# Status of Maize Lethal Necrosis Disease in Zambia

Chomba, M. D*., Msiska, K. K., Abass M. S., Mudenda, M., Mukuwa, P.S.C.
Zambia Agriculture Research Institute, Plant Quarantine and Phytosanitary Service
P/B, 7, Chilanga, Zambia.
*Corresponding Author: Doreen Malekano Chomba, Email: dchomba71@gmail.com

## Abstract

Maize is a staple food in Zambia and contributes immensely to food security for smallholder farmers. Disease outbreaks such as Maize Lethal Necrosis Disease (MLND) can be a key constraint to maize production. This disease is caused by synergistic co-infection with *Maize Chlorotic Mottle Virus* (MCMV) and any virus from the family Potyviridae, particularly, *Sugarcane Mosaic Virus* (SCMV), *Maize Dwarf Mosaic Virus* (MDMV) or *Wheat Streak Mosaic Virus* (WSMV). In 2011, an outbreak of MLND affecting almost all of the currently grown commercial varieties posed a challenge to maize production in Kenya and it has since been reported in DR Congo, Ethiopia, Kenya, Rwanda, Tanzania, and Uganda causing yield losses of up to 100%. Despite MLND having been reported in some neighboring countries, there is no information on the status of the disease in Zambia. Additionally, there is a lot of grain and seed trade between Zambia and other countries among which MLND has been reported. The aim of this study was to establish: (a) the status of MCMV; (b) agricultural practices used by farmers and (c) insect vectors associated with MLND. A survey was conducted in nine (9) provinces of Zambia during 2014/2015 and 2015/2016 cropping seasons. Farmers' maize fields were sampled at every five to ten-kilometer interval and tested using rapid diagnostic kits capable of detecting MCMV. Four hundred and nineteen samples collected all tested negative for MCMV. Zambian Agricultural Research Institute (ZARI), with all stakeholders in the maize value chain should continue implementing measures aimed at preventing the introduction of MLND in Zambia.

**Key words:** Survey, MLND, Losses, food security

## INTRODUCTION

Maize (*Zea mays* L.) is one of the principal cereal crops in Sub-Saharan Africa (SSA) and is largely produced by smallholder farmers over 35 million hectares with an estimated production quantity of over 70 million metric tons of grain (Boddupalli *et al*., 2020). The crop is critical to food security in SSA; Eastern and Southern Africa uses 85% of the maize produced as food, while Africa as a whole uses 95% as food (Shiferaw *et al*., 2011). Maize is equally a very important food crop in Zambia and according to (Chapoto *et al*., 2010) the average consumption of maize grain in Zambia has been estimated at 133 kg per year making it the most popular food crop. It is cultivated in all the provinces and its production is dominated by small scale farmers who constitute an important and invaluable component of the Zambian economy (Chiona *et al*., 2014). According to the analysis of this study regarding the trend of maize production based on the crop forecasting survey estimates made available by the national Central Statistical Office (CSO) for the period 2011 to 2015, the country produced a cumulative total of 14.37 million MT. Zambia's small to medium holder farmers accounted for 89% of the total production over this period.

In terms of trade, Zambia has been a hub of seed and grain exports to her neighboring countries. A total of 120,000 MT of seed maize was exported within the Southern African Development Community (SADC) region and COMESA member countries (ACTESA, 2015), implying that maize has a significant contribution to the Zambia's Gross Domestic Product (GDP) and thereby to the National economy.

Although maize is widely grown, it is faced with several biotic constraints such as weeds, pathogens and insect pests thereby affecting its productivity (Oerke, 2006). In September 2011, a high incidence of a new maize disease called Maize Lethal Necrosis Disease (MLND) was reported at lower elevations (1,900 m a.s.l) in the Longisa division of Bomet County, Southern Rift Valley in Kenya. Since then, the disease has spread to many countries of East Africa rapidly due to insufficient knowledge on how the disease should be managed (Mahuku *et al*., 2015). MLND is caused by the synergistic co-infection of maize with *Maize Chlorotic*

