# Maize lethal necrosis disease: Investigating Risks and Pre-emptive Management in West Africa

**By**

Dr. M. T. Salaudeen
Department of Crop Production,
Federal University of Technology (FUT), Minna, Niger State, Nigeria
mtsalaudeen.fut@gmail.com

Dr. P. Lava Kumar
Virology and Molecular Diagnostics Unit,
International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria
L.kumar@cgiar.org

Dr. A. Menkir, Maize Breeding Unit,
International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria
a.menkir@cgiar.org

## A Technical Report Submitted to the International Maize and Wheat Improvement Center (CIMMYT), Mexico

March, 2015

## IMPORTANCE OF MAIZE IN NIGERIA AND JUSTIFICATION

Maize (*Zea mays* L.) is one of the major cereal crops in different agro-ecological zones of Nigeria (Iken and Amusa, 2004). Maize is consumed by humans and also forms an important feed element for livestock feeds. In addition, it has a lot of applications in the agro-based industries. It is an integral component of the traditional cropping systems in the country. Nigeria with an annual production of close to 8 million metric tonnes in 2013 is the largest producer in Africa. Maize is the third most widely grown crop in Nigeria, following sorghum and millet. Maize is highly productive, cheap, less rigorous to produce and adapts to wide range of agro ecological zones (Babatunde *et al*., 2008). Besides, the popularity of maize is not limited to the value of its output but also based on the number of farmers that produced it, as well as for its economic value (Olaniyi and Adewale, 2012). An estimated 5.2 million hectares were harvested in 2013 with an average yield of 2 mt ha/ha. In Nigeria, the increase in maize production from 1965 to date has been largely attributed to increase in land area cultivated (Table 1).

An understanding of the spread and associated yield reduction induced by spread of MLND would contribute in a way for effective control. This will ultimately result in high productivity and food insecurity would be minimal. Because of the possibility of identifying MLND-resistant or tolerant maize varieties that are locally adapted and high-yielding insecticidal control of the insect vectors would be reduced. This would reduce chemical health hazards to farmers, residual effects on the crops and consumers, environmental contamination and destruction of beneficial insects.

### Maize Production Agro-ecologies and Planting Seasons in Nigeria

The greatest quantity of green maize comes from the rainforest agro-ecological zone of Nigeria while the savanna zone in northern Nigeria which consists of Derived Savanna, Guinea Savanna and Sahel agro-ecological zones account for substantial quantity of the pod (Ogunlade *et al*., 2010). Although large proportion of the green maize is still produced in the south-Western part of the country, there has been a conspicuous shift of dry grain production to the savanna, especially the Northern Guinea savanna. This agro-ecological zone is now regarded as the maize belt of Nigeria. In the zone, farmers prefer maize to sorghum owing to several reasons including availability of streak resistant varieties for all ecological zones in Nigeria, availability of high-yielding hybrid varieties, increase in maize demand coupled with the federal Government imposed ban on importation of rice, maize and wheat. Local production had to be geared up to meet the demand for direct human consumption and industries (Iken and Amusa, 2004). Some of the major maize-producing States in the savanna zone of Nigeria include Benue, Kogi, Kwara, Niger, Kaduna, Kano, Katsina and Plateau.

Planting dates and the number of times the crop can be grown within the cropping season vary according to agro-ecological zone (Table 2) (Iken and Amusa, 2004). The major organizations involved in maize improvement and protection in Nigeria include Institute of Agricultural Research and Training (IAR&T), Osun State; Institute for Agricultural Research (IAR), Zaria, Kaduna State; National Agricultural Extension Research and Liaison Services (NAERLS) of Ahmadu Bello University, Zaria, Kaduna State; National Agricultural Seed Council (NASC), Abuja; and the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State.

**Table 1. Maize production statistics for Nigeria from 1965 – 2013**

| Year | Area harvested (ha) | Yield (kg/ha) | Production (tonnes) |
|---|---|---|---|
| 1965 | 1404000 | 8262 | 1160000 |
| 1966 | 1145000 | 8515 | 975000 |
| 1967 | 1163000 | 8169 | 950000 |
| 1968 | 1095000 | 10283 | 1126000 |
| 1969 | 1371000 | 9154 | 1255000 |
| 1970 | 1449000 | 9959 | 1443000 |
| 1971 | 1218000 | 10460 | 1274000 |
| 1972 | 1115000 | 5731 | 639000 |
| 1973 | 1246000 | 6485 | 808000 |
| 1974 | 579000 | 9119 | 528000 |
| 1975 | 971000 | 13718 | 1332000 |
| 1976 | 892000 | 11973 | 1068000 |
| 1977 | 610000 | 10656 | 650000 |
| 1978 | 519000 | 12678 | 658000 |
| 1979 | 425000 | 11482 | 488000 |
| 1980 | 465000 | 13161 | 612000 |
| 1981 | 438000 | 16438 | 720000 |
| 1982 | 556000 | 13777 | 766000 |
| 1983 | 1058000 | 9707 | 1027000 |
| 1984 | 1050000 | 11390 | 1196000 |
| 1985 | 1556000 | 11735 | 1826000 |
| 1986 | 2800000 | 12679 | 3550000 |
| 1987 | 3408000 | 13533 | 4612000 |
| 1988 | 3212000 | 16401 | 5268000 |
| 1989 | 3590000 | 13950 | 5008000 |
| 1990 | 5104000 | 11301 | 5768000 |
| 1991 | 5142000 | 11299 | 5810000 |
| 1992 | 5223000 | 11181 | 5840000 |
| 1993 | 5309000 | 11848 | 6290000 |
| 1994 | 5426000 | 12720 | 6902000 |
| 1995 | 5472000 | 12666 | 6931000 |
| 1996 | 4273400 | 13261 | 5667000 |
| 1997 | 4200000 | 12510 | 5254000 |
| 1998 | 3884000 | 13200 | 5127000 |
| 1999 | 3423000 | 15998 | 5476000 |
| 2000 | 3159000 | 13001 | 4107000 |
| 2001 | 3283000 | 13999 | 4596000 |
| 2002 | 3282000 | 14899 | 4890000 |
| 2003 | 3469000 | 14999 | 5203000 |
| 2004 | 3479000 | 16002 | 5567000 |
| 2005 | 3589000 | 16598 | 5957000 |
| 2006 | 3905000 | 18182 | 7100000 |
| 2007 | 3944000 | 17049 | 6724000 |
| 2008 | 3845000 | 19571 | 7525000 |
| 2009 | 3350560 | 21961 | 7358260 |
| 2010 | 4149310 | 18502 | 7676850 |
| 2011 | 6008470 | 15279 | 9180270 |
| 2012 | 5200000 | 18096 | 9410000 |
| 2013 | 5200000 | 20000 | 10400000 |

Source: FAO (2013)

**Table 2. Maize seasons in different agro-ecological zones of Nigeria**

| S/No. | Agro-ecological zone | State (s) | Season 1 | Season 2 |
|---|---|---|---|---|
| 1 | Forest | Akwa-Ibom, Cross River, Lagos | Mid April to 2nd week in May | July to August |
| 2 | Forest - Savanna transition | Oyo, Edo | 3rd week in April to 3rd week in May | July to August |
| 3 | Southern Guinea Savanna | Kwara, Niger | Las week in April to 3rd week in May | July to August |
| 4 | Northern Guinea Savanna | Kaduna, Niger | Last week in May to 1st week in June | |
| 5. | Sudan Savanna | Kano, Katsina | First two weeks in June | |

