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Joyce Eunice1,2*, Douglas Miano2, Muiru W.M2, Eunice Mutitu2 and Isaac Macharia1

Abstract: Maize production in Kenya is now under threat from the devastating maize lethal necrosis (MLN) disease in the field. This study was conducted to assess status of MLN in maize seed production fields from both small- and large-scale producers in Kenya. The survey was conducted in five Agro-ecological Zones (AEZs) in 13 counties in 2015, 2017, 2018, and 2019. Sampling for asymptomatic and non-symptomatic was done using standardized protocol. On-site maize chlorotic mottle virus (MCMV) testing was performed by immunostrips followed by laboratory qRT-PCR test. A total of 2,550 ha was surveyed with 21% having MLN disease and varying levels of severity. The MLN incidence and severity was not significantly different (P > 0.05) in the various Kenyan sampled agro-ecological zones, counties, maize varieties, and maize growth stages. Elevated MLN incidences and severities were observed in sub-humid AEZs comprising Embu, Uasin Gishu, Nakuru, and Elegeyo Marakwet counties that form the hotspots for MLN disease. The main MLN-causing viruses detected using q-RT-PCR were MCMV and sugarcane mosaic virus (SCMV). Out of the total samples analyzed using qRT-PCR, 38% were found to have MCMV, 14% with SCMV, and 18% with both MCMV and SCMV. From the 185 sample analyzed with immunostrip from 2017 to 2019, 29 (16%) were positive for MCMV. Phytosanitary programs should be included in seed legislation for legal adoption and strategies to control the spread of MLN disease should focus on high-risk hotspots agro-ecological zones and counties.

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PUBLIC INTEREST STATEMENT

Maize lethal necrosis is an emerging disease and becoming a threat to maize production and productivity in sub-Saharan Africa. Maize farming is the backbone of food and nutrition security in Kenya. The spread of transboundary pests and diseases has increased significantly in recent years, affecting food and income security of several million resource-poor farmers, especially in sub-Saharan Africa. An intensive multidisciplinary and multi-institutional strategy is being implemented to curb the spread of MLN in sub-Saharan Africa and mitigate the impact of the disease. Monitoring of the disease in maize seed crops can be enhanced by use of Agristrips, which can effectively be adopted in field inspections.
1. Introduction
Maize is a strategic cereal crop in sub-Saharan Africa and a staple crop for more than 70 million people, with its cultivation covering more than 25 million ha in sub-Saharan Africa (Mudde et al., 2018). In Kenya, maize constitutes a significant basis of food security and is cultivated by both large and small-scale farmers. More than 90% of the Kenyan population relies on it for income, human consumption, and raw material for industrial uses (Manje et al., 2015). Among the diseases, maize lethal necrosis (MLN) disease has emerged as the single most important production constraint in maize (Demissie et al., 2016; X. Xie et al., 2016; Qin Yang et al., 2017).

Maize lethal disease is caused by synergistic co-infection of maize chlorotic mottle virus (MCMV) (Machlomovirus, Tombusviridae) and a maize-infecting potyvirus such as maize dwarf mosaic virus (MDMV) or wheat streak mosaic virus (WSMV) or sugarcane mosaic virus (SCMV) (Wangai et al., 2012).

MLN was first reported in the USA and symptoms such as chlorosis, mosaic, and necrosis were seen in infected plants, resulting in either plant-stunted growth or death (Niblett & Claflin, 1978; Uyemoto et al., 1980). In Africa, the disease was reported in Rwanda, the Democratic Republic of Congo in 2014 (Adams et al., 2014; Lukanda et al., 2014), and in the border districts of Uganda (ASARECA, 2013). Currently, MLN disease has spread to all the eastern Africa countries and most of the sub-Saharan countries, with significant effect on maize production (Lukanda et al., 2014; Mahuku et al., 2015a; Manje et al., 2015).

