

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 their 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 most common weed species while *Phaseolus vulgaris*, *Manihot esculenta*, *Arachis hypogaea*, *Musa sp.*, *Glycine max* and *Ipomoea batatas* were 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 *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: Alternative 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. In Uganda, it 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

Comment [E1]: Suggestion: caused

50 epidemiology of virus diseases and should be considered in development of an integrated disease
51 management strategy.
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53 Several studies on the significance of weeds as reservoirs of MLN causing viruses notably MCMV [8, 11,
54 7, 10] and MDMV and SCMV [12, 13, 14] have been conducted in maize production agro-ecologies in
55 temperate regions of North America. Little or no related studies have been conducted in tropical regions
56 [9, 15, 16]. Differences in agroecosystems and agro-ecologies result in differences in the biology and
57 occurrence of different weed species, which may cause variation in their relation to MLN causing viruses
58 and their vectors. Most of these alternative weed hosts found growing in association with maize
59 agroecosystems in these countries where first studies on MLN were conducted may have acted as
60 alternative hosts for vector feeding or reproduction of virus vectors, reservoirs of the MLN causing viruses
61 or both. However, it is not known whether similar grass weed species that are hosts of MLN are present in
62 Uganda and if they could have had a role in enhancing the spread of MLN. The alternative host status of
63 related plant species and common intercrops grown with maize in Uganda for the Ugandan strain(s) of
64 MLN causing viruses and their role in the spread of MLN in Uganda is unknown. Some maize viruses
65 have been known to have different reactions on the same alternative host due to variation in the strains.
66 There have been no studies conducted on MLN causing viruses and their natural or artificial hosts in
67 Uganda. Some non-chemical methods of managing MLN such as crop rotation and fallowing focus on the
68 removal of the maize host from the field for a defined period. It is not known whether crop rotation is a
69 feasible MLN disease control measure. The success of such cultural methods depends on the duration of
70 survival of MLN causing viruses without a maize host or alternate hosts. In most cases no attention is
71 given to weeds which could be alternative hosts and sources of inoculum for MLN viruses. In order to
72 develop an MLN management strategy that is effective, information should be generated through studies
73 conducted to establish the host range of Ugandan strain(s) of MLN causing viruses. The aim of this study
74 was to **establish survey** the weeds and cultivated crop species occurring in maize agroecological zones
75 of Uganda. **In addition, and** to determine the existence of natural alternative weed and cultivated crop
76 hosts of MLN causing viruses. It was hypothesized that potential alternative hosts of Maize Lethal
77 Necrosis-causing viruses are present in major maize growing agroecological zones of Uganda and act as
78 natural sources of inoculum to maize fields.
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80 2. MATERIALS AND METHODS

81 2.1 Description of the Study Area

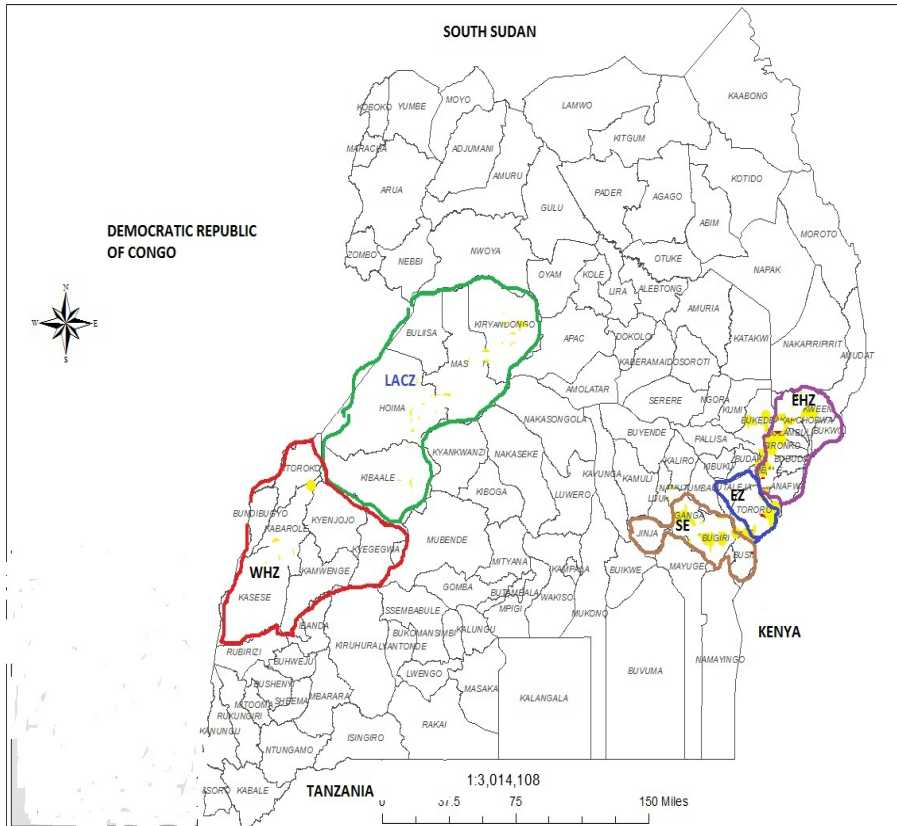
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83
84 The study was conducted over three seasons from 2014A, 2014B and 2015A and covered 14 major
85 maize growing districts from five agroecological zones (AEZ) of Uganda (Fig. 1). The study agroecological
86 zones were classified according to the National Agricultural Research Organization [17] based on distinct
87 vegetation type, elevation and climatic conditions. The Eastern Agro-ecological Zone (EAZ) covered the
88 districts of Tororo and Bukedea and is characterized by an annual average rainfall of 1197 mm with
89 temperature ranges from 15-32.5 °C. The Lake Albert Crescent Agro-ecological Zone (LACZ) covered the
90 districts of Hoima, Masindi, Kibaale and Kiryandongo and is characterized by an annual average rainfall
91 of 1259mm with temperature ranging from 17.5-32.5°C. The South Eastern Agro-ecological Zone (SEAZ)
92 covered the districts of Iganga, Busia and Bugiri with average annual rainfall ranging from 1215-1328mm
93 and temperature ranging from 15 to 32.5°C. The Eastern Highlands Agro-ecological Zone (EHAZ)
94 covered the districts of Mbale, Bulambuli, Sironko, Kween and Kapchorwa with average annual rainfall
95 more than 1,400 mm and temperature ranging from 7.5 to 27.5°C. The Western Highlands Agro-
96 ecological Zone (WHAZ) covered the districts of Kasese and Kabarole with average annual rainfall of
97 1,270 mm and temperature ranging from 15 to 30 °C.