*Mottle Virus* (MCMV) of the genus *Machlomovirus* and any of the *Potyviruses* such as: *Maize Dwarf Mosaic Virus* (MDMV), *Sugarcane Mosaic Virus* (SCMV), and *Wheat Streak Mosaic Virus* (WSMV) (Achon *et al*., 2017). In Eastern Africa, MLND was found to have resulted from co-infection of maize with MCMV and SCMV, although MCMV alone appeared to cause significant crop losses. SCMV has been known to be distributed widely in Africa since the 1970s (Mahuku *et al*., 2015). Therefore, this makes the detection of MCMV important as it is the only single virus needed together with SCMV to cause MLND (Mahuku *et al*., 2015). The disease is naturally known to affect all varieties of maize resulting in chlorotic mottling of the leaves, severe stunting and necrosis. This subsequently hinders the physiological processes of the plant such as photosynthesis, chlorophyll formation as well as denaturing enzymes necessary for the crop to produce. This further leads to low maize yields or plant death (Wangai *et al*., 2012). MLND is an economically devastating disease in maize growing areas of the world and is currently becoming an emerging threat in Africa and Asia (Achon *et al*., 2017). In Kenya, field losses for all commercial maize varieties were estimated at 30 to 100% depending on the stage of disease onset and severity (Mahuku *et al*., 2015). In 2012, MLND affected 77,000 ha in Kenya, translating into an estimated yield loss of 126 million MT valued at U.S.$52 million (Wangai *et al*., 2012a; Mahuku *et al*., 2015). Further annual losses due to MLN were estimated at 0.5 million MT/year (22%) or $187M in Kenya (De Groote *et al*., 2016). The transmission of MCMV occurs through insect vectors, mechanically, and also via seed at very low rates of about 0.04% (Jensen *et al*., 1991). This poses a challenge to detect this virus to prevent its introduction, infection and transmission (Liu *et al*., 2015). Infected soil has also been shown to transmit the viruses that cause MLN (Mahuku *et al*., 2015). Weeds such as Bermudagrass *(Cynodon dactylon)* Napier grass (*Pennisetum purpureum)* have been known to be hosts of viruses that causes MLND. It is further reported that insects such as thrips, beetles and aphids carry the viruses from one plant to another in the field (Mahuku *et al*., 2015).

Despite MLND being reported in some of Zambia's neighboring countries such as DR Congo and Tanzania, so far, no study has been carried out to establish its status in Zambia. In the light of this, a survey was conducted from 2015 to 2016 in order to investigate and establish if there was any occurrence of MLND. The objective of this study was to determine: (1) the presence or absence of Maize Chlorotic Mottle Virus (MCMV) in Zambia; (2) some of the agro cultural practices conducted by farmers which may predispose them to the attack of MLN in the study area in an event of an outbreak; (3) the presence or absence of insect vectors and weeds known to be hosts of MLND causing viruses.

## MATERIALS AND METHODS

### Study Location

The surveys were conducted from 2014/2015 to 2015/2016 cropping seasons and covered the following provinces: Copperbelt, Muchinga Northern, Lusaka, Luapula, North-Western, Southern, Central and Eastern. Areas along the border between Zambia, Tanzania and DR Congo covering the Copperbelt, Muchinga and Northern Provinces were targeted in 2014/2015. The survey was later extended to include other provinces covering Lusaka, Luapula, North-Western, Southern, Central and Eastern Provinces in the 2015/2016 cropping season (Fig. 1).