In Kenya, the disease was first reported in 2011 in Bomet County (Wangai et al., 2012), with an estimated loss of maize valued at US$ 67 M in 2012 (Prasanna, 2014). Since then, the disease has spread to other parts of the country, including the Central, Nyanza, Western, and Rift Valley parts of the country (Wangai et al., 2012; Miano, 2014). In Kenya, MLN disease is caused by a synergy between MCMV and SCMV (Adams et al., 2014; Wangai et al., 2012).

Under field conditions, MCMV and SCMV are known to be transmitted from plant to plant by several insect vectors (Jiang et al., 1992; Cabana et al., 2013; Ford et al., 2004) with alternative host plants in maize fields acting as inoculum sources of the MLN-causing viruses (Nelson et al., 2011). MCMV is transmitted by thrip and chrysomelid beetles (Zhao et al., 2014) and while SCMV is transmitted by aphids mainly Myzus persicae and Aphis gossypii (Cabana et al., 2013). Research also indicates that contaminated seed and soil transmit MCMV and SCMV though at very low rate (Jensen et al., 1991a; Zhang et al. 2011b; Mahuku et al., 2015b). Transmission of MCMV through seed was first reported in America by Jensen et al. (1991b) and recently in Africa by Mahuku et al. (2015a). Transmission of SCMV has been reported (Li et al., 2011) but it is not being considered as a major concern (Redinbaugh & Stewart, 2018). MCMV and SCMV either appear singly or in mixed infections (Guadie et al., 2018).

Development and implementation of integrated disease management systems against any disease in a geographical area is guided by precise and accurate information on the disease and factors that influence its development. However, because no quantifiable information is readily available on the distribution, incidence and severity of the MLN disease or MLN-causing viruses in major maize seed production areas in Kenya, it has been difficult to develop an appropriate Integrated Disease Management System for the disease. Phytosanitary procedures need to be strengthened to enhance the delivery of virus-free seeds due to correlation between vectors presence and MLN epidemics (Prasanna et al., 2020). The use of field validated protocols and
techniques like immunostrips can also enhance on monitoring of the disease. This research documents the status of MLN in seed maize crop fields in Kenya.

2. Materials and methods

2.1. Study sites

Surveillance was conducted in 2015, 2017, 2018, and 2019 to evaluate the MLN disease status in key maize seed production zones in Kenya from small- and large-scale seed companies and producers. This study covered 13 Kenyan counties in five AEZs categorized on the basis of nature of vegetation, altitude, and climatic conditions (FAO, 1996). The humid zone covered the following counties: Kakamega, Transnzoia, and Meru with elevation between 1980 and 2700 m and minimum rainfall of 1000 mm. The sub-humid zone covered Embu, Uasin Gishu, Nakuru, and Elgeyo Marakwet counties with elevation between 900 and 1800 m and annual rainfall of between 950 and 1500 mm. The semi-humid zone covered West Pokot and Machakos counties with elevation of 900–1800 m and annual rainfall of 500–1000 mm. The semi-arid covered Makueni and Kajiado with an annual rainfall of 300–600 mm. The arid covered Baringo and Taita Taveta counties with an annual rainfall of 200–400 mm.

2.2. Survey design

Maize seed fields were directly inspected and growers were interviewed to capture the status of the fields, the geographic and temporal distribution of MLN disease in the counties. Survey fields in the seed production areas were purposefully selected after every 10–20 km. In the assessment of MLN incidence and severity, maize variety, agro-ecological, and growth stages were considered during the survey periods. These stages were V1–V9, vegetative stages; VT, Tassling; R1, flowering/silking; R2, blistering; R3, milky; R4, dough; R5, physiologically mature; and R6, harvesting stage. Each seed field evaluation for incidence and severity scoring was collected along a quadrat counting 20 plants and crossing the field in two diagonals forming an X pattern. The counts were defined in each field by the size of the field used to calculate the number of plants within the transect. Disease severity was reported on a rating scale of 1–5, where 1 = no disease symptoms, 2 = fine chlorotic streaks/mottling on lower leaves, 3 = chlorotic mottling and mosaic throughout the whole plant, 4 = excessive chlorotic mottling, mosaic, plant necrosis, and/or dead heart, and 5 = dead plant and complete plant necrosis (Beyene et al., 2017). Disease severity scores were converted into percentage severity index (PSI) for analysis (Wheeler, 1969).