Comment [E2]: Suggestion: These alternative weed hosts growing in association with maize agroecosystems in countries where first studies on MLN were conducted may have acted as alternative hosts for vectors or as reservoirs of the MLN causing viruses or both.

Comment [E3]: Please put one or more references to the sentence

Comment [E4]: Is there previous work demonstrating the existence of Ugandan strain?

Comment [E5]: Please explain what is A and B



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

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 104 **2.2 Field surveys and sampling**

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 106 The five major maize growing agroecological zones of Uganda (namely, the Eastern, Eastern Highland,
 107 South Eastern, Western Highland and Lake Albert Crescent Agroecological zones) were surveyed for
 108 alternative host plants of MLN. From each agroecological zone, at least two districts were chosen
 109 purposively based on maize production data and surveyed for plants that were displaying virus-like
 110 symptoms or were apparently healthy looking. The surveys were carried out during the first season of
 111 2014, second season of 2014 and first season of 2015 from 16 major maize growing districts. Fields were
 112 selected at regular intervals along major and feeder roads traversing the sampling area. Average distance
 113 between sampled fields was about 5km. Weeds and cultivated crops grown as intercrops or near maize
 114 were collected from near maize fields on either side of the road while alternating after every 5 km. In all
 115 cases, the site of collection was a maize field in which the plants were growing naturally as weeds.
 116 Sampling activities commenced in the early morning hours just before sunrise (between 0630 and 0700
 117 h) to minimize the impact of wilting. Cultivated crop species and weeds growing within the vicinity of the
 118 maize crop and one meter from the maize plant were collected together with their rooting system and
 119 crown. At each stop the 3 plants from each weed species and 3 plants from each intercrop species grown

120 with maize in a 1-meter square area was sampled using a quadrat. The samples collected were put in
121 separate bags to avoid cross contamination. With a total of 10 sampling sites located 5 to 10 km from
122 each other.

123

124 **2.3 Plant materials and taxonomic identification**

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

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131 **2.4 Detection of Viruses in in weeds and cultivated crops from five major maize growing** 132 **agroecological zones of Uganda**

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134 **2.4.1 Serological detection of Maize lethal necrosis causing viruses in weeds and cultivated crops**

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

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

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159 **2.4.2 Molecular detection of Maize lethal necrosis causing viruses in weeds and cultivated crops**

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161 Total RNA was extracted from leaves of weeds and cultivated crops with Trizol Reagent (Bioneer, South
162 Korea) according to the manufacturer's instructions and subsequently used for cDNA synthesis by RT-
163 PCR using AccuPower® Reverse Transcription Polymerase Chain Reaction (RT-PCR) PreMix kit
164 (Bioneer Corporation, Korea) following manufacturer's instructions. MCMV and SCMV primers which
165 flank the coat protein gene of each virus and amplify a fragment of approximately 550bp for MCMV and
166 900 bp for SCMV were used for RT-PCR [3]. Electrophoresis was done on the RT-PCR product using
167 1.5% agarose gels for 45 minutes at 120V and current of 400 mA in TAE agarose gel. The amplified DNA
168 fragments were visualized on a 1.5% agarose gels under UV light. A 100b DNA Ladder (Bioneer®) was
169 used as the standard.

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171 **2.5 Data collection and analysis**

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Comment [E6]: Why did you not test for MDMV, since in the introduction it was listed as related to MLN?

