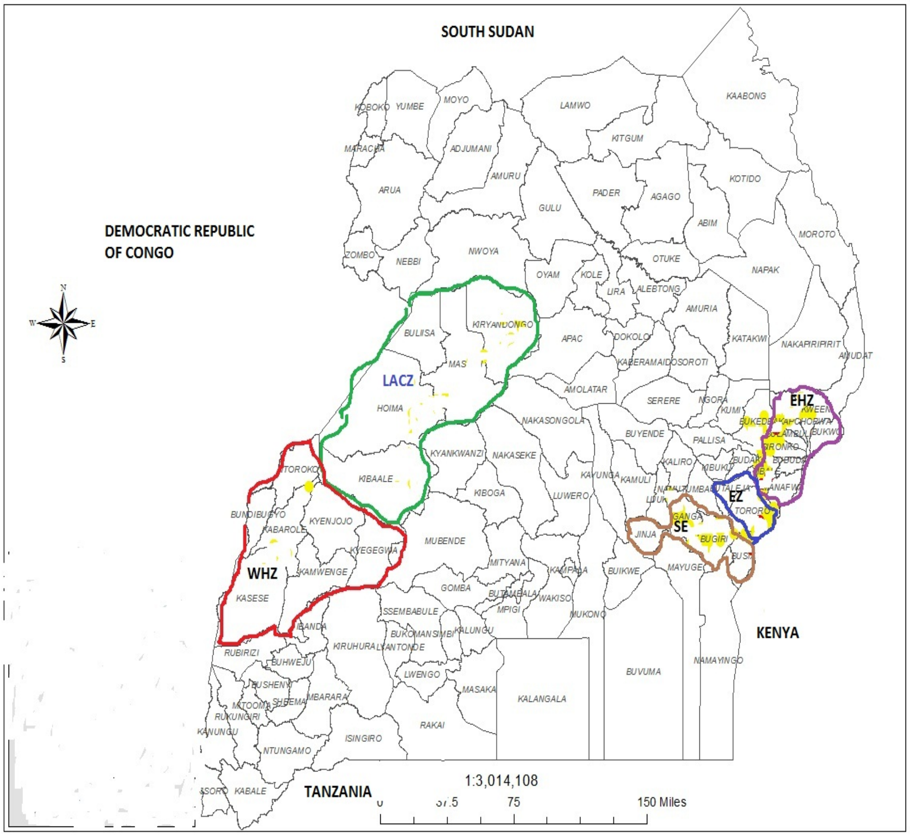

Fig. 1. The location of major maize growing agroecological zones surveyed during the study. Where WHZ: Western highland agroecological zone, SE= South Eastern Agreoecological zone, EZ= Eastern Agroecological Zone, EHZ= Eastern highland agroecological zone, LACZ= Lake Albert Crescent Agroecological zone.

2.2 Field surveys and sampling

The five major maize growing agroecological zones of Uganda (namely, the Eastern, Eastern Highland, South Eastern, Western Highland and Lake Albert Crescent Agroecological zones) were surveyed for alternative host plants of MLN. From each agroecological zone, at least two districts were chosen purposively based on maize production data and surveyed for plants that were displaying virus-like symptoms or were apparently healthy looking. The surveys were carried out during the first season of 2014, second season of 2014 and first season of 2015 from 16 major maize growing districts. Fields were selected at regular intervals along major and feeder roads traversing the sampling area. Average distance between sampled fields was about 5km. Weeds and cultivated crops grown as intercrops or near maize were collected from near maize fields on either side of the road while alternating after every 5 km. In all cases, the site of collection was a maize field in which the plants were growing naturally as weeds. Sampling activities commenced in the early morning hours just before sunrise (between 0630 and 0700 h) to minimize the impact of wilting. Cultivated crop species and weeds growing within the vicinity of the maize crop and one meter from the maize plant were collected together with their rooting system and crown. At each stop the 3 plants from each weed species and 3 plants from each intercrop species grown with maize in a 1-meter square area was sampled using a quadrant. The samples collected were put in separate bags to avoid cross contamination. **With a**A total of 10 sampling sites were located 5 to 10 km from each other.

2.3 Plant materials and taxonomic identification

Plants were initially identified during the field surveys only by close examination for distinguishing features of common weed and crop families. The plants were later identified to species level according to the taxonomic keys using reference herbarium collections available at the Department of Botany Herbarium, Makerere University using reference identification keys from [18].

2.4 Detection of Viruses in in weeds and cultivated crops from five major maize growing agroecological zones of Uganda

2.4.1 Serological detection of Maize lethal necrosis causing viruses in weeds and cultivated crops

Between 5 and 10 leaves (preferably those with virus-like symptoms) were sampled from taxonomically identified plants. Leaves were used for serological testing of MLN-causing viruses. Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was used to test for the presence of SCMV and MCMV in weed and crop leaf samples collected during the survey [19]. The antisera were purchased from Agdia Inc. (Elkhart, IN, USA). The polyclonal antibodies used included anti-Sugarcane mosaic virus and anti- Maize chlorotic mottle virus. In the test all the buffers were prepared according to the manufacturer's specifications from Agdia Inc. (Elkhart, IN, USA).

Plant leaf samples were crashed 1:20 (w/v) in extraction buffer using a motor and pestle. DAS-ELISA plates were prepared by adding 200µl coating antibody for each specific MLN causing virus into each well of microtitre pate (dilution 1:200 v/v of antibody: buffer) followed by 2 hours of incubation at 37^{o}C. Plates were washed three times in PBS-T (Phosphate Buffered Saline-Tween 20 pH 7.4). A total of 200µl of the test samples were added into each well in duplicates and incubated at 4^{o}C overnight. Plates were washed three times and 200µl enzyme conjugate diluted in ECL buffer 1:200 (v/v) added to each well. Plates were incubated at 37^{o}C for 3 hours and washed three times. A total 200µl freshly prepared substrate (1 mg/ml para-nitrophenyl-phosphate in substrate buffer) was added to each well, incubated at 37˚C for 60 minutes. In the microtitre plates positive and negative control tests of healthy maize plants were included. The positive controls were purchased from Agdia Inc. (Elkhart, IN, USA). Plates were then assessed visually for colour change and absorbance measured at 405 nm wavelength using a BIO-RAD® microtitre plate reader Model 680 (BIO-RAD Laboratories, Hercules, California, USA). All samples were assayed in duplicate and the results judged to be positive if the absorbance was greater than or equal to twice the average reading of the negative (healthy) controls.