2.3. Detection of MCMV using immuno strip in the field

Surveys were carried out using CIMMYT and partners standardized protocols for MLN in which an average of six leaves were obtained from each field and screened on site using immunostrips (Bioreba) for MCMV. The immunostrip kits were used on homogenized leaf tissue in the field. The advantage of these kits is that they are simple to use (no specialist knowledge required), give a rapid in-field result, stable in a wide range of climates, and relatively inexpensive. Samples were ground in the immuno-strip-loaded buffer sap and left to stand for 5 min. The result is an easy-to-read pattern of bands (single band for negative, double band for positive). Using GPS, all sampled fields were geo-referenced and mapping information for sampling points and associated incidence and severity was created.

2.4. Detection of MLN viruses by qPCR

2.4.1. Isolation of RNA

By using modified cetyl trimethylammonium bromide (CTAB) method, total RNA was extracted from samples of maize leaves (I. P. Adams et al., 2009). Leaves approximately 100 mg were crushed in 1 mL of 0.1 M Tris base (pH 8) CTAB buffer, 2% CTAB w/v, 0.02 M EDTA and 1.4 M NaCl, 2% polyvinylpyrrolidone (PVP), and 1% Na2SO4 added before use. The extract of leaves were loaded into a 1.5 mL sterile Eppendorf tube and incubated for 10 min at 65°C. The extracts were diluted with an equivalent amount of chloroform: isoamyl alcohol (24:1) after incubation and centrifuged at 12,000 g for 10 min.
The intermediate aqueous phase was transferred in sterile Eppendorf tube mixed with an equivalent amount of 4 M LiCl and incubated at –20°C for 1 h. Samples were vortexed and centrifuged for 10 min at 12,000 rpm once again. In a separate sterile microfuge tube, 450 μL supernatant was obtained and 300 μL of cold iso-propanol was transferred to the tube and incubated for 1 h at –20°C. The samples were then centrifuged at 12,000 g for 25 min. The resultant pellets were washed in 70% ethanol, air-dried, and re-suspended in 50 μL of water and stored at –20°C for further analysis. Quantity and quality checks were implemented using a NanoDrop (Thermo Fisher Scientific, Madison, USA) at 260/280 and 260/230 absorbance ratio.

2.4.2. Polymerase chain reaction (qPCR)
The RNA from the positively identified samples by immunostrips were analyzed to confirm the presence of MCMV and SCMV by real-time qPCR as described by Adams et al. (2012). Real-time qPCR was assayed in 1 μL of RNA of 25 μL reaction volume containing 2.5 μL of 10× PCR buffer, 5.5 μL of 25 mM MgCl₂, 2.0 μL of 6.25 Mm of dNTPs, 1.1 μL of 7.5 μM of forward primer and reverse primer, 0.5 μL of Taqman probe, 0.05 μL of MMLV, and 0.125 μL of Taq polymerase enzyme. Thermal requirements for PCR amplification were set as follows: 48°C for 30 min for cDNA synthesis, 95°C for 10 min to deactivate MMLV and activate taq polymerase proceeded by denaturation at 95°C for 15 s and annealing/extension at 60°C for 1 min for 40 cycles. Appropriate positive and negative controls were included in the assay.