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

185 **3. RESULTS**

186 **3.1 Survey of weed and cultivated crop species found growing in association with maize** 187 **in major maize growing agroecological zones in Uganda**

188 A total of 16 species of weeds representing 8 families were found in the major maize growing
189 agroecological zones in Uganda (Table 1). The family Poaceae had the highest (8) number of species
190 followed by Asteraceae with two species. The other families namely Amaranthaceae, Commelinaceae,
191 Cyperaceae, Euphorbiaceae and Oxalidaceae each had one species. A total of 17 cultivated crops
192 species representing 9 families were found in the major maize growing agroecological zones of Uganda
193 (Table 2). The family Fabaceae had the highest (5) number of species followed by Solanaceae with three
194 species and Poaceae with three species. The other families namely Musaceae, Pedaliaceae,
195 Euphorbiaceae, Convolvulaceae, Cucurbitaceae each had one species. Most of the crop species
196 identified were annuals (15 species) while 2 were perennials (Table 2).
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203 **Table 1. Potential weed hosts of MLN viruses identified in 5 major maize agroecological zones in**
 204 **Uganda during surveys conducted from 2014 to 2015**

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

Comment [E7]: It is a cultivated crop, isn't ?

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

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213 *Digitaria abyssinica*, *Bidens pilosa* and *Commelina benghalensis* were the most frequently found weed
 214 species across the five major maize growing agroecological zones in Uganda (Table 3). The frequency of
 215 occurrence of all weed species was significantly different across the 5 agroecological zones except for
 216 *Euphorbia heterophylla* Linn species which was not significantly different ($\chi^2 = 4.246$, $df = 4$, $P > 0.05$). The
 217 highest frequency of occurrence of weed species was found in the Eastern Highland Agroecological Zone
 218 (432), followed by the Lake Albert Crescent Zone (237), South Eastern (182), Eastern Agroecological
 219 Zone (170) and Western Highland agroecological zone (158). Among the observed weeds, 10 weed
 220 species occurred in all the agroecological zones and they included *Pennisetum purpureum*, *Pennisetum*
 221 *clandestinum*, *Euphorbia spp*, *Imperata cylindrica*, *Amaranthus spinosus*, *Eleusine indica*, *Striga*
 222 *hermonthica*, *Commelina benghalensis*, *Bidens pilosa* and *Digitaria abyssinica*(Table 3).
 223

224 As regards cultivated crops, Beans (*Phaseolus vulgaris*), Cassava (*Manihot esculenta*), Groundnuts
 225 (*Arachis hypogaea*), Bananas (*Musa sp*), Soybeans (*Glycine max*) and Sweet potato (*Ipomoea batatas*)
 226 were the most frequently found cultivated crop species grown with maize over the three seasons
 227 surveys across the five major maize agroecological zones (Table 4). They were used as 'indicators' in
 228 subsequent screen house studies to determine the MLN virus host range of cultivated crops grown with
 229 maize. The highest number of crop species (136) was found in the Eastern Highland Agroecological
 230 Zone, followed by South Eastern Agroecological Zone (74), Lake Albert Crescent Zone (70), Eastern
 231 Agroecological Zone (51) and Western Highland Agroecological Zone (40).
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 233

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

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

236 EAZ= Eastern Agroecological zone, EHZ= Eastern highland Agroecological zone, LAZ= Lake Albert

237 Crescent Zone, SEZ= South Eastern Agroecological zone, WHZ= Western Highland Agroecological zone.

238 ^aTotal number of samples of specific weed species identified and tested

239

240 **Table 4. Distribution and frequency of occurrence of cultivated crops grown as intercrops with**
 241 **maize in five major maize growing agroecological zones in Uganda during 3 consecutive seasons**
 242 **(2014A, 2014B and 2015A)**

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

243 EAZ= Eastern Agroecological zone, EHZ= Eastern highland Agroecological zone, LAZ= Lake Albert

244 Crescent Zone, SEZ= South Eastern Agroecological zone, WHZ= Western Highland Agroecological zone.

245 ^aTotal number of samples of specific weed species identified and tested

246 **3.2 Occurrence of MLN causing viruses in alternative host weeds found in maize during**
 247 **surveys in major agroecological zones in 2014A, 2014B and 2015A**