2.4.2 Molecular detection of Maize lethal necrosis causing viruses in weeds and cultivated crops

Total RNA was extracted from leaves of weeds and cultivated crops with Trizol Reagent (Bioneer, South Korea) according to the manufacturer's instructions and subsequently used for cDNA synthesis by RT-PCR using *AccuPower*® Reverse Transcription Polymerase Chain Reaction (RT-PCR) PreMix kit (Bioneer Corporation, Korea) following manufacturer's instructions. MCMV and SCMV primers which flank the coat protein gene of each virus and amplify a fragment of approximately 550bp for MCMV and 900 bp for SCMV were used for RT-PCR [3]. Electrophoresis was done on the RT-PCR product using 1.5% agarose gels for 45 minutes at 120V and current of 400 mA in TAE agarose gel. The amplified DNA fragments were visualized on a 1.5% agarose gels under UV light. A 100bp? DNA Ladder (Bioneer®) was used as the standard.

2.5 Data collection and analysis

Data was collected on the frequency of occurrence of weeds and cultivated crop species in each sampled field. In order to determine disease incidence, data on disease incidence (%) was expressed as being equal to the total number of infected plants as a percentage of the total number of plants tested [20]. The collected data wereas arranged using Excel to generate datasets. The datasets were then imported into SPSS. The survey data obtained wereas analyzed using Statistical Package for Social Sciences (SPSS, version 20.0. Armonk, New York: IBM Corporation). The frequency of occurrence of weeds and crops for each agroecological region and district were analyzed using cross tabulation in the Statistical Package for Social Sciences (SPSS, version 20.0. Armonk, New York: IBM Corporation). In addition to descriptive statistics, further analysis was required to establish significant differences in existif present in frequency of occurrence of weed species between agroecological zones. Therefore differences in frequency of occurrence of weed species between agroecological zones were tested using Pearson's chi-square tests and cross tabulations.

I don't think the statisitical tests are meaningful for these types of data, especially since there were so many cases of 0 or 1 weeds or positive pathogen tests. The data are OK but trying to define statistical differences is very questionable. What logical hyopothesis are Chi square based on? How are 0 classes handled?

3. RESULTS

3.1 Survey of weed and cultivated crop species found growing in association with maize in major maize growing agroecological zones in Uganda

A total of 16 species of weeds representing 8 families were found in the major maize growing agroecological zones in Uganda (Table 1). The family Poaceae had the highest (8) number of species followed by Asteraceae with two species. The other families namely Amaranthaceae, Commelinaceae, Cyperaceae, Euphorbiaceae and Oxalidaceae each had one species. A total of 17 cultivated crops species representing 9 families were found in the major maize growing agroecological zones of Uganda (Table 2). The family Fabaceae had the highest (5) number of species followed by Solanaceae with three species and Poaceae with three species. The other families namely Musaceae, Pedaliaceae, Euphorbiaceae, Convolvulaceae, Cucurbitaceae each had one species. Most of the crop species identified were annuals (15 species) while 2 were perennials (Table 2).

Table 1. Potential weed hosts of MLN viruses identified in 5 major maize agroecological zones in Uganda during surveys conducted from 2014 to 2015

Family	Species	Common name	Life cycle	Type of weed
Amaranthaceae	*Amaranthus spinosus* Linn	Thorny pigweed	Annual	Broad leaves
Asteraceae	*Bidens pilosa* Linn.	Black jack	Annual	Broad leaves
	Galinsonga parviflora Cav.	Gallant soldier	Annual	Broad leaves
Commelinaceae	*Commelina benghalensis* (L.)	Wandering Jew	Annual	Broad leaves
Euphorbiaceae	*Euphorbia heterohylla* Linn	Purge weed	Annual	Broad leaves
Poaceae	*Eleusine indica* (L.) Gaert	Wild Finger Millet	Annual	Grasses
Scrophulariaceae	*Striga hermonthica*	purple witch weed	Annual	Broad leaves
Cyperaceae	*Cyperus rotundus* (L.)	Nutgrass	Perennial	Sedges
Oxalidaceae	*Oxalis latifolia* Kunth	Broadleaf Woodsorrel	Perennial	Broad leaves
Poaceae	*Pennisetum purpureum*	Elephant Grass	Perennial	Grasses
	Panicum maximum Jacq.	Common Guinea Grass	Perennial	Grasses
	Imperata cylindrica (L.)	Sword or Spear Grass	Perennial	Grasses
	Cynodon dactylon (L.) Pers.	Common Star Grass	Perennial	Grasses
	*Pennisetum clandestinum*Chiov	Kikuyu Grass	Perennial	Grasses
	Saccharum officinarum	Sugar cane	Perennial	Grasses
	Digitaria abyssinica (A.Rich) Stapf	African Couch Grass	Perennial	Grasses

Table 2. Potential cultivated crops hosts of MLN viruses found growing as intercrops with maize in 5 major maize agroecological zones of Uganda over 3 seasons 2014-2015.

Family	Common name	Botanical name	Life cycle	Total	Proportion
Amaryllidaceae	Onions	*Allium cepa*	Annual	3	0.8
Convolvulaceae	Sweet potato	*Ipomoea batatas*	Annual	13	3.2
Cucurbitaceae	Pumpkin	*Cucurbita sp*	Annual	3	0.8
Euphorbiaceae	Cassava	*Manihot esculenta*	Perennial	49	13.2
Fabaceae	Groundnuts	*Arachis hypogaea*	Annual	37	10
	Soybeans	*Glycine max*	Annual	13	3.5
	Common Beans	*Phaseolus vulgaris*	Annual	189	50.9
	Mung bean	*Vigna radiate*	Annual	1	0.3
	Cowpea	*Vigna unguiculata*	Annual	5	1.3
Musaceae	Bananas	*Musa sp*	Perennial	35	9.4
Pedaliaceae	Simsim	*Sesamum indicum*	Annual	2	0.5
Poaceae	Finger millet	*Eleusine coracana*	Annual	6	1.6
	Rice	*Oryza sativa*	Annual	3	0.8
	Sorghum	*Sorghum bicolor*	Annual	9	2.4
Solanaceae	Bitter tomato	*Solanum incanum*	Annual	1	0.3
	Potato	*Solanum tuberosum*	Annual	1	0.3
	Tomatoes	*Lycopersicumesculentum*	Annual	2	0.5
	Total			**372**	**100**