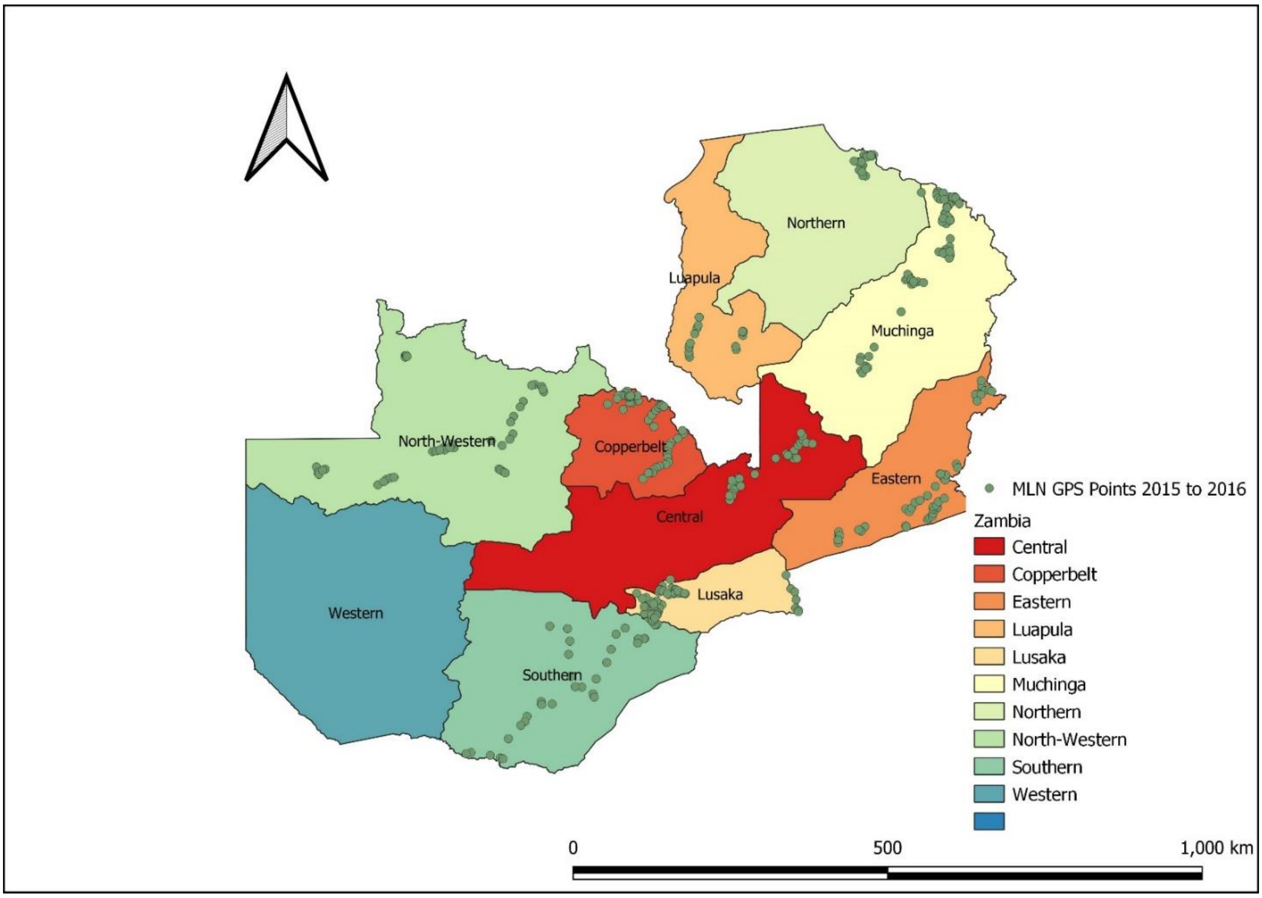


Figure 1: Map of Zambia showing areas surveyed.

## 2.2 Data collection using questionnaires

The questionnaires used in the survey were designed by the International Maize and Wheat Improvement Center (CIMMYT) and adopted to be utilized during the survey. The information collected included: name and sex of farmer, location, type and source of seed, planting date, field size, variety, stage of crop, crop rotation history, frequency of visits from extension agents, pesticides use, type of insects, type of diseases, symptoms and weeds.

The global positioning system (GPS) latitude, longitude and elevation points were also recorded to help with the production of maps.

The agro cultural practices were collected by means of questionnaires. Farmers were asked to whether they practiced crop rotation, planted local varieties; and whether they used recycled seed among other questions. The farmer's responses were recorded on questionnaires. The information on agro cultural practices was collected because, in countries were MLN has been reported, research has shown that crop rotation, use of certified seed are among interventions being used for disease management. Therefore, such information would be used by policy makers and extension staff to intensify the awareness on good agricultural practices that help to prevent the disease. Similarly, insects and weeds such as thrips, aphids and beetles among others have been reported to be vectors that aid the transmission of MLN causing viruses. Consequently, farmers would be advised on the control methods for such pests.

### 2.3 Field leaf sampling

Maize fields were inspected and sampled for detection of MCMV between January and March of each cropping season. The plants sampled were of varying growth stages ranging from flowering to the dough stages. Samples were picked at an interval of every five to ten (5-10) km distances between maize fields depending on the availability of maize fields in a particular area.