## Major Maize Seed Companies in Nigeria

The major maize seed companies in Nigeria include Premier Seed Ltd in Zaria, Kaduna State. This is the largest seed company in the country, founded in 1989 as Parental Line Seed Limited, which merged with the Pioneer Hi-bred Seed Company of the USA in 1990. Premier produces its own inbred lines for hybrid maize. It also produces seed of open pollinated variety (OPV) maize. Others include Agricultural Seed Nigeria Limited (AgSeed), founded in 1984 by the Leventis Foundation; and Romarey Seeds Venture Nigeria Limited located in Jos, Plateau State

## Maize Production Constraints

Despite the annual increase in area under production, yield per hectare for maize in Nigeria is as low as 2.0 mt $ha^{-1}$ far lower than world average which is 5.1 mt ha/ha. This could be attributed to biotic and abiotic factors. Abiotic factor includes poor soil fertility, inadequate physical infrastructure, and poor resources (Ibrahim *et al.*, 2014). The major biotic constraints to maize production includes insect pests and disease attack (Table 3)

Table 3. Major insect pests and diseases of maize in Nigeria

| S/No. | Insect pests | Reference (s) |
|---|---|---|
| 1 | Maize borers (*Busseola fusca* Fuller) | Daramola (1991) |
| 2 | Armyworm (*Spodoptera exempta* Walker) | Ditto |
| 3 | Silkworm (Bombyx mori L.) | Ditto |
| 4 | Grasshopper (*Zonocerus variegatus* L.) | Ditto |
| 5 | Termites (*Odontotermes smeathmani* Fuller) | Ditto |
| 6 | Weevil (*Sitophilus zeamais* Motsch) | Ditto |
| | **Diseases** | |
| 1 | Maize rust | Fajemisin et al. (1976); Oladipo et al. (1993) |
| 2 | Leaf blight | Ditto |
| 3 | Curvularia leaf spot | Ditto |
| 4 | Downy mildew | Ditto |
| 5 | Stalk & ear rots | Ditto |
| 6 | *Maize streak virus* | Ditto |
| 7 | *Maize mottle/ Chlorotic stunt* | Ditto |

**Maize Lethal Necrosis outbreak in Africa**

Maize lethal necrosis disease (MLND), first reported in 2011 in Kenya, is a new viral disease in Africa that devastated maize production reducing yields by 30 to 100 % in the affected farms (Wangi *et al*., 2012). This disease has since spread to Uganda, Tanzania, Rwanda and South Sudan. Great risk exists for further spread within Africa.

MLND is a synergistic disease caused by co-infection of *Maize chlorotic mottle virus* (MCMoV, genus *Machlomovirus*) and *Sugarcane mosaic virus* (SCMV, genus *Potyvirus*) (Jiang *et al*., 1992). However, other potyviruses, such as *Maize dwarf mosaic virus* and *Wheat streak mosaic virus*, can also interact with MCMoV in causing MLND. Single infection of any of these viruses does not lead to lethal necrosis. MCMoV is transmitted by thrips and beetles, whereas potyviruses are transmitted by aphids. Low rate (1:2500) of MCMoV transmission through seed has also been reported (Jensen *et al*., 1991). Although, this is yet to be proven for African MCMoV isolates, but seed-transmission represents a means for long distance virus spread in the continent.

Sustainable MLND control requires a thorough understanding of its epidemiology, potential vectors, virus strains and identification of resistant sources. In Eastern Africa studies are already on-going to understand and curb the MLND menace but such attempt has not been initiated in West and Central Africa. Spread of MCMoV can trigger onset of MLND epidemics in any region due to the ubiquitous occurrence of complimenting potyviruses and insect vectors.

Nigeria has been selected because it is a major maize–producing country in Africa. Selected states represent diverse agro-ecologies and farming systems, and represent a typical cross section of maize cultivating zones for assessing virus and vector diversity. Additionally, Nigeria as a principal maize-growing country in Africa (IITA, 2013) is

surrounded by Chad and Cameroon which serve as a link between West and Eastern Africa countries (where MLND has been confirmed). Because the disease is transmitted by insect vectors, trans-border spread of MLND is not impossible. Therefore, a survey of some Nigerian states sharing border with the aforementioned countries would provide information on the incidence and severity of the disease. This information also could serve as a basis for further investigation of the prevalence of MLND in other West African countries.

**Specific objectives**

This project was conducted to:

1. Determine the incidence and diversity of potyviruses (e.g. SCMV) with potential to interact synergistically with MCMoV in causing MLND.
2. Identify occurrence and diversity of potential vectors of MLND prevalent in Nigeria.
3. Establish a phenotyping system for evaluating maize germplasm for SCMV resistance.
4. Establish capacity to diagnose MLND and its causal agents in Nigeria.
5. Create awareness about MLND.
6. Develop linkages with MLND control programs in East Africa for information sharing and augmenting control efforts

The investigation was expected to provide vital information on the risks and development of pre-emptive control measures. West and Central African breeding program of IITA, and several commercial seed companies are based in Nigeria that have active international seed exchange programs. Spread of MLND can prove catastrophic in Nigeria and in West African sub-region. Outputs of this project would contribute to preparedness and prevention of MLND pandemic extendable to other countries in West and Central Africa.

## MATERIALS AND METHODS

A survey protocol was developed and used for field assessment for virus diseases. Surveys were conducted in the following states during the early season: Akwa-Ibom and Cross Rivers (25$^{th}$ April – 7$^{th}$ May, 2014); Kaduna, Kano and Niger (6 – 15 August, 2014). A follow up survey was conducted in the following states during the late season: Niger (26$^{th}$ September – 1 October, 2014); Ogun, Osun and Oyo (3$^{rd}$ – 16$^{th}$ October, 2014). In addition, surveys were conducted in Kwara, Niger and Ogun States from 6$^{th}$ – 19$^{th}$ November, 2014. Fields were surveyed when plants were at the mid reproductive phase (*ca.* 10 weeks after sowing). Information on each field was captured using the Data Collection Sheet. This basically includes the date and time of visit, field size, cropping system, crops in neighbouring fields, etc. The geographical coordinates/positioning of each field was obtained using a Global Positioning System (GPS), for virus distribution map.

In each site, between twenty and thirty plants were examined for general disease symptoms along intersecting field diagonals. Symptom types and severity (on a scale of 1 to 5, where 1 means mild infection and 5 represents severe disease condition) were recorded. In addition, photographs were taken of plants with varied morphological dispositions, including healthy ones. For each field, disease incidence was determined as

the percentage of plants with at least a severity score of 2. Disease severity range and mean were also computed. Leaf samples collected were preserved over silica gels in vial bottles until analyzed. Leaf samples were tested for for MCMV, SCMV and potyviruses using Enzyme-Linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR). Altogether, 1438 samples were assayed. The data obtained have been used to generate MSV distribution map.