Digitaria abyssinica, Bidens pilosa and *Commelina benghalensis* were the most frequently found weed species across the five major maize growing agroecological zones in Uganda (Table 3). The frequency of occurrence of all weed species was significantly different across the 5 agroecological zones except for *Euphorbia heterohylla* Linn species which was not significantly different (x^2 = 4.246, df =4, P > 0.05). The highest frequency of occurrence of weed species was found in the Eastern Highland Agroecological Zone (432), followed by the Lake Albert Crescent Zone (237), South Eastern (182), Eastern Agroecological Zone (170) and Western Highland agroecological zone (158). Among the observed weeds, 10 weed species occurred in all the agroecological zones and they included *Pennisetum purpureum, Pennisetum clandestinum, Euphorbia spp, Imperata cylindrica, Amaranthus spinosus, Eleusine indica, Striga hermonthica, Commelina benghalensis, Bidens pilosa* and *Digitaria abyssinica*(Table 3).

As regards cultivated crops, Beans (*Phaseolus vulgaris*), Cassava (*Manihot esculenta*), Groundnuts (*Arachis hypogaea*), Bananas (*Musa sp*),Soybeans(*Glycine max*) *and* Sweet potato (I*pomoea batatas*) were the most frequently found cultivated crop species grown with maize over the three seasons surveys across the five major maize agroecological zones (Table 4). They were used as ‘indicators’ in subsequent screen house studies to determine the MLN virus host range of cultivated crops grown with maize. The highest number of crop species (136) was found in the Eastern Highland Agroecological Zone, followed by South Eastern Agroecological Zone (74), Lake Albert Crescent Zone (70), Eastern Agroecological Zone (51) and Western Highland Agroecological Zone (40).

Table 3, Distribution and frequency of occurrence of weed species in five major maize growing agroecological zones in Uganda during 3 seasons 2014, 2014 and 2015A

Weed species	EAZ	EHZ	LAZ	SEZ	WHZ	Total[a]	Proportion (%)	χ2	P??	df
Striga hermonthica	24	7	6	68	4	109	9	173.897	0.0001	4
Digitaria abyssinica	51	119	59	37	28	294	25	35.397	0.0001	4
Amaranthus spinosus	5	26	3	5	30	69	6	86.302	0.0001	4
Bidens pilosa	16	82	72	22	25	217	18	49.372	0.0001	4
Pennisetum purpureum	2	8	19	1	4	34	3	28.462	0.0001	4
Panicum maximum	0	3	8	0	0	11	1	19.300	0.001	4
Oxalis latifolia	4	13	1	0	0	18	2	17.388	0.02	4
Commelina benghalensis	20	61	10	16	15	122	10	29.136	0.001	4
Imperata cylindrica	10	11	28	4	4	57	5	35.061	0.001	4
Cyperus rotundus	4	6	0	0	0	10	1	13.326	0.01	4
Chloris gayana	12	9	0	7	3	31	3	24.408	0.001	4
Galinsonga parviflora	0	3	0	0	6	9	1	28.607	0.001	4
Euphorbia spp	11	19	8	10	5	53	4	4.246	0.374??	4
Pennisetum clandestinum	2	27	1	4	11	45	4	30.513	0.001	4
Eleusine indica	9	32	22	8	16	87	7	11.732	0.019	4
Saccharum officinarum	0	6	0	0	7	13	1	28.018	0.001	4
Total	**170**	**432**	**237**	**182**	**158**	**1179**	**100**			

EAZ= Eastern Agroecological zone, EHZ= Eastern highland Agroecological zone, LAZ= Lake Albert Crescent Zone, SEZ= South Eastern Agroecological zone, WHZ= Western Highland Agroecological zone.
[a]Total number of samples of specific weed species identified and tested

Table 4. Distribution and frequency of occurrence of cultivated crops gown as intercrops with maize in five major maize growing agroecological zones in Uganda during 3 consecutive seasons (2014A, 2014B and 2015A)

Species	EAZ	EHZ	LAZ	SEZ	WHZ	Total[a]	Proportion (%)	χ2	P	df
Phaseolus vulgaris	21	90	19	44	15	189	50.9	37.676	0.001	4
Manihot esculenta	6	10	20	11	2	49	13.2	14.905	0.005	4
Arachis hypogaea	1	24	3	3	6	37	10	21.542	0.001	4
Musa sp	0	6	17	1	11	35	9.4	38.004	0.001	4
Glycine max	4	1	2	5	1	13	3.5	8.485	0.075	4
Ipomoea batatas	2	2	4	3	1	13	3.2	2.238	0.692	4
Sorghum bicolor	7	0	0	2	0	9	2.4	36.102	0.001	4
Eleusine coracana	4	0	0	2	0	6	1.6	18.208	0.001	4
Vigna unguiculata	4	0	1	0	0	5	1.3	21.030	0.001	4
Oryza sativa	0	0	0	1	2	3	0.8	9.895	0.042	4
Cucurbita sp	0	0	3	0	0	3	0.8	11.075	0.026	4
Allium cepa	0	3	0	0	0	3	0.8	6.000	0.199	4
Lycopersicum esculentum	0	0	1	0	1	2	0.5	4.558	0.336	4
Sesamum indicum	2	0	0	0	0	2	0.5	13.623	0.009	4
Solanum tuberosum	0	0	0	0	1	1	0.3	7.425	0.115	4
Vigna radiata	0	0	0	1	0	1	0.3	3.898	0.420	4
Solanum incanum	0	0	0	1	0	1	0.3	3.898	0.420	4
Total	**51**	**136**	**70**	**74**	**40**	**373**	**100**			

EAZ= Eastern Agroecological zone, EHZ= Eastern highland Agroecological zone, LAZ= Lake Albert Crescent Zone, SEZ= South Eastern Agroecological zone, WHZ= Western Highland Agroecological zone.
[a]Total number of samples of specific weed species identified and tested

3.2 Occurrence of MLN causing viruses in alternative host weeds found in maize during surveys in major agroecological zones in 2014A, 2014B and 2015A