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 249 The entire potential alternate host weed collected from 16 districts in five major maize agroecological
 250 zones of Uganda were identified as 16 different species (Table 5). These weeds belonged to eight
 251 families namely: Scrophulariaceae, Poaceae, Amaranthaceae, Asteraceae, Oxalidaceae,
 252 Commelinaceae, Cyperaceae and Euphorbiaceae. Of these 16 species, some showed symptoms
 253 suggesting viral infection (chlorotic mosaic), whereas others showed no symptoms. All the weed species
 254 collected were tested for MCMV and SCMV by DAS-ELISA. Of these weeds, five tested positive for
 255 MCMV. These included *Digitaria abyssinica*, *Amaranthus spinosus* and *Pennisetum purpureum*, *Cyperus*
 256 *rotundus* and *Commelina benghalensis* (Tables 5, 6 and 7). The ELISA test indicated that, in 2014A, only
 257 *Pennisetum purpureum* tested positive for MCMV with 1/1 species or 100% infected from Bulambuli
 258 district in Eastern Highland Agroecological zone (Table 5). During season 2014B, The ELISA test
 259 indicated that only *Pennisetum purpureum* was positive for MCMV with 2/2 species or 100% infected from
 260 Bulambuli district in Eastern Highland Agroecological zone (Table 6). In 2015A, *Digitaria abyssinica* (2/46
 261 or 4.34%) and *Cyperus rotundus* (2/2 or 100%) from Bulambuli district in Eastern highland AEZ and (1/10
 262 or 10%) from Tororo district in Eastern Agroecological zone, *Amaranthus spinosus*(2/7 or 28.57%)
 263 *Pennisetum purpureum* (2/5 or 40%) and *Commelina benghalensis* (1/19 or 5.26%) from Bulambuli
 264 district in Eastern Highland agroecological zone, tested positive for MCMV. *Eleusine indica* (2/6 or 30%)
 265 from Tororo district in Eastern Agroecological zone also tested positive for MCMV in 2015A (Table 7).
 266 None of the grasses tested positive for SCMV during all the three surveys conducted (Tables 5, 6 and 7).
 267 *Digitaria abyssinica*, *Commelina benghalensis*, *Amaranthus spinosus* and *Pennisetum purpureum*
 268 expressed virus symptoms, including chlorosis of leaves typical of MCMV (Fig. 2).
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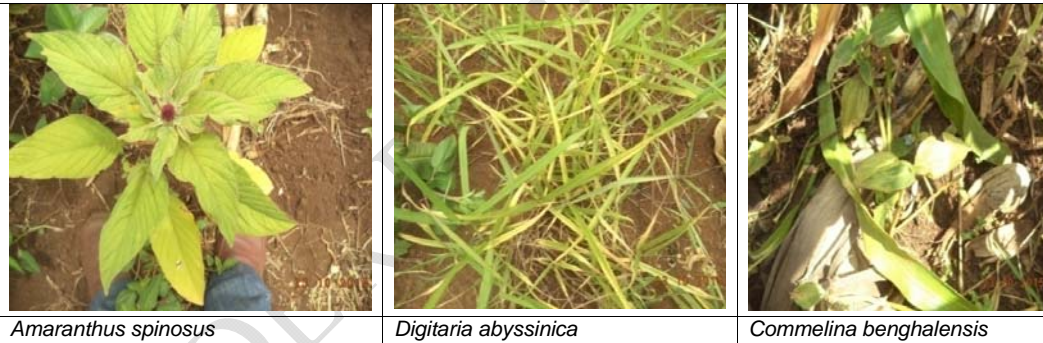


Fig. 2. Examples of MCMV virus-like chlorotic symptoms observed in alternative weed hosts found in MLN infected fields in Bulambuli district eastern Uganda

271 **Table 5. Occurrence of *Maize chlorotic mottle virus*(MCMV) and *Sugarcane mosaic virus*(SCMV) in weed species collected from five**
 272 **agroecological zones in Uganda during first season 2014**
 273

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
<i>Striga hermonthica</i>	38	15(0)	15(0)	2(0)	2(0)	2(0)	2(0)	18(0)	18(0)	1(0)	1(0)
<i>Digitaria abyssinica</i>	106	26(0)	26(0)	42(0)	42(0)	22(0)	22(0)	11(0)	11(0)	5(0)	5(0)
<i>Amaranthus spinosus</i>	24	2(0)	2(0)	11(0)	11(0)	0(0)	0(0)	2(0)	2(0)	9(0)	9(0)
<i>Bidens pilosa</i>	75	6(0)	6(0)	34(0)	34(0)	26(0)	26(0)	6(0)	6(0)	3(0)	3(0)
<i>Pennisetum purpureum</i>	7	1(0)	1(0)	1(1)	1(0)	4(0)	4(0)	0(0)	0(0)	1(0)	1(0)
<i>Panicum maximum</i>	4	0(0)	0(0)	1(0)	1(0)	3(0)	3(0)	0(0)	0(0)	0(0)	0(0)
<i>Oxalis latifolia</i>	4	0(0)	0(0)	4(0)	4(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
<i>Commelina benghalensis</i>	58	11(0)	11(0)	29(0)	29(0)	6(0)	6(0)	8(0)	8(0)	4(0)	4(0)
<i>Imperata cylindrica</i>	16	4(0)	4(0)	4(0)	4(0)	3(0)	3(0)	3(0)	3(0)	2(0)	2(0)
<i>Cyperus rotundus</i>	3	1(0)	1(0)	2(0)	2(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
<i>Cynodon dactylon</i>	13	7(0)	7(0)	3(0)	3(0)	0(0)	0(0)	2(0)	2(0)	1(0)	1(0)
<i>Galinsoga parviflora</i>	3	0(0)	0(0)	1(0)	1(0)	0(0)	0(0)	0(0)	0(0)	2(0)	2(0)
<i>Euphorbia heterophylla</i>	14	3(0)	3(0)	5(0)	5(0)	3(0)	3(0)	2(0)	2(0)	1(0)	1(0)
<i>Pennisetum clandestinum</i>	13	0(0)	0(0)	10(0)	10(0)	0(0)	0(0)	0(0)	0(0)	3(0)	3(0)
<i>Eleusine indica</i>	26	2(0)	2(0)	11(0)	11(0)	9(0)	9(0)	2(0)	2(0)	2(0)	2(0)
<i>Saccharum officinarum</i>	4	0(0)	0(0)	2(0)	2(0)	0(0)	0(0)	0(0)	0(0)	2(0)	2(0)