**Enzyme-Linked Immunosorbent Assay (ELISA) Protocols**

Samples were subjected to serological test using the standard laboratory Antigen coated plate (ACP) Enzyme Linked immunosorbent Assay procedure. One hundred µL of individual saps were loaded into microtitre plates and was incubated for 1 hr at $37^0$ C. Plates were washed using PBS-T thrice and tap dried. One gram of healthy maize leaf was ground in 20 mL conjugate buffer in order to get rid of all host protein i.e. 1:20 (w/v) and was incubated for 30 min at $37^0$ C just after the addition of MSV and MCMV antibodies at 1:10,000 dilution each, which was later added to the plate and was re-incubated. Plates were washed and tap dried 1 hr after incubation. 1:15,000 dilution of goat anti rabbit enzyme was subsequently added to individual plates and re-incubated. Finally, 1:10,000 dilution of substrate buffer was prepared and dispensed and was kept in a dark chamber for colour reaction. Readings were taken both at 1 hr and overnight and data were analyzed by multiplying the healthy value by two and comparing with surveyed samples.

**Procedure for the extraction of nucleic acid and virus indexing**

Five leaf tissues were selected per field for the extraction of total nucleic acid using CTAB protocol. Sample selection was based on severity scores, giving highly infectious plants priority. One hundred milligram each of the collected leaf sample was ground thoroughly using sterile mortal in 10 volumes (1 ml) of CTAB buffer (2% CTAB (w/v), 1.4M NaCl, 0.2% 2-mercaptoethanol (v/v), 20mM EDTA, 100 mM Tris–HCl, pH 8.0). 750µL of each sample was transferred to a 1.5 ml eppendorf tube, mixed and incubated in a water bath at 60 $^0$C for 10min. The extract was mixed with an equal volume (750µLl) of phenol: chloroform: isoamyl alcohol (25:24:1), mixed thoroughly and centrifuged at >12,000×g for 10 min. The supernatant was transferred to a new 1.5 mL eppendorf tube and nucleic acid was precipitated by adding 0.6 volumes (300µl) of ice cold ($-20^0$C) isopropanol.

Samples were then incubated at $-20^0$C for 1 hr and centrifuged finally at 12,000×g for 10 min at $4^0$ C. The pellet was washed in 0.5 ml 70% ethanol, centrifuged for 5 min and air dried. The pellet was finally dissolved in 50 µl of sterile distilled water and stored at $-20^0$ C. The detergent CTAB was also added in order to aid the release of DNA into the extraction buffer and to protect the DNA from endogenous nucleases. The additive EDTA (ethylene diamine tetra acetic acid) which served as chelating agent was also added as acofactor to bind magnesium ions. The phenol-chloroform was used to denature and separate the proteins from the DNA in the buffer- tissue mixture. A pH of 8.0 was maintained since at lower pH DNA is selectively retained in the organic phase leaving the RNA in the aqueous phase. Suspended DNA samples were then diluted at 1:50v/v (stock DNA solution: sterile distilled water) and were used to set up PCR using the following set of reagents for the preparation of cocktail.

Ten pmol each of the primers, 5× GoTaq green reaction buffer (10mM Tris-HCl (pH 8.8), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% Triton-X 100), 10 mM each dNTPs, 25mM $MgCl_2$, and 0.3 units Taq DNA polymerase. PCR was carried out in a 96-well Applied Biosystem Veritti Thermalcycler (Applied Biosystems Inc., USA) with the following thermocyclic conditions

$44^0$C for 30 min,

$94^0$C for 1 min
$52^0$C for 2 min
$72^0$C for 3 min
$94^0$ C for 1 min
$54^0$C for 2 min 35 cycles
$72^0$C for 1 min 33 sec
$72^0$C for 5 min

This was used for the detection of Potyvirus while the following thermocyclic condition was used for the detection of multiple viruses (*Maize streak virus* (MSV), *Maize chlorotic mottle virus* (MCMV) *and Sugarcane mosaic virus* (SCMV).

$42^0$C for 30 min,
$94^0$C for 3 min
$94^0$ C for 30 sec
$54^0$C for 30 sec 40 cycles
$68^0$C for 1 min
$72^0$C for 10 min

1.5 g of agarose powder was mixed with 100 mL of the electrophoresis buffer which was then heated in a microwave oven until completely melted. After cooling the solution to about 6 $^0$C, it was poured into a casting tray containing a sample comb and allowed to solidify at room temperature. After the gel had solidified, the comb was removed, using care not to rip the bottom of the wells. The gel, still in its plastic tray, was inserted horizontally into the electrophoresis chamber and just covered with buffer. Samples containing DNA mixed with loading buffer were then pipetted into the sample wells, the lid and power leads were placed on the apparatus and a current was applied. DNA migrated towards the positive electrode, which is usually coloured red.

The distance of the DNA migration in the gel was judged by visually monitoring the migration of the tracking dyes. Bromophenol blue and xylene cyanol dyes migrated through the agarose gels at roughly the same rate as the DNA fragments. Finally, the gel was placed on the ultraviolet transilluminator for gel picture documentation. Gel pictures were subsequently used for the scoring of viruses as leaf samples that showed expected band sizes of 1304 bp, 500 bp and 900 bp for MSV, MCMV and SCMV, respectively were considered to be virus infected.

## RESULTS

In all the agro-ecologies surveyed majority of the farmers grew maize on less than 1 ha. of land. In the eastern part of the country although maize is cultivated twice annually most farmers preferred cassava as number one crop. Farmers confirmed that double cropping of maize was facilitated by the bimodal nature of rainfall. Maize was grown in mixtures with other principal food crops such as cassava, yam, sugarcane, banana and plantain, cocoyam, fluted pumpkin, okra, pepper etc. The neighbouring crops around maize were predominantly maize and cassava or cassava, yam and vegetables such as fluted pumpkin and okra.

All the farmers interviewed in Akwa Ibom and Cross River States testified that they usually purchased maize seeds from the market, however, actual variety names were not known. Similarly, they never used pesticides to control insect pests and diseases; they normally replenished tired soils through the application of poultry droppings and careful management of plant debris after harvesting. Some farmers confirmed the presence of some disease conditions on yearly basis but attributed such to low level of soil fertility. All the farmers interviewed were ready to acquire seeds from reliable sources such as research institutes, Agricultural Development Projects (ADPs), ministries of agriculture etc.

In Kaduna, Kano and Niger situated in the northern part of the country, rainfall is monomodal and maize is the principal cereal crop. In most instances maize was intercropped with guinea corn or millet. The neighbouring crops around maize were predominantly maize, cowpea, soybean, guinea corn and vegetables.. Most farmers stated that they sourced for maize seeds from Research Institutes such as IITA, and ADPs. Some farmers attested to pesticide application only for weed control of insect pests in cowpea fields. Soil improvement is mainly through application of inorganic fertilizers (NPK and Urea). Some farmers also apply cow dung or poultry droppings on the farm in order to boost yield. There were farmers who confirmed the presence of some disease conditions on yearly basis. All the farmers interviewed were ready to acquire maize and sugarcane seeds/cuttings from reliable sources such as research institutes, ADPs, Ministry of Agriculture etc.

In south western Nigeria, rainfall peaks twice as in Akwa Ibom and Cross River States. Maize fields were surrounded by cassava, yam and vegetables. Farmers usually obtain seeds fro IITA, IAR&T (Institute of Agricultural Research and Training), ADPs and Ministry of Agriculture. Disease symptoms were generally mild or moderate to severe mottling. Some plants exhibited mosaic symptom, stunting, deformation and there were cases of plant death (Plate 1). By visual observation, disease incidence was highest in Gbako (63.3 %) Local Government of Niger State, whereas the lowest was observed in Owode (7.1 %) Local Government Area of Osun State. In ELISA and PCR tests none of the samples tested positive to SCMV or MCMV. However, about 19 % of the samples tested positive to *Maize streak virus* (Fig. 1 – 11 and Table 3) an endemic virus in Nigeria and several parts of Africa. The data in Table 1 also reveal that MSV incidence was highest in Kano State (45.7 %) while the lowest was recorded in Cross River State (8.3 %).