The entire potential alternate host weed collected from 16 districts in five major maize agroecological zones of Uganda were identified as 16 different species (Table 5). These weeds belonged to eight families namely: Scrophulariaceae, Poaceae, Amaranthaceae, Asteraceae, Oxalidaceae, Commelinaceae, Cyperaceae and Euphorbiaceae. Of these 16 species, some showed symptoms suggesting viral infection (chlorotic mosaic), whereas others showed no symptoms. All the weed species collected were tested for MCMV and SCMV by DAS-ELISA. Of these weeds, five tested positive for MCMV. These included *Digitaria abyssinica*, *Amaranthus spinosus* and *Pennisetum purpureum, Cyperus rotundus* and *Commelina benghalensis* (Tables 5, 6 and 7). The ELISA test indicated that, in 2014A, only *Pennisetum purpureum* tested positive for MCMV with 1/1 species? Samples? or 100% infected from Bulambuli district in Eastern Highland Agroecological zone (Table 5). During season 2014B, The ELISA test indicated that only *Pennisetum purpureum* was positive for MCMV with 2/2 species or 100% infected from Bulambuli district in Eastern Highland Agroecological zone (Table 6). In 2015A, *Digitaria abyssinica* (2/46 or 4.34%) and *Cyperus rotundus* (2/2 or 100%) from Bulambuli district in Eastern highland AEZ and (1/10 or 10%) from Tororo district in Eastern Agroecological zone*, Amaranthus spinosus*(2/7 or 28.57%) *Pennisetum purpureum* (2/5 or 40%) and *Commelina benghalensis* (1/19 or 5.26%) from Bulambuli district in Eastern Highland agroecological zone, tested positive for MCMV*. Eleusine indica* (2/6 or 330%) from Tororo district in Eastern Agroecological zone also tested positive for MCMV in 2015A (Table 7). None of the grasses tested positive for SCMV during all the three surveys conducted (Tables 5, 6 and 7). *Digitaria abyssinica*, *Commelina benghalensis, Amaranthus spinosus* and *Pennisetum purpureum* expressed virus symptoms, including chlorosis of leaves typical of MCMV (Fig. 2). (for these small sample sizes, % is not needed

Amaranthus spinosus	*Digitaria abyssinica*	*Commelina benghalensis*

Fig. 2. Examples of MCMV virus-like chlorotic symptoms observed in alternative weed hosts found in MLN infected fields in Bulambuli district eastern Uganda

Table 5. Occurrence of *Maize chlorotic mottle virus*(MCMV) and *Sugarcane mosaic virus*(SCMV) in weed species collected from five agroecological zones in Uganda during first season 2014

Weed Species	Total no. of samples[a]	Number of samples in different agroecological zones (/Number of virus positive samples)									
		EAZ[b]		EHZ[b]		LAZ[b]		SEZ[b]		WHZ[b]	
		MCMV[c]	SCMV[c]	MCMV[c]	SCMV[c]	MCMV[c]	SCMV[c]	MCMV[c]	SCMV[c]	MCMV[c]	SCMV[c]
Striga hermonthica	38	15(0)	15(0)	2(0)	2(0)	2(0)	2(0)	18(0)	18(0)	1(0)	1(0)
Digitaria abyssinica	106	26(0)	26(0)	42(0)	42(0)	22(0)	22(0)	11(0)	11(0)	5(0)	5(0)
Amaranthus spinosus	24	2(0)	2(0)	11(0)	11(0)	0(0)	0(0)	2(0)	2(0)	9(0)	9(0)
Bidens pilosa	75	6(0)	6(0)	34(0)	34(0)	26(0)	26(0)	6(0)	6(0)	3(0)	3(0)
Pennisetum purpureum	7	1(0)	1(0)	1(1)	1(0)	4(0)	4(0)	0(0)	0(0)	1(0)	1(0)
Panicum maximum	4	0(0)	0(0)	1(0)	1(0)	3(0)	3(0)	0(0)	0(0)	0(0)	0(0)
Oxalis latifolia	4	0(0)	0(0)	4(0)	4(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Commelina benghalensis	58	11(0)	11(0)	29(0)	29(0)	6(0)	6(0)	8(0)	8(0)	4(0)	4(0)
Imperata cylindrica	16	4(0)	4(0)	4(0)	4(0)	3(0)	3(0)	3(0)	3(0)	2(0)	2(0)
Cyperus rotundus	3	1(0)	1(0)	2(0)	2(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Cynodon dactylon	13	7(0)	7(0)	3(0)	3(0)	0(0)	0(0)	2(0)	2(0)	1(0)	1(0)
Galinsonga parviflora	3	0(0)	0(0)	1(0)	1(0)	0(0)	0(0)	0(0)	0(0)	2(0)	2(0)
Euphorbia heterohylla	14	3(0)	3(0)	5(0)	5(0)	3(0)	3(0)	2(0)	2(0)	1(0)	1(0)
Pennisetum clandestinum	13	0(0)	0(0)	10(0)	10(0)	0(0)	0(0)	0(0)	0(0)	3(0)	3(0)
Eleusine indica	26	2(0)	2(0)	11(0)	11(0)	9(0)	9(0)	2(0)	2(0)	2(0)	2(0)
Saccharum officinarum	4	0(0)	0(0)	2(0)	2(0)	0(0)	0(0)	0(0)	0(0)	2(0)	2(0)

[a]Total number of samples of specific weed species identified and tested: [b]Agroecological zones where; EAZ= Eastern Agroecological zone, EHZ= Eastern highland Agroecological zone, LAZ= Lake Albert Crescent Zone, SEZ= South Eastern Agroecological zone, WHZ= Western Highland Agroecological zone. [c]Numbers in parenthesis represent the number of ELISA positive samples for MLN virus. MCMV =*Maize chlorotic mottle virus*, SCMV= *Sugarcane mosaic virus*.