### 2.4 Sample collection

The survey team followed the X pattern (Fig. 2) to sample the field crops in order to maximize coverage and to have a thorough examination of the field (Muliokela, 1995). A total of six plants were randomly selected and inspected for the identification of MLN virus symptoms along each path within the X pattern (Suresh and Mezzalama, 2016).

A total of six flag leaf samples per field were cut out using scissors previously disinfected with bleach targeting both the symptomatic and non-symptomatic plants for general virus related symptoms. Depending on the size of the field the sampling was done as follows: for field of 5 ha one X pattern, 5 to 20 ha two X pattern and over 20 ha six or more. According to the CIMMYT MCMV detection protocol, it is recommended to test using flag leaves because these leaves are more succulent than the rest of the leaves, and more importantly the immunostrips are sensitive enough to detect the virus during the surveillance. Tissue paper towels were used to hold the leaves when cutting in order to avoid contamination during sampling. Each of the six individual wrapped leaves were placed in individual paper bags with uniquely identified barcodes placed on each sample. In order to avoid contamination, the scissors were disinfected in between fields.

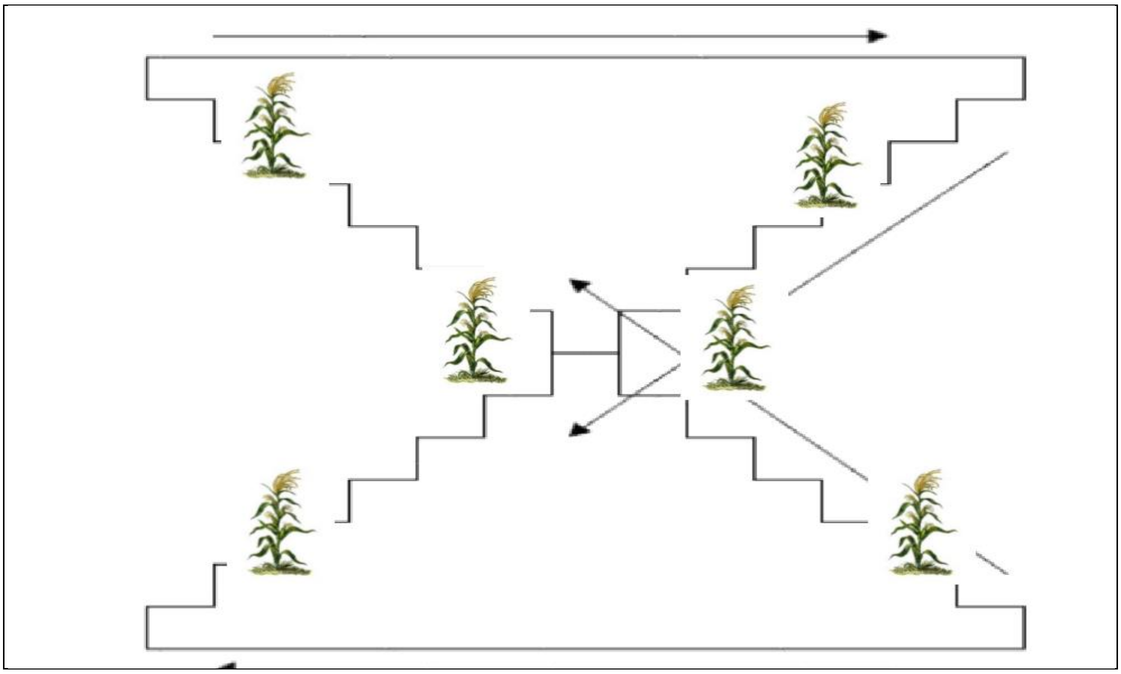

Figure 2: Schematic diagram on how sampling of plants was done.

**Diagnostic procedure: Testing the leaves using the Immunostrips**

With clean gloves, coin size leaf pieces were cut off from each of the six leaf samples collected and placed in a small size zip lock bags containing 4 mls of extraction buffer before crashing the leaves. One drop of extracted plant sap using a pipette was collected and placed in another small clean vial with three drops of extraction buffer added and mixed thoroughly. Thereafter, MCMV immunostrips was inserted into a test bag on its end marked sample and left for 10 to 15 minutes before reading the results.