Plate 1. Representatives of the diverse symptoms observed on maize and sugarcane plants during the surveys of Rainforest and Savanna agro-ecological zones of Nigeria in 2014

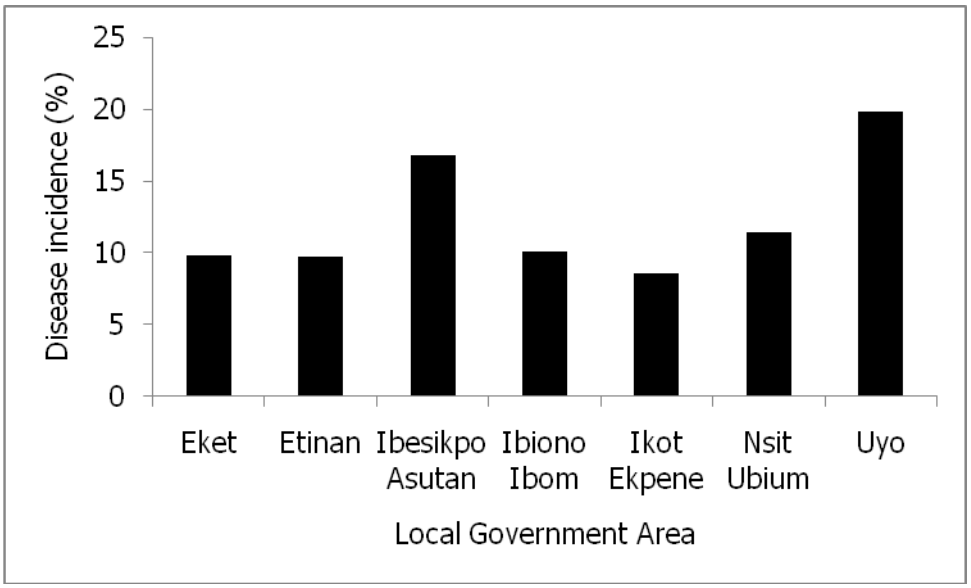


Fig. 1: Incidence of virus diseases in different Local Government Areas of Akwa Ibom State, 2014

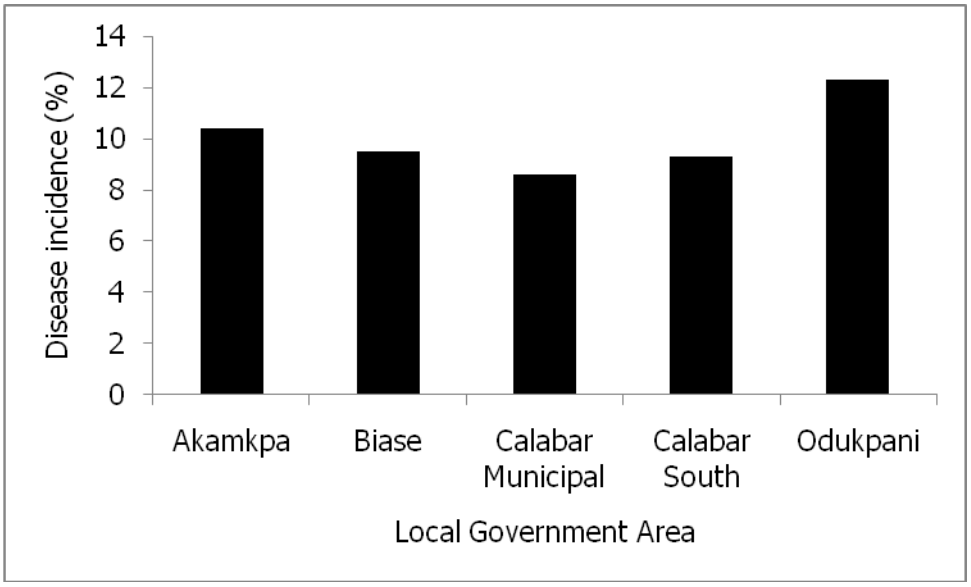


Fig. 2: Incidence of virus diseases in different Local Government Areas of Cross River State, 2014

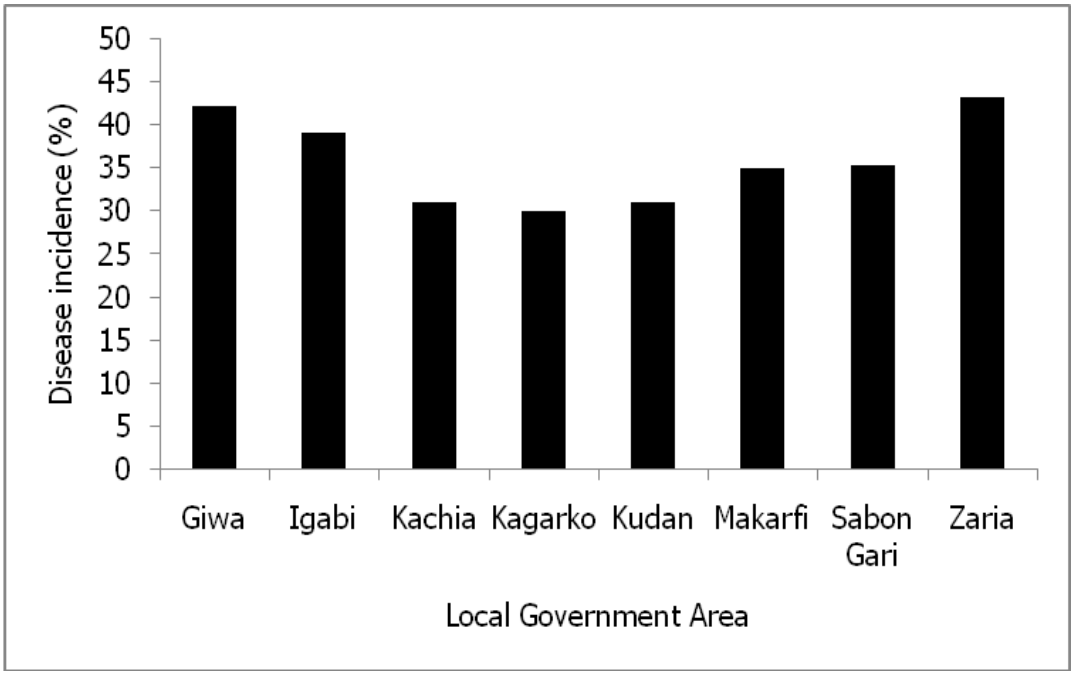


Fig. 3: Incidence of virus diseases from maize fields in different Local Government Areas of Kaduna State, 2014