Table 6. Occurrence of *Maize chlorotic mottle virus* (MCMV) and *Sugarcane mosaic virus* (SCMV) in weed species collected from five agroecological zones in Uganda during second season 2014

Number of samples in different agroecological zones/Number of virus positive samples

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Weed Species List alphabericaaly or by freq	Total no. of samples[a]	EAZ[b]		EHZ[b]		LAZ[b]		SEZ[b]		WHZ[b]	
		MCMV[c]	SCMV[c]	MCMV[c]	SCMV[c]	MCMV[c]	SCMV[c]	MCMV[c]	SCMV[c]	MCMV[c]	SCMV[c]
Striga hermonthica	30	6 (0)	6 (0)	2(0)	2(0)	2(0)	2(0)	18(0)	18(0)	2(0)	2(0)
Digitaria abyssinica	102	15(0)	15(0)	31(0)	31(0)	22(0)	22(0)	16(0)	16(0)	18(0)	18(0)
Amaranthus spinosus	25	2(0)	2(0)	8(0)	8(0)	2(0)	2(0)	1(0)	1(0)	12(0)	12(0)
Bidens pilosa	94	7(0)	7(0)	29(0)	29(0)	32(0)	32(0)	8(0)	8(0)	18(0)	18(0)
Pennisetum purpureum	15	1(0)	1(0)	2(2)	2(0)	9(0)	9(0)	1(0)	1(0)	2(0)	2(0)
Panicum maximum	5	0(0)	0(0)	1(0)	1(0)	4(0)	4(0)	0(0)	0(0)	0(0)	0(0)
Oxalis latifolia	9	1(0)	1(0)	7(0)	7(0)	1(0)	1(0)	0(0)	0(0)	0(0)	0(0)
Commelina benghalensis	36	7(0)	7(0)	13(0)	13(0)	3(0)	3(0)	6(0)	6(0)	7(0)	7(0)
Imperata cylindrica	24	3(0)	3(0)	4(0)	4(0)	17(0)	17(0)	0(0)	0(0)	0(0)	0(0)
Cyperus rotundus	3	1(0)	1(0)	2(0)	2(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Cynodon dactylon	10	2(0)	2(0)	5(0)	5(0)	0(0)	0(0)	2(0)	2(0)	1(0)	1(0)
Galinsonga parviflora	3	0(0)	0(0)	1(0)	1(0)	0(0)	0(0)	0(0)	0(0)	2(0)	2(0)
Euphorbia heterohylla	20	4(0)	4(0)	9(0)	9(0)	1(0)	1(0)	3(0)	3(0)	3(0)	3(0)
Pennisetum clandestinum	15	2(0)	2(0)	4(0)	4(0)	1(0)	1(0)	4(0)	4(0)	4(0)	4(0)
Eleusine indica	28	1(0)	1(0)	11(0)	11(0)	7(0)	7(0)	2(0)	2(0)	7(0)	7(0)
Saccharum officinarum	4	0(0)	0(0)	2(0)	2(0)	0(0)	0(0)	0(0)	0(0)	2(0)	2(0)

[a]Total number of samples of specific weed species identified and tested: [b]Agroecological zones where; EAZ= Eastern Agroecological zone, EHZ= Eastern highland Agroecological zone, LAZ= Lake Albert Crescent Zone, SEZ= South Eastern Agroecological zone, WHZ= Western Highland Agroecological zone. [c]Numbers in parenthesis represent the number of ELISA positive samples for MLN virus. MCMV =*Maize chlorotic mottle virus*, SCMV= *Sugarcane mosaic virus*.

Table 7. Occurrence of *Maize chlorotic mottle virus*(MCMV) and *Sugarcane mosaic virus*(SCMV) in weed species collected from five agroecological zones in Uganda during first season 2015

Weed Species	Total no. of samples[a]	Number of samples in different agroecological zones/Number of virus positive samples									
		EAZ[b]		EHZ[b]		LAZ[b]		SEZ[b]		WHZ[b]	
		MCMV[c]	SCMV[c]	MCMV[c]	SCMV[c]	MCMV[c]	SCMV[c]	MCMV[c]	SCMV[c]	MCMV[c]	SCMV[c]
Striga hermonthica	41	3(0)	3(0)	3(0)	3(0)	2(0)	2(0)	32 (0)	32 (0)	1(0)	1(0)

Digitaria abyssinica	86	10(1)	10(0)	46(2)	46(0)	15(0)	15(0)	10(0)	10(0)	5(0)	5(0)
Amaranthus spinosus	20	1(0)	1(0)	7(2)	7(0)	1(0)	1(0)	2(0)	2(0)	9(0)	9(0)
Bidens pilosa	48	3(0)	3(0)	19(0)	19(0)	14(0)	14(0)	8(0)	8(0)	4(0)	4(0)
Pennisetum purpureum	12	0(0)	0(0)	5(2)	5(0)	6(0)	6(0)	0(0)	0(0)	1(0)	1(0)
Panicum maximum	2	0(0)	0(0)	1(0)	1(0)	1(0)	1(0)	0(0)	0(0)	0(0)	0(0)
Oxalis latifolia	5	3(0)	3(0)	2(0)	2(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Commelina benghalensis	28	2(0)	2(0)	19(1)	19(0)	1(0)	1(0)	2(0)	2(0)	4(0)	4(0)
Imperata cylindrica	17	3(0)	3(0)	3(0)	3(0)	8(0)	8(0)	1(0)	1(0)	2(0)	2(0)
Cyperus rotundus	4	2(0)	2(0)	2(2)	2(0)	0(0)	0(0)	0(0)	0(0)	0(0)	**0(0)**
Cynodon dactylon	8	3(0)	3(0)	1(0)	1(0)	0(0)	0(0)	3(0)	3(0)	1(0)	1(0)
Galinsonga parviflora	3	0(0)	0(0)	1(0)	1(0)	0(0)	0(0)	0(0)	0(0)	2(0)	2(0)
Euphorbia heterohylla	19	4(0)	4(0)	5(0)	5(0)	4(0)	4(0)	5(0)	5(0)	1(0)	1(0)
Pennisetum clandestinum	17	0(0)	0(0)	13(0)	13(0)	0(0)	0(0)	0(0)	0(0)	4(0)	4(0)
Eleusine indica	33	6(2)	6(0)	10(0)	10(0)	6(0)	6(0)	4(0)	4(0)	7(0)	7(0)
Saccharum officinarum	5	0(0)	0(0)	2(0)	2(0)	0(0)	0(0)	0(0)	0(0)	3(0)	3(0)

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[a]Total number of samples of specific weed species identified and tested: [b]Agroecological zones where; EAZ= Eastern Agroecological zone, EHZ= Eastern highland Agroecological zone, LAZ= Lake Albert Crescent Zone, SEZ= South Eastern Agroecological zone, WHZ= Western Highland Agroecological zone. [c]Numbers in parenthesis represent the number of ELISA positive samples for MLN virus. MCMV =*Maize chlorotic mottle virus*, SCMV= *Sugarcane mosaic virus*.