**Vector Pest Survey**

General vector pest surveillance was conducted in order to check for presence or absence of all vectors associated with MLND such as aphids, thrips and stem borers without determining the numbers occurring per host. Pests found were noted and samples submitted to the entomology laboratory for identification.

**Surveillance of Maize Aphid**

The upper leaves were examined from each plant and a total of 50 plants were sampled per field. Both winged and wingless aphids were collected and preserved in 70% ethyl alcohol and later submitted to the Entomology Laboratory for identification.

**Surveillance of Maize Thrips**

Observations for the presence of thrips was done along the path of the X pattern on all the internal plant parts, as well as in sheaths, under cob husks, on silk, between kernels, and in tassels, including individual spikelet. Plants were cut down, packed into sealed plastic bags and transported to the laboratory, where they were inspected under a microscope. All the collected thrips were preserved in 70% alcohol and later submitted to the Entomology Laboratory for identification.

**Surveillance of Maize Stem Borer**

The field was scanned for the presence of the stem borers. All the infested plants were dissected and the larvae found where collected. The collected larvae were preserved in 70% alcohol and later submitted to the Entomology Laboratory for identification.

**Surveillance of weeds**

The field was checked for the presence of weeds occurring using the weed identification book. Weed samples were collected and matched in line with the descriptors outlined in the weed pocket book and identified accordingly. This was done in order to check for the presence of weeds reported as hosts for MLN causing viruses

**Results**

Table 1 below indicates results for a total number of 419 samples obtained from the surveyed provinces of Zambia. Sampling sites per province ranged from 31 to 76. Muchinga Province had the highest number of samples with 76 while southern province had the lowest. The provinces such as Lusaka, Muchinga and Copperbelt had higher samples tested because they relatively have high interactions with countries outside Zambia. Muchinga and Copperbelt share borders with Tanzania and the Democratic Republic Congo, respectively while Lusaka is the hub for all grain and seed import and export activities. All samples collected and tested for MCMV were negative.

Table 1: Table of results for rapid diagnostic tests during surveillance for 2014 to 2016.

| SN | Province | No of fields surveyed | Bulk MCMV | AgriStrip (+/-) | Result |
|---|---|---|---|---|---|
| 1 | Eastern | 51 | Negative | | |
| 2 | Central | 33 | Negative | | |
| 3 | Southern | 31 | Negative | | |
| 4 | Lusaka | 73 | Negative | | |
| 5 | Muchinga | 76 | Negative | | |
| 6 | Copperbelt | 69 | Negative | | |
| 8 | North -western | 41 | Negative | | |
| 9 | Northern & Luapula | 45 | Negative | | |
| **Total** | | **419** | | | |

All samples showed only one red line, which implied no presence of MLND in all the samples tested.

**Information obtained from the questionnaires.**

Results obtained from the questionnaires regarding farmers not implementing the agro-cultural practices are shown in figure 3 below. Two hundred and twelve farmers were planting local varieties while 164 farmers were not practicing crop rotation with 85 using recycled seed.