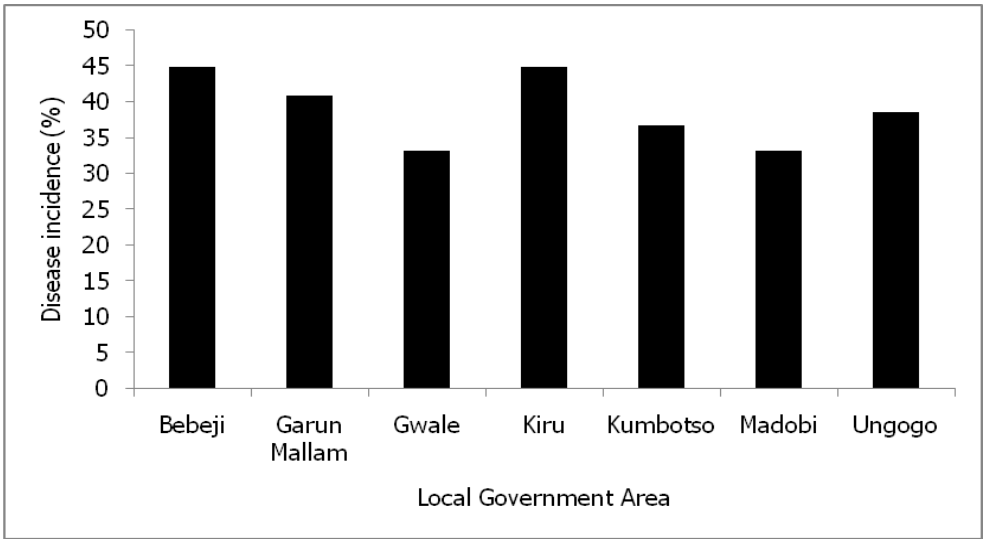


Fig. 4: Incidence of virus diseases from maize fields in different Local Government Areas of Kano State, 2014

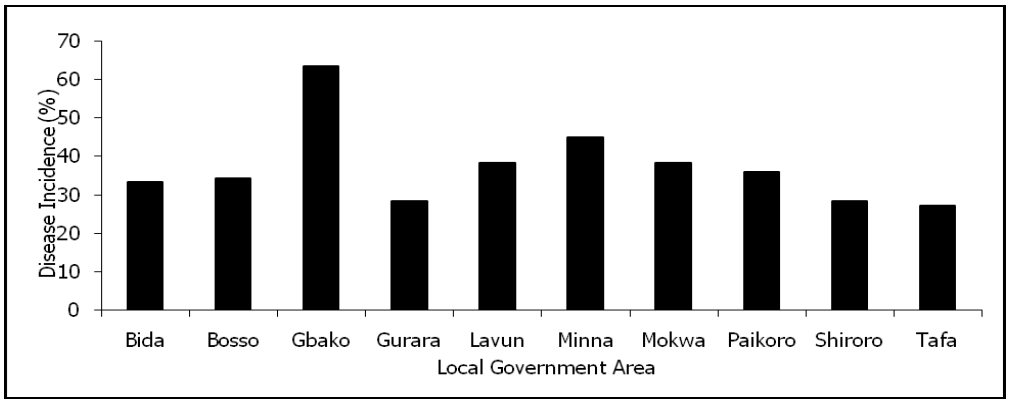


Fig. 5: Incidence of virus diseases from maize fields in different Local Government Areas of Niger State during the early cropping season (first survey), 2014

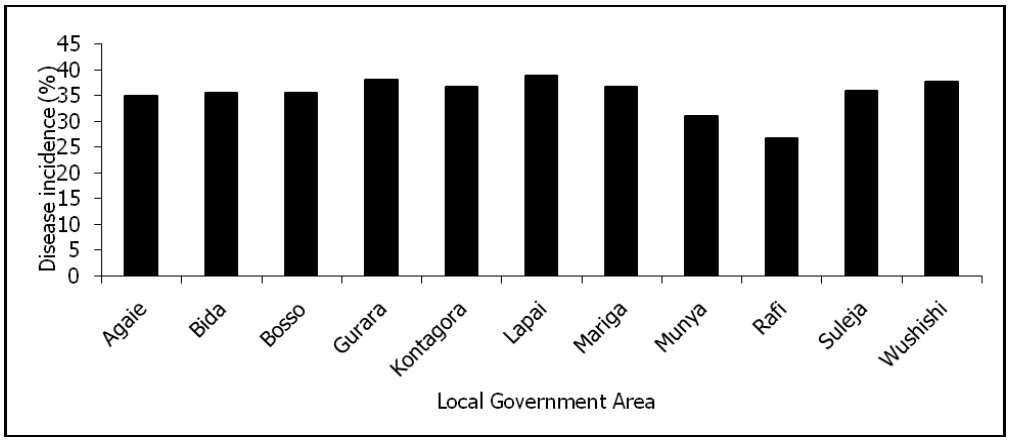


Fig. 6: Incidence of virus diseases from maize fields in different Local Government Areas of Niger State during the late cropping season (second survey), 2014

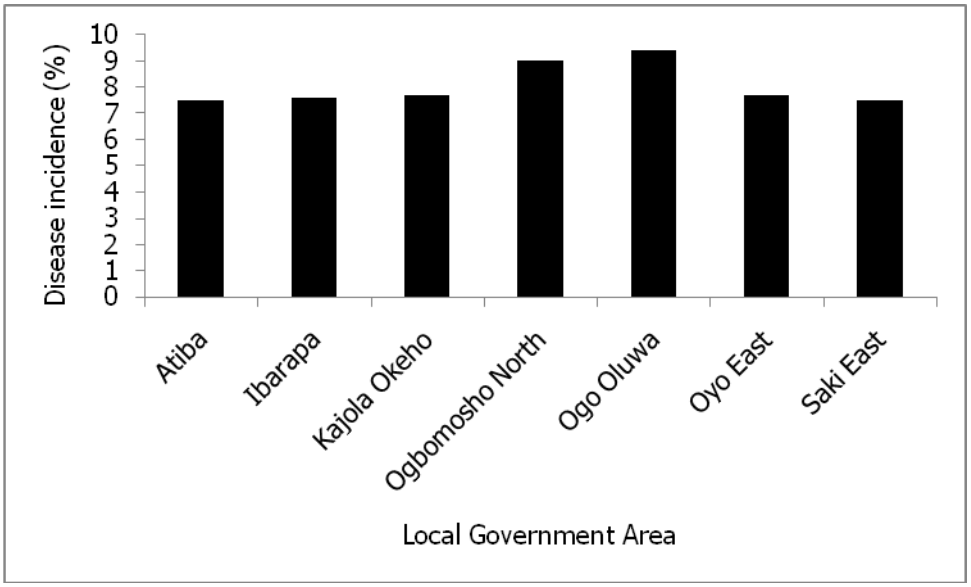


Fig. 7: Incidence of virus diseases from maize fields in different Local Government Areas of Oyo and Ogun States during the late cropping season (first survey), 2014