The investigation was performed using primer and probe targeting protein coat region, which are MCMV F: 5′—CCGCTTACCCGAGTGGAA—3′ MCMV R: 5′—TGCTCAATAGCTCTGGATT T—3′. The Probe involved were MCMV Pe 5′—[FAM]—CAGCGGACGTAGCTGCAG—[BHQ1]—3′; SCMV F: 5′ CCA GGC CAA CTT GTA ACA AAG C—3′; SCMV R: 5′—CAT CAT GTG TGG ATA AAT ACA GTT GAA—3′ and SCMV pe (FAM)—TGT CGT TAA AGG CCC ATG TCC GCA-BHQ1. Data from the tests were obtained in the form of values of Ct (cycle thresholds), which is typically inversely proportional to the amount of virus in the samples. Ct values less than 29 indicated strong positive reactions showing high amounts of the target MCMV virus concentrations whereas Ct of 30–35 indicated low virus concentration. All samples with Ct values less than 35 were deemed positive and those with value above 35 were classified as being negative for the virus (Zhang et al., 2011).

2.5. Data analysis
General analysis of variance (ANOVA) was used for detection of significance of the effects of type of agro-ecological condition, type of maize variety, growth stage on the observed MLN disease severities, and incidence using GenStat 15th edition statistical software (VSN International, UK). The test statistic took into account the sample sizes, sample means, and sample standard deviations in each of the comparison groups.

3. Results

3.1. MLN disease incidence and severity in Kenyan agro-ecological zones
In the year 2019, where there was no MLN disease occurrence noted in all agro-ecological zones sampled in major seed production fields. The study reported no MLN disease incidence and severity of MLN in semi-arid in 2015 and arid zones in 2018 (Figure 3). The mean MLN incidence across the agro-ecological zones in Kenya was highest in sub-humid that recorded 2.5, followed semi-humid zone with 1.98, semi-arid agro-ecological zone had 0.59, humid with 0.43 and least MLN incidence was documented in arid zone with of 0.23 (Figure 1). Sub-humid agro-ecological zone recorded the highest mean severity index of 1.55, then the semi-humid with 1.525, arid with 1.475, humid with 1.35, and lowest mean severity index of 1.175 was noted in semi-arid agro-ecological zone (Figure 2). The highest MLN incidence of 6.8 was recorded in the year 2015 in the semi-humid agro-ecological zone, and the lowest incidence of zero was recorded in all agro-ecological zone of Kenya (Figure 3). The highest MLN severity index of 2.3 was documented in the year 2015 in semi-humid agro-ecological zone while the lowest severity index of 1 was recorded in all agro-ecological zones of Kenya (Figure 4). There was no significant difference (P > 0.05) on MLN incidence across the
agro-ecological zones of Kenya for the five agro-ecological zones \( F(4, 15) = 1.05, P = 0.416 \). There was no significant difference \( (P > 0.05) \) on severity across the agro-ecological zones of Kenya for the five agro-ecological zones \( F(4, 15) = 0.42, P = 0.789 \).

### 3.2. Incidence and severity of MLN in major maize production counties in Kenya

For the 4 years, a total of 13 counties consistently produced seed during this duration. Embu County had the highest MLN mean incidence of 5.32 while Kajiado County had zero MLN mean incidence (Figure 5). Elegeyo Marakwet had the highest mean MLN severity index of 1.8 followed by Embu County with 1.7. The lowest mean MLN severity index of 1 was documented in Kajiado County (Figure 6). The highest MLN incidence of 21 was documented in the year 2015 in Embu County while the lowest MLN incidence of zero was recorded in all counties of Kenya in the year 2019 (Figure 7). Embu and Pokot counties recorded the highest MLN severity index of 2.6 in the year 2015 followed by Elegeyo Marakwet with 2.5. The least MLN severity of 1 was noted in all the counties in the year 2019 (Figure 8). Distribution of viruses within the counties in the consecutive years showed decline in MLN disease (Figure 17). There was no significant difference \( (P > 0.05) \) on incidence among the counties in Kenya for the 13 counties \( F(12, 39) = 0.71, P = 0.733 \). There was no significant difference on MLN severity \( (P > 0.05) \) among the counties in Kenya for the 13 counties \( F(12, 39) = 0.6, P = 0.828 \).