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 275 ^aTotal number of samples of specific weed species identified and tested: ^bAgroecological zones where; EAZ= Eastern Agroecological zone, EHZ=
 276 Eastern highland Agroecological zone, LAZ= Lake Albert Crescent Zone, SEZ= South Eastern Agroecological zone, WHZ= Western Highland
 277 Agroecological zone. ^cNumbers in parenthesis represent the number of ELISA positive samples for MLN virus. MCMV =*Maize chlorotic mottle*
 278 *virus*, SCMV= *Sugarcane mosaic virus*.

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

Weed 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
<i>Striga hermonthica</i>	30	6 (0)	6 (0)	2(0)	2(0)	2(0)	2(0)	18(0)	18(0)	2(0)	2(0)
<i>Digitaria abyssinica</i>	102	15(0)	15(0)	31(0)	31(0)	22(0)	22(0)	16(0)	16(0)	18(0)	18(0)
<i>Amaranthus spinosus</i>	25	2(0)	2(0)	8(0)	8(0)	2(0)	2(0)	1(0)	1(0)	12(0)	12(0)
<i>Bidens pilosa</i>	94	7(0)	7(0)	29(0)	29(0)	32(0)	32(0)	8(0)	8(0)	18(0)	18(0)
<i>Pennisetum purpureum</i>	15	1(0)	1(0)	2(2)	2(0)	9(0)	9(0)	1(0)	1(0)	2(0)	2(0)
<i>Panicum maximum</i>	5	0(0)	0(0)	1(0)	1(0)	4(0)	4(0)	0(0)	0(0)	0(0)	0(0)
<i>Oxalis latifolia</i>	9	1(0)	1(0)	7(0)	7(0)	1(0)	1(0)	0(0)	0(0)	0(0)	0(0)
<i>Commelina benghalensis</i>	36	7(0)	7(0)	13(0)	13(0)	3(0)	3(0)	6(0)	6(0)	7(0)	7(0)
<i>Imperata cylindrica</i>	24	3(0)	3(0)	4(0)	4(0)	17(0)	17(0)	0(0)	0(0)	0(0)	0(0)
<i>Cyperus rotundus</i>	3	1(0)	1(0)	2(0)	2(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
<i>Cynodon dactylon</i>	10	2(0)	2(0)	5(0)	5(0)	0(0)	0(0)	2(0)	2(0)	1(0)	1(0)
<i>Galinsonga parviflora</i>	3	0(0)	0(0)	1(0)	1(0)	0(0)	0(0)	0(0)	0(0)	2(0)	2(0)
<i>Euphorbia heterophylla</i>	20	4(0)	4(0)	9(0)	9(0)	1(0)	1(0)	3(0)	3(0)	3(0)	3(0)
<i>Pennisetum clandestinum</i>	15	2(0)	2(0)	4(0)	4(0)	1(0)	1(0)	4(0)	4(0)	4(0)	4(0)
<i>Eleusine indica</i>	28	1(0)	1(0)	11(0)	11(0)	7(0)	7(0)	2(0)	2(0)	7(0)	7(0)
<i>Saccharum officinarum</i>	4	0(0)	0(0)	2(0)	2(0)	0(0)	0(0)	0(0)	0(0)	2(0)	2(0)

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^aTotal number of samples of specific weed species identified and tested: ^bAgroecological 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. ^cNumbers 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
<i>Striga hermonthica</i>	41	3(0)	3(0)	3(0)	3(0)	2(0)	2(0)	32 (0)	32 (0)	1(0)	1(0)

<i>Digitaria abyssinica</i>	86	10(1)	10(0)	46(2)	46(0)	15(0)	15(0)	10(0)	10(0)	5(0)	5(0)
<i>Amaranthus spinosus</i>	20	1(0)	1(0)	7(2)	7(0)	1(0)	1(0)	2(0)	2(0)	9(0)	9(0)
<i>Bidens pilosa</i>	48	3(0)	3(0)	19(0)	19(0)	14(0)	14(0)	8(0)	8(0)	4(0)	4(0)
<i>Pennisetum purpureum</i>	12	0(0)	0(0)	5(2)	5(0)	6(0)	6(0)	0(0)	0(0)	1(0)	1(0)
<i>Panicum maximum</i>	2	0(0)	0(0)	1(0)	1(0)	1(0)	1(0)	0(0)	0(0)	0(0)	0(0)
<i>Oxalis latifolia</i>	5	3(0)	3(0)	2(0)	2(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
<i>Commelina benghalensis</i>	28	2(0)	2(0)	19(1)	19(0)	1(0)	1(0)	2(0)	2(0)	4(0)	4(0)
<i>Imperata cylindrica</i>	17	3(0)	3(0)	3(0)	3(0)	8(0)	8(0)	1(0)	1(0)	2(0)	2(0)
<i>Cyperus rotundus</i>	4	2(0)	2(0)	2(2)	2(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
<i>Cynodon dactylon</i>	8	3(0)	3(0)	1(0)	1(0)	0(0)	0(0)	3(0)	3(0)	1(0)	1(0)
<i>Galinsonga parviflora</i>	3	0(0)	0(0)	1(0)	1(0)	0(0)	0(0)	0(0)	0(0)	2(0)	2(0)
<i>Euphorbia heterophylla</i>	19	4(0)	4(0)	5(0)	5(0)	4(0)	4(0)	5(0)	5(0)	1(0)	1(0)
<i>Pennisetum clandestinum</i>	17	0(0)	0(0)	13(0)	13(0)	0(0)	0(0)	0(0)	0(0)	4(0)	4(0)
<i>Eleusine indica</i>	33	6(2)	6(0)	10(0)	10(0)	6(0)	6(0)	4(0)	4(0)	7(0)	7(0)
<i>Saccharum officinarum</i>	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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^aTotal number of samples of specific weed species identified and tested: ^bAgroecological 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. ^cNumbers in parenthesis represent the number of ELISA positive samples for MLN virus. MCMV =Maize chlorotic mottle virus, SCMV= Sugarcane mosaic virus.