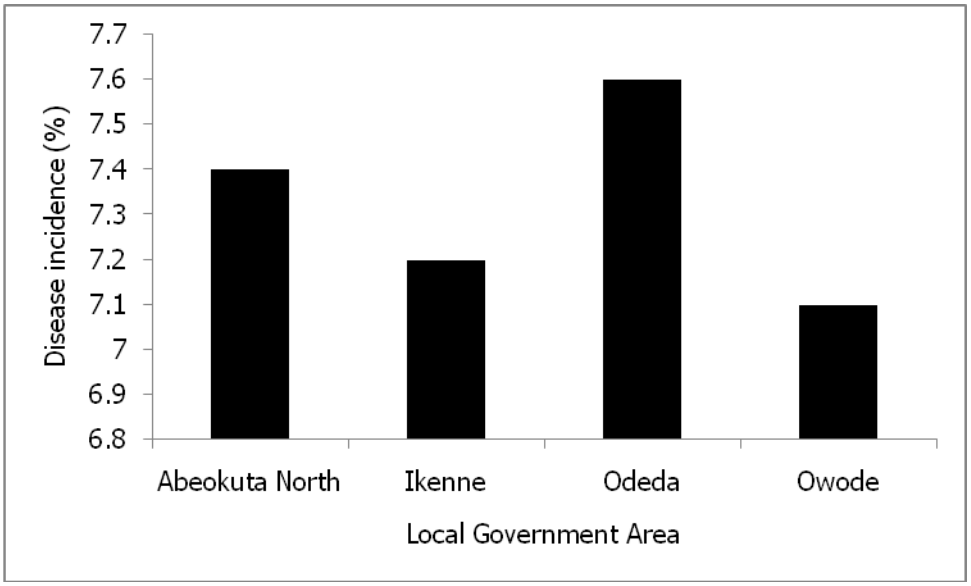


Fig. 8: Incidence of virus diseases from maize fields in different Local Government Areas of Ogun States during the late cropping season (first survey), 2014

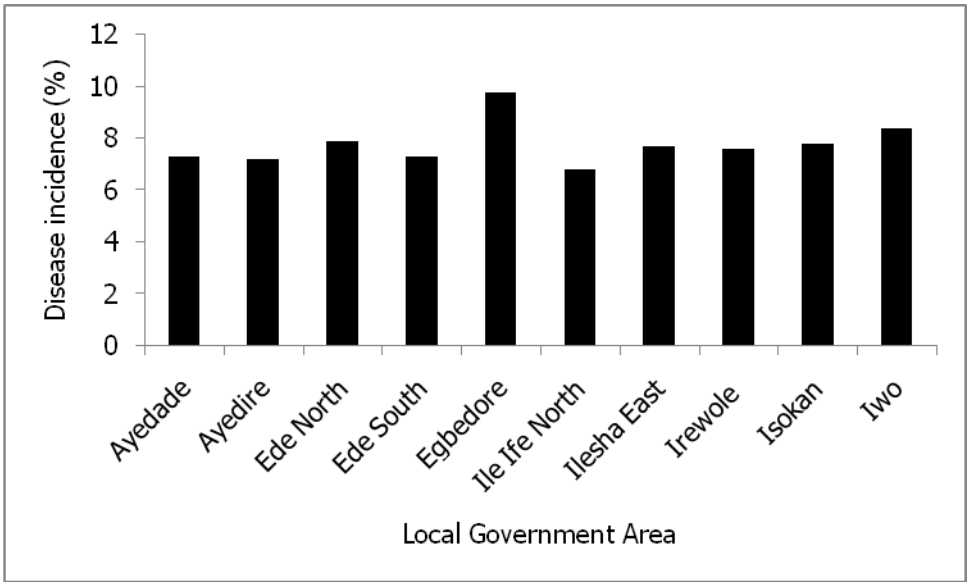


Fig. 9: Incidence of virus diseases from maize fields in different Local Government Areas of Osun State during the late cropping season, 2014

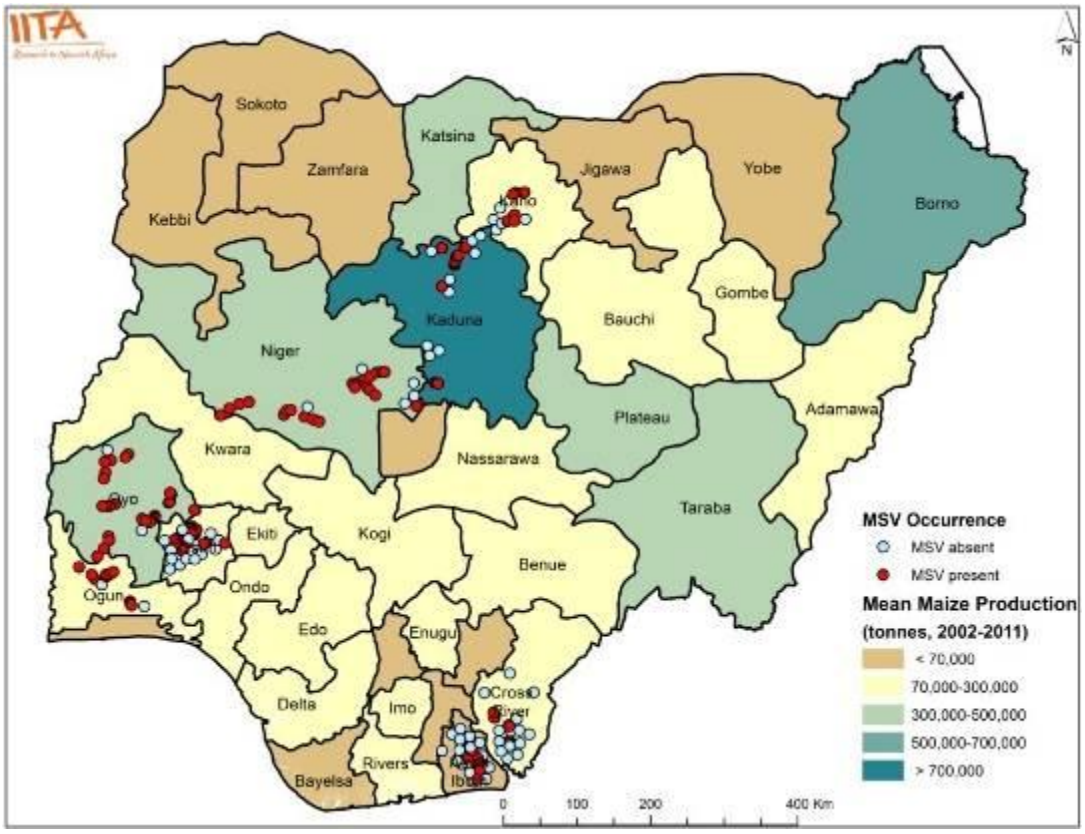


Fig 10. Survey locations and occurrence of maize streak disease from farmers' fields in 7 states of Nigeria.