### 3.3. Disease incidence and severity in maize varieties under maize seed production

A total of eight maize varieties that were consistently produced were sampled during the survey. The highest mean MLN incidence of 10.9 was recorded in DK 8031 maize variety while Duma 43 and DH04 recorded the least mean incidence of zero (Figure 9). The MLN severity of 1.7 was the highest in DK 8031 maize variety while MLN severity index of 1 was recorded in DH04 (Figure 10). In the year 2018, DK 8031 maize variety recorded the highest incidence of 42 followed by WE1101 variety that recorded 20.1 MLN incidence in 2015 (Figure 11). The MLN severity index of 3 was highest for DK 8031 maize variety in the year 2018 followed by severity index of 2.5 for H624 in the year 2015 (Figure 12). There was no significant difference \( (P > 0.05) \) on MLN incidence among maize varieties for the eight varieties sampled \( F(7, 24) = 0.89, P = 0.057 \). There was no significant difference \( (P > 0.05) \) on MLN severity among maize varieties in Kenya for the eight varieties sampled \( F(7, 24) = 0.62, P = 0.732 \).

### 3.4. Disease incidence and severity in different maize growth stages

In the assessment of MLN incidence and severity, different growth stages were considered during the survey periods. These stage were V1–V9, vegetative stages; VT, tasselling; R1, flowering/silking; R2, blistering; R3, milky; R4, dough; R5, physiologically mature; and R6, harvesting stage. Majority of the crops surveyed were at (R4) dough stage (23%) followed by (R3) milk stage (17%). Flowering and physiologically mature stages were also recorded during the surveys covering 14% of the total growth stages. Growth stage (R5) physiologically mature recorded the highest incidence of 2 followed by (R3) with MLN mean incidence of 1.8. The least MLN mean incidence was seen in V3, V6, V7, and V8 maize growth stage that recorded zero MLN mean incidence (Figure 13). Growth stage (R4) dough stage recorded the highest severity 1.8 with the lowest at (V3) vegetative stage (Figure 14). There was no significant difference on MLN incidence \( (P > 0.05) \) among maize growth stages for all the growth stages sampled \( F(13, 42) = 0.71, P = 0.723 \). There was no significant difference \( (P > 0.05) \) on MLN severity among maize growth stages in Kenya for the 14 growth stages sampled \( F(13, 42) = 0.61, P = 0.807 \).

### 3.5. Detection of MLN-causing viruses by real-time PCR assays

In 2015, samples were analyzed using q-PCR in the presence of MCMV and SCMV. A total of 118 samples were collected, of which 38% were found to have MCMV, 14% with SCMV, 18% with both MCMV and SCMV, and 30% did not have any of the viruses (Figure 15). Figure 16 shows Ct value some samples that were positive while others negative using specific primer and labeled primer probes.
4. Discussion

The objective of this study was to establish incidence, severity, and distribution of MLN in major maize-growing agro-ecological zones of Kenya. No significant differences in MLN disease incidences and severity among agro-ecological zones were observed in this study (Figure 17). Considering the relatively elevated incidence and severity of infection observed in maize in the subhumid and semi-humid agro-ecological zones, these zones therefore deserve the highest priority for research and control. The prevalence and persistence of plant viruses in the tropics and subtropics are aided by optimal conditions of tropical relative humidity and temperature that facilitate the perpetuation of both the viruses and their insect vectors (Macauley & Ramadjita, 2015; Sharma & Misra, 2011). Thus, there is a need for an area-wide MLN disease management strategy for areas at higher MLN disease risk, e.g., growers in regions at higher risk should apply strategies that reduce MLN disease build-up in the fields such as controlling the insect vectors; timely weeding; fertilizer application and roguing of infected plants, while those in areas at lesser risk should apply strategies such as quarantine and planting MLN disease free seed to prevent spread of MLN disease to other areas.