295 **3.3 Occurrence of MLN causing viruses in cultivated crops found in maize agroecosystems**
296 **during surveys of major agroecological zones in 2014A, 2014B and 2015A**
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298 Of these crops, the following tested positive for MCMV; beans (*Phaseolus vulgaris*) from MLN infected
299 field in Bulambuli district (Eastern Highland Agroecological zone), cassava (*Manihot esculenta*) from
300 MLN infected field in Bulambuli district (Eastern Highland Agroecological zone) and simsim (*Sesamum*
301 *indicum*) from MLN infected field in Tororo district found in Eastern Agroecological zone (Tables 8 and 9).
302 Of these crops, only (*Sorghum bicolor*) from MLN infected field in Tororo district (Eastern Agroecological
303 zone) and sweet potato (*Ipomoea batatas*) from Nabongo subcounty, Bulambuli district (Eastern highland
304 agroecological zone) tested positive for SCMV using DAS ELISA. The ELISA test indicated that, in
305 2014A, none of the cultivated crops tested positive for MCMV. In 2014B, two samples of simsim
306 (*Sesamum indicum*) tested positive for MCMV from Molo Sub County, Tororo district in Eastern
307 Agroecological zone. In 2015A, two samples of beans from Simu subcounty, Bulambuli district in Eastern
308 highland agroecological zone tested positive for MCMV. In addition, two cassava (*Manihot esculenta*)
309 samples collected from the same location in Eastern highland agroecological zone tested positive for
310 MCMV. In 2015, two sorghum (*Sorghum bicolor*) samples collected from Molo subcounty in Tororo district
311 (Eastern highland agroecological zone) and one sample of sweet potato (*Ipomoea batatas*) from
312 Nabongo subcounty, Bulambuli district (Eastern Highland Agroecological Zone) tested positive for SCMV.
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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)

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^aTotal number of samples of specific crop identified and tested: ^bAgroecological 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. ^cNumbers in parenthesis represent the number of ELISA positive samples for MLN virus. MCMV= *Maize chlorotic mottle virus*. SCMV= *Sugarcane mosaic virus*

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

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	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)

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 326 ^aTotal number of samples of specific crop identified and tested: ^bAgroecological zones where; EAZ= Eastern Agroecological zone, EHZ= Eastern
 327 highland Agroecological zone, LAZ= Lake Albert Crescent Zone, SEZ= South Eastern Agroecological zone, WHZ= Western Highland
 328 Agroecological zone. ^cNumbers in parenthesis represent the number of ELISA positive samples for MLN virus. MCMV= *Maize chlorotic mottle*
 329 *virus*. SCMV= *Sugarcane mosaic virus*
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331 **3.4 Molecular detection of MLN causing viruses from collected weeds**

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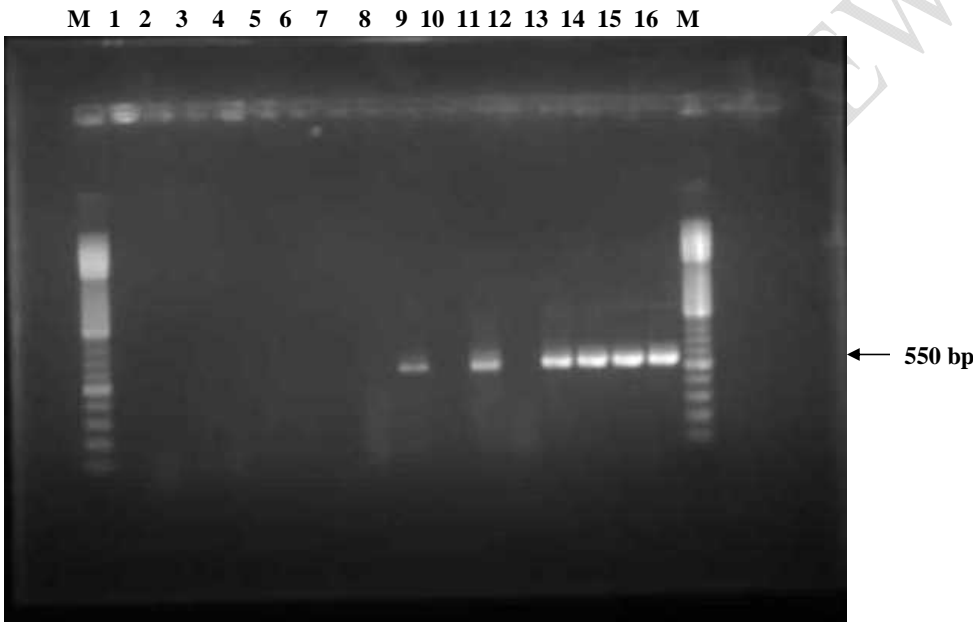