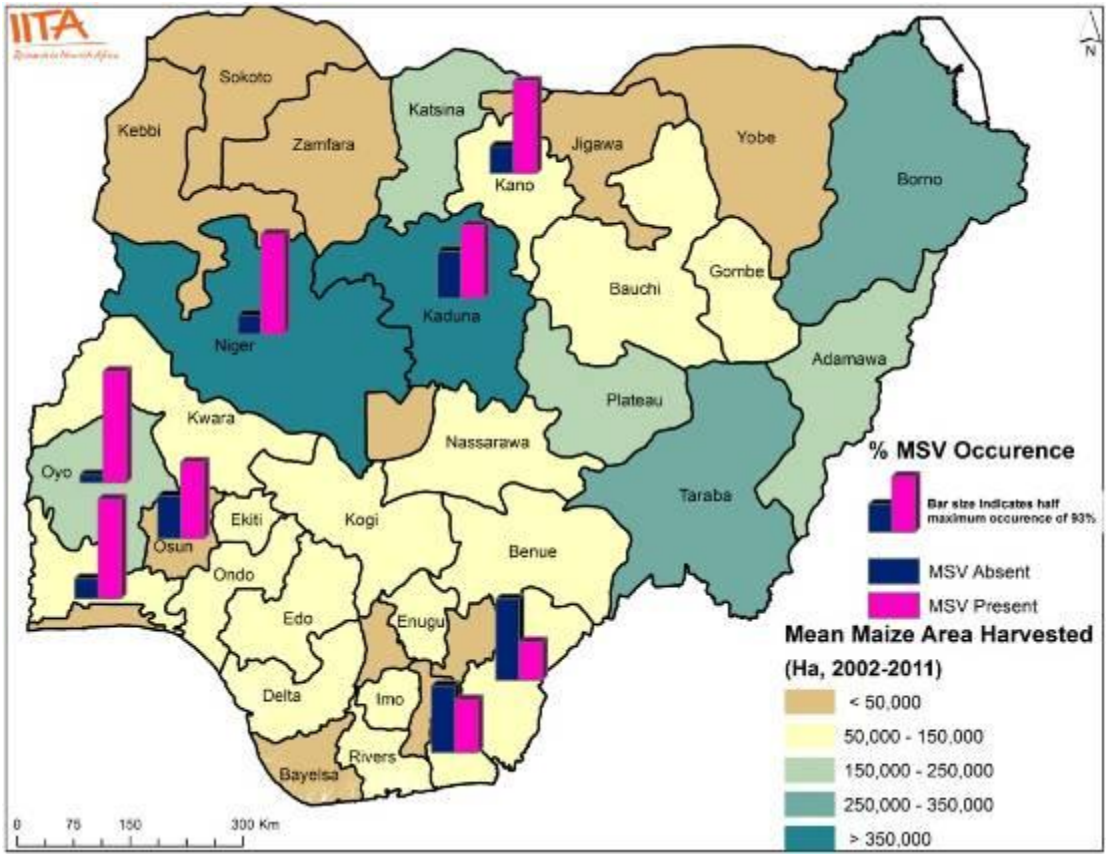


Fig 11. Percent *Maize streak virus* (MSV) occurrence from different states in Nigeria

**Table 3: Reactions of maize and sugarcane leaf samples to MSV and MCMV in Enzyme-Linked Immunosorbent Assay (ELISA)**

| State of Nigeria | Total number of Maize samples indexed for virus | Total number of samples Negative to virus | Total number of samples Positive to virus | % MSV incidence | % MCMV incidence |
|---|---|---|---|---|---|
| **AKW** | 609 | 553 | 76 | 12.5 | 0 |
| **CRS** | 408 | 374 | 34 | 8.3 | 0 |
| **NRS** | 123 | 77 | 46 | 37.4 | 0 |
| **KDS** | 50 | 31 | 19 | 38 | 0 |
| **KNS** | 35 | 19 | 16 | 45.7 | 0 |
| **OSS** | 89 | 58 | 31 | 34.8 | 0 |
| **OYS** | 89 | 52 | 37 | 41.6 | 0 |
| **OGS** | 35 | 22 | 13 | 37.1 | 0 |

*AKW- Akwa Ibom State, CRS- Cross River State, NRS- Niger State, KDS- Kaduna State and KNS- Kano State. OSS- Osun state, OGS- Ogun state and OYS- Oyo state.

## Discussion

Several viruses cause significant reductions in maize in sub-Saharan Africa with attendant severe losses and threat to food security The detection of MSV in all the surveyed sites suggests the prevalence of the virus in Nigeria. This implies that the virus is endemic in the country. Hitherto, streak infection in maize was of little importance until 1970s. The prevalence of MSV disease could have been facilitated by a number of factors. Notable among them is the mode of spread. *Maize streak virus* is obligately transmitted by leafhoppers in a persistent manner (Alegbejo *et al*., 2002). Therefore, the infected plants become sources of primary inoculum and virus refuge to infect other plants. Because of the active nature of the vector infection is not usually restricted to the nearby plants but those in far distance are prone to infection risk.

The observation that farmers were not disposed to insecticide applications seems to have worsened the situation. However, even if adopted, investigations have revealed that the leafhopper vectors subsist on several alternative crop plants including weed species (Ouwafemi *et al*., 2011). Besides, application of insecticide is partially effective because the recurring influx of migrant hopper populations re-infect the crop after each application (Magenya *et al*., 2008). Low level of streak incidence was encountered in some maize fields due to late infection. The incidence of streak disease was high in the Forest and Forest – Savanna transition agro-ecologies due to bimodal nature of rainfall which encourages relay cropping of maize within the cropping seasons. In the Savanna agro-ecology, streak disease was prevalent probably because of the intensive cultivation. Iken and Amusa (2004) reported that maize production is now greatest in the Savanna agro-ecology of Nigeria. Unlike the Forest zone which is characterized by trees, the Savanna region is generally hone to grass plants which enable leafhopper vectors to survive between seasons. Besides, the observation that maize is commonly grown in mixture with guinea corn and millet was possibly responsible for high rate of infection because these crops are also important host plants for leafhoppers.

Weather is another factor which enhances leafhopper population. Disease incidence is particularly aggravated under favourable temperature. For instance Alegbejo *et al*. (2005) reported a significant positive correlation between leafhopper population and MSV disease incidence in the Savanna agro-ecology of Nigeria. Similarly there were reports of MSV disease epidemics in 1966, 1971, 1973, 1976, 1983 and 1984 (Eseman, 1966; Fajemisin and Shoyinka, 1976; Kim *et al*., 1981; Efron *et al*., 1989). Streak severity was not uniform in the study area due to a number of reasons. As clearly confirmed, by the farmers most of them sourced seeds from open markets. This practice does not guarantee the use of streak-resistant/tolerant varieties. However, adoption of varieties with MSV resistance genes offers insurance against total crop failure. The differences in MSV disease severity was also variable as a result of differences in genetic architecture of the cultivars, plants' age at the time of infection, strain and virulence of the invading. Previous studies revealed that 11 strains of MSV have been confirmed from different parts of Africa and its neighbouring Island (Martin *et al.*, 2001; Schnippenkoetter *et al.*, 2001; Willment *et al*., 2002; Varsani *et al*., 2008; Oluwafemi *et al*., 2011). Therefore, these and the associated attributes probably contributed to the variation in the level of severity on the plants.

## CONCLUSION AND RECOMMENDATIONS

The results of this investigation show that MLND is not yet in Nigeria. Even in the States that have close proximity to the East African countries where the disease has reached an alarming rate, none of the samples tested for the viruses that perpetuate MLND. However, continuous surveillance is essential in order to forestall any eventuality. In the main time strategies which are currently in use in the countries where the virus has been confirmed are hereby recommended:

1. Adoption of good cultural practices such as crop rotation, crop diversification, conservation agriculture and timely weed control.
2. Judicious use of systemic and contact pesticides to control possible vectors of MLND.
3. Enforcement of local quarantine procedures at the borders.
4. Use of clean certified seed.
5. Application of manure, basal and top dressing fertilizers to enhance growth and development.

**REFERENCES**


Alegbejo, M. D, Olojede, S. O, Kashina, B. D. and Abo, M. E. (2002). *Maize streak Mastrevirus* in Africa: distribution, transmission, epidemiology, economic significance and management strategies. J. Sust. Agric. 19(4): 35 - 45.