3.3 Occurrence of MLN causing viruses in cultivated crops found in maize agroecosystems during surveys of major agroecological zones in 2014A, 2014B and 2015A

Of these crops, the following tested positive for MCMV; beans (*Phaseolus vulgaris*) from MLN infected field in Bulambuli district (Eastern Highland Agroecological zone), cassava (*Manihot esculenta*) from MLN infected field in Bulambuli district (Eastern Highland Agroecological zone) and simsim (*Sesamum indicum*) from MLN infected field in Tororo district found in Eastern Agroecological zone (Tables 8 and 9). Of these crops, only (*Sorghum bicolor*) from MLN infected field in Tororo district (Eastern Agroecological zone) and sweet potato (*Ipomoea batatas*) from Nabongo subcounty, Bulambuli district (Eastern highland agroecological zone) tested positive for SCMV using DAS ELISA. The ELISA test indicated that, in 2014A, none of the cultivated crops tested positive for MCMV. In 2014B, two samples of simsim (*Sesamum indicum*) tested positive for MCMV from Molo Sub County, Tororo district in Eastern Agroecological zone. In 2015A, two samples of beans from Simu subcounty, Bulambuli district in Eastern highland agroecological zone tested positive for MCMV. In addition, two cassava (*Manihot esculenta*) samples collected from the same location in Eastern highland agroecological zone tested positive for MCMV. In 2015, two sorghum (*Sorghum bicolor*) samples collected from Molo subcounty in Tororo district (Eastern highland agroecological zone) and one sample of sweet potato (*Ipomoea batatas*) from Nabongo subcounty, Bulambuli district (Eastern Highland Agroecological Zone) tested positive for SCMV.

Table 8. Occurrence of *Maize chlorotic mottle virus*(MCMV) and *Sugarcane mosaic virus*(SCMV) in cultivated crop species grown as intercrops with maize collected from five agroecological zones of Uganda during second season 2014

Crop species	Total no. of samples[a]	Number of samples in different agroecological zones/Number of virus positive samples									
		EAZ[b]		EHZ[b]		LAZ[b]		SEZ[b]		WHZ[b]	
		MCMV[c]	SCMV[c]	MCMV[c]	SCMV[c]	MCMV[c]	SCMV[c]	MCMV[c]	SCMV[c]	MCMV[c]	SCMV[c]
Bananas	23	0 (0)	0 (0)	2 (0)	2 (0)	11(0)	11(0)	1(0)	1(0)	9(0)	9(0)
Beans	49	6 (0)	6 (0)	28 (0)	28 (0)	5 (0)	5 (0)	6(0)	6(0)	4(0)	4(0)
Cassava	18	0 (0)	0 (0)	4(0)	4(0)	9 (0)	9 (0)	5(0)	5(0)	0(0)	0(0)
Cowpea	3	2 (0)	2 (0)	0 (0)	0 (0)	1(0)	1(0)	0(0)	0(0)	0(0)	0(0)
Groundnuts	5	0 (0)	0 (0)	3 (0)	3 (0)	2 (0)	2 (0)	0(0)	0(0)	0(0)	0(0)
Irish potato	1	0 (0)	0 (0)	0 (0)	0 (0)	0(0)	0(0)	0(0)	0(0)	1(0)	1(0)
Millet	3	2 (0)	2 (0)	0 (0)	0 (0)	0(0)	0(0)	1(0)	1(0)	0(0)	0(0)
Rice	3	0 (0)	0 (0)	0 (0)	0 (0)	0(0)	0(0)	1(0)	1(0)	2(0)	2(0)
Simsim	2	2 (2)	2 (0)	0 (0)	0 (0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Soybeans	2	0 (0)	0 (0)	0 (0)	0 (0)	0(0)	0(0)	1(0)	1(0)	1(0)	1(0)
Sorghum	3	3 (0)	3 (0)	0 (0)	0 (0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Sweet potato	7	0 (0)	0 (0)	1 (0)	1 (0)	2(0)	2(0)	3(0)	3(0)	1(0)	1(0)
Tomatoes	2	0 (0)	0 (0)	0 (0)	0 (0)	1(0)	1(0)	0(0)	0(0)	1(0)	1(0)
Yam	1	0 (0)	0 (0)	0 (0)	0 (0)	0(0)	0(0)	0(0)	0(0)	1(0)	1(0)

[a]Total number of samples of specific crop identified and tested: [b]Agroecological zones where; EAZ= Eastern Agroecological zone, EHZ= Eastern highland Agroecological zone, LAZ= Lake Albert Crescent Zone, SEZ= South Eastern Agroecological zone, WHZ= Western Highland Agroecological zone. [c]Numbers in parenthesis represent the number of ELISA positive samples for MLN virus. MCMV= *Maize chlorotic mottle virus*. SCMV= *Sugarcane mosaic virus*

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Table 9. Occurrence of *Maize chlorotic mottle virus*(MCMV) and *Sugarcane mosaic virus*(SCMV) in food crop species grown as intercrops with maize collected from five agroecological zones of Uganda during first season 2015

		Number of samples in different agroecological zones/Number of virus positive samples									
Crop species	**Total no. of samples[a]**	**EAZ[b]**		**EHZ[b]**		**LAZ[b]**		**SEZ[b]**		**WHZ[b]**	
		MCMV[c]	**SCMV[c]**	**MCMV[c]**	**SCMV[c]**	**MCMV[c]**	**SCMV[c]**	**MCMV[c]**	**SCMV[c]**	**MCMV[c]**	**SCMV[c]**
Bananas	6	0 (0)	0 (0)	2(0)	2(0)	3(0)	3(0)	0 (0)	0 (0)	1(0)	1(0)
Beans	107	11(0)	11(0)	42(2)	42(0)	10(0)	10(0)	37(0)	37(0)	7(0)	7(0)
Cassava	21	4(0)	4(0)	5(2)	5(0)	7(0)	7(0)	4(0)	4(0)	1(0)	1(0)
Cowpea	1	1(0)	1(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Groundnuts	26	0(0)	0(0)	18(0)	18(0)	1(0)	1(0)	3(0)	3(0)	4(0)	4(0)
Millet	3	2(0)	2(0)	0(0)	0(0)	0(0)	0(0)	1(0)	1(0)	0(0)	0(0)
Onions	1	0(0)	0(0)	1(0)	1(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Pumpkin	1	0(0)	0(0)	0(0)	0(0)	1(0)	1(0)	0(0)	0(0)	0(0)	0(0)
Sorghum	6	4(0)	4(2)	0(0)	0(0)	0(0)	0(0)	2(0)	2(0)	0(0)	0(0)
Soybeans	10	4(0)	4(0)	1(0)	1(0)	1(0)	1(0)	4(0)	4(0)	0(0)	0(0)
Sweet potato	3	1(1)	1(0)	0(0)	1(1)	1(0)	1(0)	0(0)	0(0)	0(0)	0(0)