Fig. 3. RT-PCR products of MCMV in weed samples collected from major maize agroecological zones in Uganda. Lane M represents 100bp DNA ladder (Bioneer). Lane 1= *Striga hermonthica*, 2= *Bidens pilosa*, 3= *Oxalis latifolia*, 4= *Galinsonga parviflora*, 5= *Saccharum officinarum*, 6= *Euphorbia spp*, 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).

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344 **4. DISCUSSION**

345
346 This study represents the first survey of potential and known alternative hosts of viruses causing MLN
347 from a large geographic area covering five major maize agroecological zones of Uganda. As regards
348 occurrence of potential weed hosts in major maize growing agroecological zones, this study showed
349 occurrence of a wide range of weeds in the five major maize growing agroecological zones of Uganda.
350 *Digitaria abyssinica*, *Bidens pilosa* and *Commelina benghalensis* were the most commonly occurring
351 weed species in the major maize growing agroecological zones in Uganda. Similarly earlier studies have
352 reported that *Digitaria spp.*, *B. pilosa*, *C. benghalensis*, *I. cylindrica*, and *P. maximum*, were the major
353 weeds in Uganda [21]. The weeds represented eight plant families. Of these families, Poaceae had the
354 highest number of species recorded. Most of the weed species that had previously been identified as
355 hosts of *Maize chlorotic mottle virus* were also in the family Poaceae which is consistent with
356 observations that that this family contains large numbers of plants susceptible to MLN causing viruses
357 [3,7]. The study also identified a number of known alternate hosts of SCMV as reported by [13] notably
358 *Chloris gayana*, *Cynodondactylon*, *Oryza sativa*, *Panicum maximum*, *Saccharum officinarum*, *Sorghum*
359 *bicolor* and *Zea mays* found present in the major maize agroecological zones in Uganda. Most of these
360 weed species are perennial in nature and can hence act as sources of inoculum when the maize crop is
361 harvested. The large number of potential weed-hosts identified suggests the availability of favorable
362 hosts with the ability to harbor the MLN causing viruses and serve as sources of inoculum to its vectors.
363 Perennial weed species can act as continuous endemic source of inoculum of virus and can be
364 transferred to annual weeds where the virus propagates before being spread further to crops that are
365 susceptible [22, 23]. This implies that such weeds should not be overlooked when developing MLN
366 management strategies.

367
368 Results of this study indicated that, weed species from Poaceae family mainly collected from Eastern
369 Highland and Eastern Agroecological Zones, had most prevalent species susceptible to MLN causing
370 viruses estimated as incidence. In the field survey conducted, *Digitaria abyssinica*, *Amaranthus spinosus*,
371 *Cyperus rotundus*, *Pennisetum purpureum* and *Commelina benghalensis* were found to be naturally
372 susceptible to MCMV. These results are in conformity with earlier reports only for *Pennisetum purpureum*
373 which was reported to be a natural host for MCMV [16]. These results were not expected for the
374 Commelinaceae and Amaranthaceae since MCMV is only known to be found in Poaceae family [7]. No
375 plants in the family Commelinaceae and Amaranthaceae have hitherto been documented as hosts of
376 MCMV. Furthermore, this appears to be the first observation of a large number of naturally MCMV-
377 infected species next to maize crops in the field notably from the MLN hotspot districts of Bulambuli and
378 Tororo. However there is no existence of MLN in weeds found in the areas **under nowwithout** disease
379 pressure. These facts suggest that the continuously high incidence of this virus in these MLN hotspot
380 areas may be partially associated with large numbers of MCMV sources in this maize-producing
381 agroecological zones. Up to the recent past when MCMV was identified in sorghum [24], sugarcane [8],
382 finger millet [9], Napier grass [16], Kikuyu grass [16], the only naturally occurring host of MCMV was
383 maize [7]. This is therefore the first report of MCMV in *Digitaria abyssinica*, *Amaranthus spinosus* and
384 *Commelina benghalensis*.