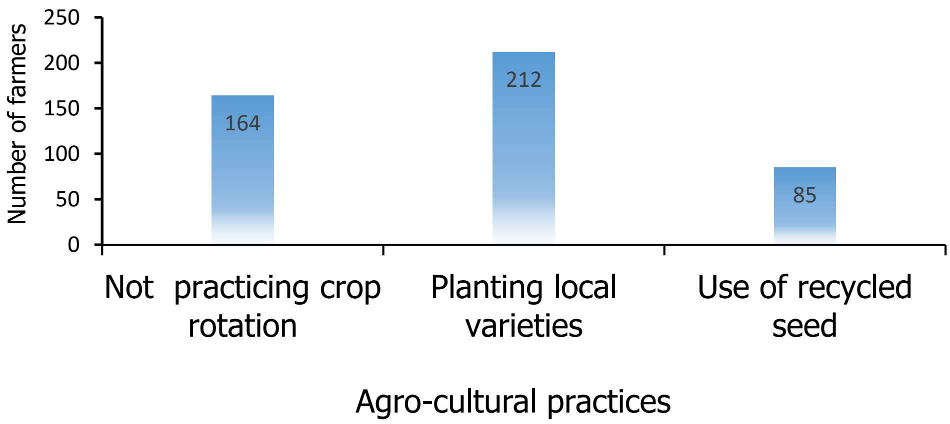


Figure 3: Number of farmers not implementing some agro cultural practices

## Observed pests in farmer's fields

The insect pests that are known to be associated with MLND were observed in the farmer's maize fields. Those observed were as follows: Aphids, thrips, stalk borers and beetles, and the weeds that may act as reservoir for the virus: Bermudagrass *(Cynodon dactylon)* and Napier grass (*Pennisetum purpureum).* In several cases maize plants in the fields had more than one type insect occurring as shown in Figure 4. The most common insect type present on plants were stalk borers followed by the combination of stalk borers and aphids. However, many farmers' fields (200 out of the 419) surveyed fields had no insects observed on plants. Generally, aphids and beetles occurred on plants which were from vegetative to tasseling stages while stalk borers and thrips were mostly observed on physiologically matured ears and stems.

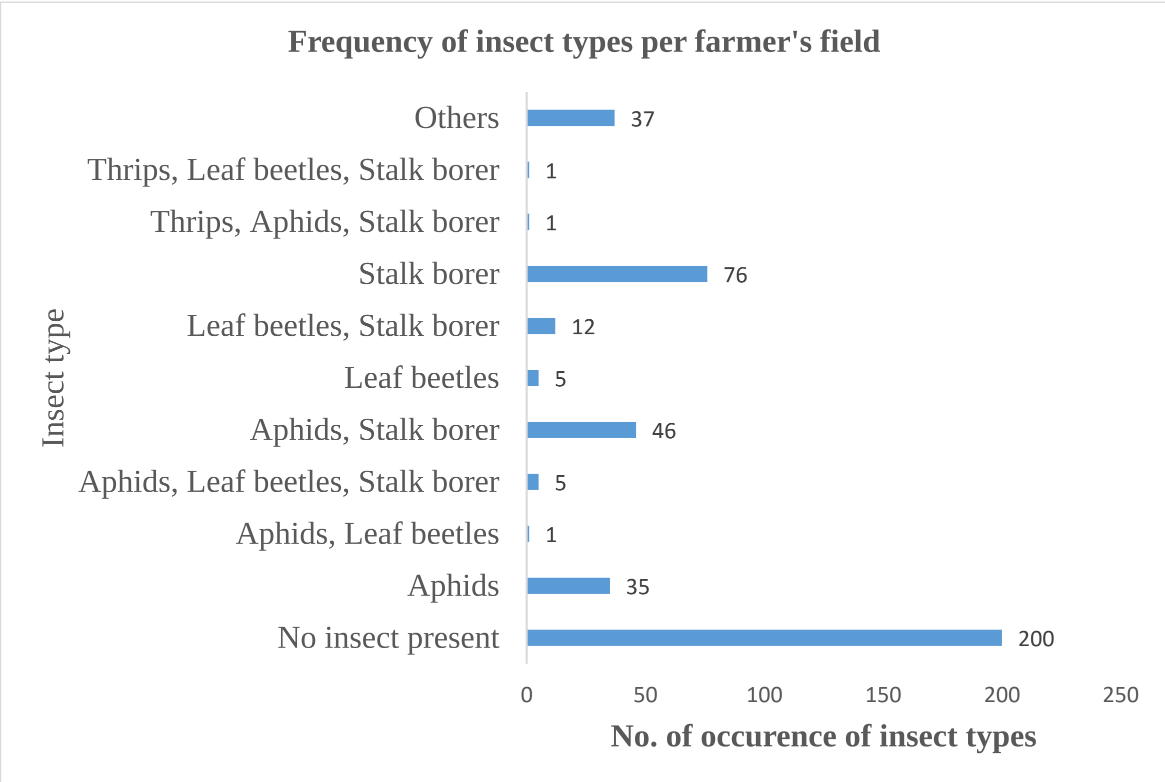


Figure 4: Frequency of insect types on maize plants in farmers' fields

**Discussion**

The survey findings revealed that the *Maize Chlorotic Mottle Virus* (MCMV) was absent. This implied that all the surveyed areas in Zambia were free from Maize Lethal Necrosis (MLN). It is important for Zambia to maintain this status if it is to continue as the hub of seed and grain exports to the neighboring countries. According to data on trade by ACTESA (2015) and CSO/MAL (2015) Crop Forecast Survey respectively, Zambia has been leading in the export of seed and it has been performing well with regards to seed and grain production.