The highest incidence of MLN recorded in Embu County could be attributed to the culture of recycling of seed in the area, extensive and continual maize production in the county. The elevated MLN incidence rate reported in Embu could also be due to the two seasons for maize cultivation (long and short rains), which can be attributed to long rains proving the inoculum disease for short rains. The MLN-causing viruses can be perpetuated between cropping cycles by farmers planting maize seeds infected with MLN-causing viruses (Jensen et al., 1991; Jones et al., 2005; Mikel et al., 1984; Shiferaw et al., 2011). Embu has favorable climate for vector propagation. The incidence of MLN disease is strongly correlated with rainfall, warm temperatures, and high relative humidity that favor the disease development (Kusia, 2014; Mude et al., 2018). Due to the close proximity of Embu county to the major maize grown counties, there is need to emphasize on local quarantine to minimize pathogen spread and disease incidence to other unaffected counties in Kenya. The
Figure 2. MLN severity across the agro-ecological zones in Kenya.

Figure 3. MLN incidence across the agro-ecological zones over the years.
Figure 4. MLN severity across the agro-ecological zones over the years.

Figure 5. MLN incidence of sampled maize-growing counties in Kenya.
The elevated disease pressure of MLN in the Nakuru, Elegeyo Marakwet, Pokot, and Transzoi Baringo counties could be attributed to their close proximity to western Kenya where the disease was first reported (Asea, 2013; IPPC, 2014; Kagoda et al., 2016; Mahuku et al., 2015a).
Low incidences in Makueni and Kajiado semi-arid AEZs may be attributed primarily to climate patterns and the cropping system. This region receives intermittent rainfall, and the production of maize is seasonal with fields left for a dry period of 3–5 months. Cropping systems, on the other hand, are known to increase insect vector population and the emergence of MLN disease epidemics in maize. In intercropped versus mono-cropped systems, a significant number of assessment studies have demonstrated disease declines (Boudreau, 2013; Gopal et al., 2010; Ramkat et al., 2008).
In the three consecutive surveys, the report suggests increased incidence of MCMV in West Pokot and Taita Taveta, which could be caused by continuous maize production through irrigation, thus increasing insect vector burden and inoculum build-up in these fields. Elevated incidence in West
Pokot and Taita Taveta also could be a result of drought, poor soil fertility, and poor agricultural practices. This is in conformity with the results of Garcia-Cano et al. (2006), Wu et al. (2013), and Isabirye and Rwomushana (2016), which found that abiotic conditions such as drought, low soil
fertility, and poor agricultural practices increase MLN severity. This also collaborates findings of Guadie et al. (2018b), who reported elevated incidence of disease incidence in key maize-growing regions that are located at low- and mid-altitude ranges. The study documented MCMV, SCMV, and combined infection of MCMV and SCMV in maize seeds. MLN-causing viruses notably MCMV, SCMV, and MDMV have been confirmed to be seed borne, although at very low levels of 0.04% (Jensen et al., 1991; Mikel et al., 1984). In main maize-growing regions in Ethiopia, studies by Guadie et al. (2018b), Fentahun et al. (2017), and Demissie et al. (2016) revealed the high prevalence of both MCMV and SCMV alone or in mixed infections. Kusia (2014) explicitly noted that MCMV and SCMV were found to be hosted in maize, wild grasses, domesticated grasses, and crop cereals either individually or in combination. This also concurs with findings of Wangai et al. (2012), who noted that mixed infections had been previously reported in Kenya.
Figure 16. Real-time image showing positive and negative results.
Figure 17. Distribution of MLN viruses in major seed production counties surveyed in 2015, 2017, 2018, and 2019.
Notably, there were viruses that were detected in some areas that had shown no disease incidence by visual observation. Symptoms caused by MLN viruses can also differ based on the age of the maize plant, the variety implicated, environmental factors, MCMV and SCMV virus strain, and various viruses may induce similar symptoms in the same plant (Kiruwa et al., 2016; Mezzalama, 2015; Wang et al., 2017). The study found that symptoms of the disease were seen in some cases during the study, even though there was no MCMV or SCMV. This result supports the likelihood of other Potyviruses and Potyviruses being involved in the outbreak of MLN disease in Kenya or incorrect symptoms (Wamaitha et al., 2018).