[a]Total number of samples of specific crop identified and tested: [b]Agroecological zones where; EAZ= Eastern Agroecological zone, EHZ= Eastern highland Agroecological zone, LAZ= Lake Albert Crescent Zone, SEZ= South Eastern Agroecological zone, WHZ= Western Highland Agroecological zone. [c]Numbers in parenthesis represent the number of ELISA positive samples for MLN virus. MCMV= *Maize chlorotic mottle virus*. SCMV= *Sugarcane mosaic virus*

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3.4 Molecular detection of MLN causing viruses from collected weeds

Weed samples that tested positive for MCMV using DAS- ELISA were confirmed positive using PCR based on presence of bands as shown in representative gels for MCMV (Fig. 3). The band size for MCMV fragment was 550bp. Amplicons of the expected size of 550 bp were amplified from RT-PCR product of the following positively tested weed species notably *Pennisetum purpureum, Digitaria abyssinica, Cyperus rotundus, Commelina benghalensis, Amaranthus spinosus, Eleusine indica.* SCMV was not detected using RT-PCR in **both** either weeds **and** or cultivated crops hence results are not presented. Why not agree with ELISA

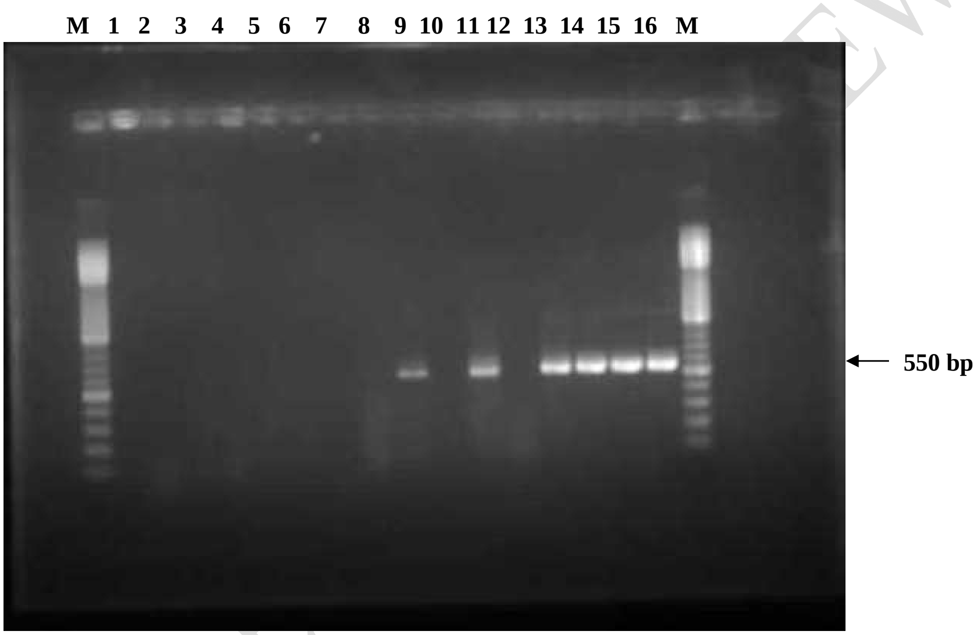

Fig. 3. RT-PCR products of MCMV in weed samples collected from major maize agroecological zones in Uganda. Lane M represents 100bp DNA ladder (Bioneer). Lane 1= ***Striga hermonthica*****, 2=** ***Bidens pilosa*****, 3=** ***Oxalis latifolia*****, 4=** ***Galinsonga parviflora*****, 5=** ***Saccharum officinarum*****, 6=** ***Euphorbia spp*****, 7=** ***Euphorbia heterohylla*** **, 8=** ***Chloris gayana*****, 9=*****Digitaria abyssinica*****, 10=Negative control (nuclease free water), 11=** ***Amaranthus spinosus*****, 12=** ***Panicum maximum*****, 13=** ***Pennisetum purpureum*****, 14=** ***Cyperus rotundus*****, 15=** ***Commelina benghalensis*****, 16= Positive control (maize).**

4. DISCUSSION

This study represents the first survey of potential and known alternative hosts of viruses causing MLN from a large geographic area covering five major maize agroecological zones of Uganda. As regards occurrence of potential weed hosts in major maize growing agroecological zones, this study showed occurrence of a wide range of weeds in the five major maize growing agroecological zones of Uganda. *Digitaria abyssinica, Bidens pilosa* and *Commelina benghalensis* were the most commonly occurring weed species in the major maize growing agroecological zones in Uganda. Similarly earlier studies have reported that *Digitaria spp*, *B. pilosa*, *C. benghalensis*, *I. cylindrica*, and *P. maximum,* were the major weeds in Uganda [21]. The weeds represented eight plant families. Of these families, Poaceae had the highest number of species recorded. Most of the weed species that had previously been identified as hosts of *Maize chlorotic mottle virus* were also in the family Poaceae which is consistent with observations that that this family contains large numbers of plants susceptible to MLN causing viruses [3,7]. The study also identified a number of known alternate hosts of SCMV as reported by [13] notably *Chloris gayana, Cynodondactylon, Oryza sativa, Panicum maximum, Saccharum officinarum, Sorghum bicolor* and *Zea mays* found present in the major maize agroeoclogical zones in Uganda. Most of these weed species are perennial in nature and can hence act as sources of inoculum **when the maize crop is harvested**. The large? number of potential weed-hosts identified suggests the availability of favorable hosts with the ability to harbor the MLN causing viruses and serve as sources of inoculum to its vectors. Perennial weed species can act as continuous endemic source of inoculum of virus and can be transferred to annual weeds where the virus propagates before being spread further to crops that are susceptible [22, 23]. This implies that such weeds should not be overlooked when developing MLN management strategies. Vector?