Babatunde, R. O., Fakayode, S. B. and Obafemi, A. A. (2008). Fadama maize production in Nigeria: Case study from Kwara State. Res. J. Agric. Biol. Sci., 4: 340-345

Efron, Y., Kim, S. K., Fajemisin, J. M., Mareck, J. H., Tang, C. Y., Dabrowski, Z. T., Rossel, H. W., Thottappilly, G. and Buddenhagen, I. W. (1989). Breeding for resistance to *Maize streak virus*: A multidisciplinary team approach. Plant Breeding. 103: 1-36.

Esenam, E. U. (1966). Some observations on the maize streak disease in Western Nigeria.
Nig. Agr. J. 3: 38 - 41.

Fajemisin, J. M. and Shoyinka, S. A. (1976). Maize streak and other maize virus diseases in West Africa. Williams, L. E., Gordon, D. T. and Nault, L. R. (eds.). In: Proceedings of International maize virus diseases colloquium and workshop. Ohio, USA. 16-19 August, 1976.

FAO (2013). Maize production in Nigeria.
http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor

Ibrahim, A., Shamsudin, M. N., Yacob, R. and Radam, A. B. (20140. Technical efficiency in maize production and its determinants: A survey of farms across agroecological zones in northern Nigeria. Trends in Agric. Econs. 7(2): 57 – 68.

IITA (International Institute of Tropical Agriculture) (2013). Maize Crop Retrived 27 September, 2013 http://www.iita.org/maize

Iken, J. E. and Amusa, N. A. (2004). Maize research and production in Nigeria. Afr. J. Biotechnol., 3: 302-307.

Jensen, S. G et al. (1991). Seed transmission of maize chlorotic mottle virus. Plant Dis. 75: 497– 498.

Jiang, X. Q et al. (1992). Maize chlorotic mottle virus in Hawaiian-grown maize – vector relations, host range and associated viruses. Crop Protect. 11:248 - 254.

Kim, S. K., Efron, Y., Singh, J., Buddenhagen, I.W., Asnani, V. L., Rossel, H. W., Bjarnason, M. and Thottappilly, G. (1981). Recent progress on maize streak resistance breeding program at 11TA. Paper presented at the 3rd OAU/STRC workshop on maize and cowpea held at IITA, Ibadan, Nigeria. February 23-27, 1981.

Magenya, O. E. V., Mueke, J. and Omwega, C. 2008. Significance and transmission of *Maize streak virus* disease in Africa and options for management: A review. Afr. J. Biotech. 7: 4897- 4910.

Martin, D. P., Willment, J. A., Billharz, R., Velders, R., Odhiambo, B., Njuguna, J., James, D. and Rybicki, E. P. 2001. Sequence diversity and virulence in *Zea mays* of *Maize streak virus* isolates. Virol. 288: 247 - 255.

Ogunlade, I., G., Tologbonse, O. D. and Alaoye, O. E. A. (2010). On-farm evaluation of drought tolerant maize varieties and hybrids in the Southern Guinea Savanna zones of Nigeria. Proceedings of the 44th Annual Conference of the South African Society for Agricultural Extension, May 4-7, 2010, Langebaan, Western Cape, South Africa, pp: 1-5.

Olaniyi, O. A. and Adewale, J. G. (2012). Information on maize production among rural youth: A solution for sustainable food security in Nigeria. Library Philos. Pract. (e-J.).

Oluwafemi, S., Alegbejo, M. D., Onasanya, A. and Olufemi, O. (2011). Relatedness of *Maize streak virus* in maize (*Zea mays* L.) to some grass isolates collected from different regions in Nigeria. Afr. J. Agric. Res. 6(27): 5878 -5883.

Schnippenkoetter, W. H., Martin, D. P., Willment, J. A. and Rybicki, E. P. (2001). Forced recombination between distinct strains of *Maize streak virus*. J. Gen. Virol. 82: 3081-3090.

Varsani, A., Shepherd, D. N., Monjane, A. L., Owor, B. E., Erdmann, J.B., Rybicki, E. P., Peterschmitt, M., Briddon, R. W., Markham, P. G., Oluwafemi, S., Windram, O. P., Lefeuvre, P., Lett, J. M. and Martin, D. P. (2008). Recombination decreased host specificity and increased mobility may have driven the emergence of *Maize streak virus* as an agricultural pathogen. J. Gen. Virol. 89: 2063 -2074.

Wangi, A et al. (2012). First Report of Maize chlorotic mottle virus and Maize Lethal Necrosis in Kenya. Plant Dis. 96: 1582.

Willment, J. A., Martin, D. P., Van der Walt, E. and Rybicki, E. P. (2002). Biological and genomic sequence characterization of *Maize streak virus* isolates from wheat. Phytopathol. 92: 81- 86.

# Appendix 1. Data Collection Sheet

| Maize Viruses Survey – 2014 | | | |
|---|---|---|---|
| Sheet # | | Field size | |
| Date/Time | | Age (wks) | |
| Location name | | Varieties | |
| District/LGA | | | |
| State | | Intercrops | |
| Agro-ecology | | | |
| Latitude | | Crops in neighbouring fields | |
| Longitude | | | |
| Altitude (m) | | Researcher (farmer) | |
| Summary | Percent infection: | Average severity: | Severity range: |

| Plant (#) | Symptoms | Severity score | Insects (thrips/rootworms/aphids/whiteflies/ Beetles/leafhoppers) | | | Details of sampled plant | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | P# | Adults | Nymphs | P# | Photo ID | Symptoms | Sev. score | Variety |
| 1 | | | 1 | | | 1 | | | | |
| 2 | | | 2 | | | 2 | | | | |
| 3 | | | 3 | | | 3 | | | | |
| 4 | | | 4 | | | 4 | | | | |
| 5 | | | 5 | | | 5 | | | | |
| 6 | | | | | | | | | | |
| 7 | | | | | | | | | | |
| 8 | | | | | | | | | | |
| 9 | | | | | | | | | | |
| 10 | | | | | | | | | | |
| 11 | | | | | | | | | | |
| 12 | | | | | | | | | | |
| 13 | | | | | | | | | | |
| 14 | | | | | | | | | | |
| 15 | | | | | | | | | | |
| 16 | | | | | | | | | | |
| 17 | | | | | | | | | | |
| 18 | | | | | | | | | | |
| 19 | | | | | | | | | | |
| 20 | | | | | | | | | | |
| 21 | | | | | | | | | | |
| 22 | | | | | | | | | | |
| 23 | | | | | | | | | | |
| 24 | | | | | | | | | | |
| 25 | | | | | | | | | | |
| 26 | | | | | | | | | | |
| 27 | | | | | | | | | | |
| 28 | | | | | | | | | | |
| 29 | | | | | | | | | | |
| 30 | | | | | | | | | | |

Abbreviations for symptom description
Prefix: m – mild; o – moderate; s – severe
Suffix: m – mosaic; mo – mottling; puc – puckering; st – stunting; d – deformation; de – death

1. No visible symptoms; plants apparently healthy
2. Mild mosaic/mild mottling on few leaves/branches of a plant (symptoms on 25% of the plant)
3. Mosaic/puckering/mottling/necrosis/vein clearing symptoms cover 50% of the plant
4. Severe mosaic/puckering/mottling/yellowing/necrosis (symptoms on entire plant) but no stunting of deformation
5. Severe mosaic/mottling/yellowing/necrosis and severe stunting (entire plant) deformation and death of the infected plants