During the survey, at least eight maize varieties were observed to be frequently grown by farmers. Out of the eight varieties observed, only two maize varieties, namely, DH04 and Duma 43, were not affected by MLN-causing viruses based on the absence of symptoms and laboratory confirmation. The absence of incidence and severity of diseases in DUMA 43 and DH 04 could be due to an effective production management approach as most Kenyan varieties are susceptible to MLN disease (Karanja et al., 2019). Previous studies by Zambrano et al. (2014) have also reported the presence in the potyviridae family of some resistance to several viruses. The involvement of passive and active defense mechanisms hinders the replication and dissemination of viruses that affect either the susceptibility or the resistance of the germplasm (Zambrano et al., 2014). The presence of MLN on virtually all commonly grown varieties in Kenya suggests high susceptibility. These findings confirm reports by Manje et al. (2015), which revealed the vulnerability of a large array (nearly 90%) of pre-commercial and commercial maize germplasm in East Africa to the MLN disease. These maize varieties will need to be tested under known inoculum pressure conditions to determine relative levels of resistance/susceptibility.

Serological assays confirmed mainly the presence of MCMV, the key MLN-causing virus driving the spread of the epidemic in Kenya. This is consistent with earlier results by Kagoda et al. (2016), who also confirmed the presence of MCMV in samples collected in Eastern Uganda. A probable hypothesis is that MCMV alone is capable of causing the expression of MLN disease symptoms. This is in agreement with reports by Mahuku et al. (2015b), who reported that MCMV alone is capable of leading to MLN disease development.

The qPCR test done on total RNA extracted from farmers fields confirmed the presence of MLN-causing viruses, notably MCMV, from samples that tested positive were 38% using real-time PCR assays. However, samples that showed a positive reaction to the SCMV were not detected using qPCR probably due to the existence of new strains with differences in the capsid protein sequence from which the primers were designed. Similarly, qPCR failed to detect SCMV from samples from Rwanda using primers designed for the Kenyan isolate of SCMV (Adams et al., 2014).

High disease incidences and severities in 2015 across most of the counties could be attributed to the lack of effective measures for regulation of MLN disease since the disease was still new and intervention measures were still in discussion. During this period, rejection of maize crops was based on 10% threshold on visual inspection and laboratory testing was not mandatory for locally produced seeds. This could have resulted in infected seed lots due to the high threshold, resulting in increased spread of the disease through seed. Seed production companies were still struggling with management options and not much had been validated to be adopted for the control of the disease. Decrease in MLN incidences in the subsequent surveys could be attributed to the efforts put in place by the National Plant Protection Organisation (NPPO) to curb the spread of MLN, which included decreasing the threshold for rejection to 1% during field inspection and zero tolerance of MLN viruses through laboratory testing. There was also mandatory testing of all locally produced seed before processing for the presence of MLN viruses.

5. Conclusion
The purpose of this study was to assess the incidence, severity, and distribution of lethal maize necrosis in Kenya’s major maize-growing agro-ecological zones. The study revealed that MLN
disease is widely spread in the study areas and is a critical impediment to maize production. The MLN incidences and severities were not statistically significant among the agro-ecological zones, counties, maize varieties, and growth stages. Efforts should be made to raise awareness of MLN disease and to enforce phytosanitary measures to control the further spread of the disease among populations and regions that have reported MLN disease. Phytosanitary measures have shown to contribute greatly to the disease decrease, and these measures should be enacted to be included in seed laws for legal adoption. Monitoring of the disease can be enhanced by use of Agristrips, which can effectively be adopted in field inspections to assist in detection, especially of latent infections.

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Data availability statement
The authors confirm that the data supporting the findings of this study are available within the article.

Disclosure statement
The authors declare that they have no conflicting interests.

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