Results of this study indicated that, several weed species from the Poaceae family, mainly collected from Eastern Highland and Eastern Agroecological Zones, **had most prevalent species** are susceptible to MLN- causing virusesviruses **estimated as incidence**. In the field survey conducted, *Digitaria abyssinica, Amaranthus spinosus, Cyperus rotundus, Pennisetum purpureum* and *Commelina benghalensis* were found to be naturally susceptible to MCMV. These results are in conformity with earlier reports only for *Pennisetum purpureum* which was reported to be a natural host for MCMV [16]. These results were not expected for the Commelinaceae and Amaranthaceae since MCMV **is** had only been **known** reported to be found in Poaceae sp.**family** [7]. No plants in the familiesy Commelinaceae and Amaranthaceae have hitherto been documented as hosts of MCMV. Furthermore, this appears to be the first observation of a large number of naturally MCMV-infected species **next** in proximity to maize crops in the field, notably from the MLN hotspot districts of Bulambuli and Tororo. However **there is no existence of** MLN was observed in weeds found in the areas **under** where there is no disease pressure. These facts suggest that the continuously high incidence of this virus in these MLN hotspot areas may be partially associated with **large numbers of**alternate MCMV sources in theseis maize-producing agroecological zones. Up to the recent past when MCMV was identified in sorghum [24], sugarcane [8], finger millet [9], Napier grass [16], Kikuyu grass [16], the only naturally occurring host of MCMV was maize [7]. This is therefore the first report of MCMV in *Digitaria abyssinica, Amaranthus spinosus* and *Commelina benghalensis*.

As regards surveys of potential natural hosts of MLN viruses in cultivated crops, the following tested positive for MCMV; beans (*Phaseolus vulgaris*) and cassava (*Manihot esculenta*), from MLN infected field in Bulambuli district (Eastern Highland Agroecological zone) and Simsim from MLN infected field in Tororo district (Eastern Agroecological zone). Out of the studied crops, only sorghum from MLN infected field in Tororo district (Eastern Agroecological zone) and sweet potato from Nabongo subcounty, Bulambuli district (Eastern highland agroecological zone) tested positive for SCMV using DAS ELISA. The findings agree with earlier reports **who also reported that**showing Sorghum is a natural host of SCMV [25]. However, plants did not show symptoms related to MCMV which suggests they could be resistant to infection. The results were not expected for thoese cultivated crops **all of** which are dicotyledonous plants from non graminae families. Prior to this, MCMV has only known to be found in the Poaceae family [7] Cassava, beans and groundnuts are dicotyledonous plants [26, 27]. These results do not support previous findings that reported that dicotyledonous species were not mechanically infected with MCMV [28, 29]. Specific isolate-host interactions could probably explain the contrasting results observed with some plant species about their host status for MLN causing viruses. However, these results are in conformity with previous studies that showed that some dicotyledonous plants can be natural and artificial hosts of MCMV [30]. Nonetheless the findings in this study suggest that these cultivated crops may carry the virus based on the virus titers that were comparable to the positive maize control. Prior to this study, no weeds and crop species were found to be naturally affected in the wild probably because they are not favorable hosts for vectors of MCMV. These ELISA based results were not confirmed in most of the weed species using PCR for SCMV but only for MCMV. These findings are in conformity to findings in Kenya that also reported low detection of SCMV using PCR despite positive results using ELISA. [1,16]. This is probably due to the emergence of new strains of SCMV with sequences in capsid protein that are different from the sequences used to design the primers used. Indeed studies have confirmed that SCMV strains in the East African region are highly divergent [1].

This study could hence provide the first evidence of the potential role of cultivated crops as hosts of MLN causing viruses. There is need for further studies on these cultivated crops to investigate whether they share vectors of MCMV with maize and these vectors can transfer the virus from the maize to these crops and vice versa. No information is available concerning the occurrence of natural sources of MLN causing viruses in cultivated crops including beans (*Phaseolus vulgaris*), cassava (*Manihot esculenta*), groundnuts (*Arachis hypogaea*) bananas (*Musa sp*), soybeans (*Glycine max*)and sweet potato (*Ipomoea batatas*) and hence these findings provide the first report of the potential role these cultivated crops could play as reservoirs of MCMV potentially increasing the amount of virus innoculum within the field. The implication of these findings is that crops like sorghum and cassava commonly grown in these areas have some varieties that are late maturing and can hence provide a source of innoculum to the next season crop of maize. Furthermore, beans are commonly grown as intercrops with maize and hence could also potentially provide a source of inoculum of MLN causing virus when grown with maize. In addition to this, sweet potato is a late planted crop in most cropping systems of Uganda and hence could also provide a source of inoculum for the MLN viruses in the subsequent season crop. However, the importance of the cultivated crops as alternate hosts needs further studies to determine if vectors that can survive on maize can also survive on these alternative hosts.

5. CONCLUSION

The overall aim of this study was to **determine** identify alternative host weeds and crops species occurring in maize and their role in the spread of maize lethal necrosis-causing viruses in Uganda. It was hypothesized that potential alternative hosts of Maize Lethal Necrosis-causing viruses are present in major maize growing agroecological zones of Uganda and act as sources of inoculum to maize fields. The study also established the following natural weed hosts of MCMV, and they included; *Digitaria abyssinica, Amaranthus spinosus, Cyperus rotundus, Pennisetum purpureum* and *Commelina benghalensis*. The natural hosts for SCMV were only Sorghum and sweet potato. No natural hosts of SCMV were detected among the weeds. Based on these observations, these could be the most likely sources of MLN virus inoculum during the period when maize has been harvested and hence contributing to the spread of the MLN disease. The study has confirmed the existence of potential natural sources of MCMV innoculum in cultivated crops beans (*Phaseolus vulgaris*), cassava (*Manihot esculenta*) and Simsim (*Sesamum indicum*) obtained from MLN hotspot districts of Bulambuli and Tororo. Mechanical inoculation studies corroborated these findings in cassava (*Manihot esculenta*), groundnuts (*Arachis hypogaea*) and beans (*Phaseolus vulgaris*).Existence of alternative hosts may explain early infection of maize plants by MCMV and SCMV and the continued occurrence of the MLN disease in the hotspot districts of eastern Uganda. Therefore, this information serves as justification for regular weed management in maize fields, as an Integrated Pest Management (IPM) option for the sustainable control of MLN.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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