Since the first report in Kenya, in 2011, the MLN has spread tremendously fast to other parts of Africa as well. The detection of MCMV and MLN in DRC and Tanzania the closest neighbors and trading partners for Zambia is of Concern. According to Lukanda (2014) MLND was detected in Kivu province in DRC in 2013 and the following year (2014), it was detected on maize affecting both the local and hybrid varieties in two provinces of Tanzania (Bini and Lubero) in and also in the northern part in Arusha. The fact that in the same period of 2014, MLN was detected in two countries confirms its fast geographical spread. This confirms the expressed concern in the report made by Isabirye and Rwomushana (2016) that MLN has the potential to spread and devastate maize production in Africa at a very fast rate. The predictive model on MLN spread shows that countries with the semi-arid and sub humid tropical type of climate in Central and Eastern Africa such as Ethiopia, Tanzania, and DRC were at risk of losing 662, 924 $Km^2$, 625, 690 $km^2$ and 615,940 $Km^2$ potential land of maize production respectively. From this information, Tanzania and DRC being Zambia's immediate neighboring countries implies, that the risk of MLN introduction to Zambia is very high. This state of affairs demands for strengthening surveillance and putting in place other measures like creating awareness to prevent further spread (Lukanda, 2014).

The practice of growing maize on a yearly basis predisposes farmers to incidences of crop diseases including

MLN. Planting the same crop on the same piece of land encourages the buildup of diseases and insect pests. Crop rotation, soil tillage, fertilization, liming and irrigation are among the agronomic practices that play an important role in preventing or reducing the risk of diseases (Heitefuss, 1989). Additionally, Mahuku *et al*. (2015) claims that some research conducted in USA and Kenya showed that interventions such as the insect vector control, crop rotation, and crop diversification are among the agronomic practices that play an important role in preventing or reducing the risk of MLND. Further, in Kenya, effective monitoring, rigorous implementation of maize-free periods and rotation with non-cereal crops have helped in minimizing MLND incidence.

Furthermore, despite the availability of many certified varieties on the market, the study revealed that 50% of farmers still planted local varieties and a further 20% used recycled seed as presented in Table 2. This type of seed in most cases results in poor yields and is highly susceptible to diseases. Mahuku *et al*. (2015) suggests that the use of resistant hybrids and cultivars, in combination with improved agronomic practices is likely to be the best solution in the long run.

Weeds such as Bermudagrass *(Cynodon dactylon)* Napier grass (*Pennisetum purpureum)* were detected in the surveyed fields as outlined in section 3.2. Mahuku *et al*. (2015) claims that these weeds are among the hosts of MLN causing viruses. Bockelman (1982) recommends that uncontrolled weeds that serve as hosts to the viruses causing MLN could act as reservoirs for the virus infection to the crops. For this reason, farmers should keep their fields weed free.

Similarly, some pests known to be vectors for MLN causing viruses such as stalkborers, aphids, thrips, and beetles were observed in the field during the survey. These pests are of concern even though seed transmission for both MCMV as well as SCMV as reported by (Wangai *et al*., 2012) is known to take place at very low rates. However, the presence of these vectors if not controlled can spread the disease very fast resulting in epidemics (Mahuku *et al*., 2015).

## Conclusion

Findings from this study clearly indicate the absence of MLND in all the 419 fields of the provinces surveyed, which suggests that MLND is not present in Zambia. The revelation of the study that some of the farmers were inclined to certain agro-cultural practices that could encourage the spread and buildup of diseases in the fields needs redress as it might increase the risk for introduction of MLND.

## Recommendation

There is need to strengthen extension services to enable farmers adopt good agronomic practices that help to prevent the spread of MLND such as crop rotation, crop diversification and controlling weeds and insects. The Government needs to continue to be proactive in conducting awareness and training on MLND. Further, the NPPO needs to put in more stringent phytosanitary measures to prevent MLND introduction and spread into Zambia by: continuing with conducting detection surveys; revision of maize phytosanitary import conditions; development and review of Standard Operating Procedures (SOPs); stakeholder consultations and sensitizations on strategies to prevent MLN and development of emergency response plan.

## Acknowledgements

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