385
386 As regards surveys of potential natural hosts of MLN viruses in cultivated crops, the following tested
387 positive for MCMV; beans (*Phaseolus vulgaris*) and cassava (*Manihot esculenta*), from MLN infected field
388 in Bulambuli district (Eastern Highland Agroecological zone) and Simsim from MLN infected field in
389 Tororo district (Eastern Agroecological zone). Out of the studied crops, only sorghum from MLN infected
390 field in Tororo district (Eastern Agroecological zone) and sweet potato from Nabongo subcounty,
391 Bulambuli district (Eastern highland agroecological zone) tested positive for SCMV using DAS ELISA.
392 The findings agree with earlier reports who also reported that Sorghum is a natural host of SCMV [25].
393 However, plants did not show symptoms related to MCMV which suggests they could be resistant to
394 infection. The results were not expected for these cultivated crops all of which are dicotyledonous plants
395 from non gramineae families. Prior to this, MCMV has only known to be found in Poaceae family [7]
396 Cassava, beans and groundnuts are dicotyledonous plants [26, 27]. These results do not support
397 previous findings that reported that dicotyledonous species were not mechanically infected with MCMV
398 [28, 29]. Specific isolate-host interactions could probably explain the contrasting results observed with
399 some plant species about their host status for MLN causing viruses. However, these results are in

Comment [E8]: Please put a sentence summarizing the results of incidence of MCMV and SCMV (total detected) in weeds/cultivated crops (total of plants)

400 conformity with previous studies that showed that some dicotyledonous plants can be natural and artificial
401 hosts of MCMV [30]. Nonetheless the findings in this study suggest that these cultivated crops may carry
402 the virus based on the virus titers that were comparable to the positive maize control. Prior to this study,
403 no weeds and crop species were found to be naturally affected in the wild probably because they are not
404 favorable hosts for vectors of MCMV. These ELISA based results were not confirmed in most of the weed
405 species using PCR for SCMV but only for MCMV. These findings are in conformity to findings in Kenya
406 that also reported low detection of SCMV using PCR despite positive results using ELISA. [1,16]. This is
407 probably due to the emergence of new strains of SCMV with sequences in capsid protein that are
408 different from the sequences used to design the primers used. Indeed studies have confirmed that SCMV
409 strains in the East African region are highly divergent [1].
410

411 This study could hence provide the first evidence of the potential role of cultivated crops as hosts of MLN
412 causing viruses. There is need for further studies on these cultivated crops to investigate whether they
413 share vectors of MCMV with maize and these vectors can transfer the virus from the maize to these crops
414 and vice versa. No information is available concerning the occurrence of natural sources of MLN causing
415 viruses in cultivated crops including beans (*Phaseolus vulgaris*), cassava (*Manihot esculenta*),
416 groundnuts (*Arachis hypogaea*) bananas (*Musa sp*), soybeans (*Glycine max*) and sweet potato (*Ipomoea*
417 *batatas*) and hence these findings provide the first report of the potential role these cultivated crops could
418 play as reservoirs of MCMV potentially increasing the amount of virus inoculum within the field. The
419 implication of these findings is that crops like sorghum and cassava commonly grown in these areas have
420 some varieties that are late maturing and can hence provide a source of inoculum to the next season
421 crop of maize. Furthermore, beans are commonly grown as intercrops with maize and hence could also
422 potentially provide a source of inoculum of MLN causing virus when grown with maize. In addition to this,
423 sweet potato is a late planted crop in most cropping systems of Uganda and hence could also provide a
424 source of inoculum for the MLN viruses in the subsequent season crop. However, the importance of the
425 cultivated crops as alternate hosts needs further studies to determine if vectors that can survive on maize
426 can also survive on these alternative hosts.
427

428 5. CONCLUSION

429
430 The overall aim of this study was to determine alternative host weeds and crops species occurring in
431 maize and their role in the spread of maize lethal necrosis-causing viruses in Uganda. It was
432 hypothesized that potential alternative hosts of Maize Lethal Necrosis-causing viruses are present in
433 major maize growing agroecological zones of Uganda and act as sources of inoculum to maize fields.
434 The study also established the following natural weed hosts of MCMV, and they included; *Digitaria*
435 *abyssinica*, *Amaranthus spinosus*, *Cyperus rotundus*, *Pennisetum purpureum* and *Commelina*
436 *benghalensis*. The natural hosts for SCMV were only Sorghum and sweet potato. No natural hosts of
437 SCMV were detected among the weeds. Based on these observations, these could be the most likely
438 sources of MLN virus inoculum during the period when maize has been harvested and hence contributing
439 to the spread of the MLN disease. The study has confirmed the existence of potential natural sources of
440 MCMV inoculum in cultivated crops beans (*Phaseolus vulgaris*), cassava (*Manihot esculenta*) and
441 Simsim (*Sesamum indicum*) obtained from MLN hotspot districts of Bulambuli and Tororo. Mechanical
442 inoculation studies corroborated these findings in cassava (*Manihot esculenta*), groundnuts (*Arachis*
443 *hypogaea*) and beans (*Phaseolus vulgaris*). Existence of alternative hosts may explain early infection of
444 maize plants by MCMV and SCMV and the continued occurrence of the MLN disease in the hotspot
445 districts of eastern Uganda. Therefore, this information serves as justification for regular weed
446 management in maize fields, as an Integrated Pest Management (IPM) option for the sustainable control
447 of MLN.
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449 . 450 COMPETING INTERESTS

451
452 Authors have declared that no competing interests exist.
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