Prevalence, Distribution and Impact of Maize Lethal Necrosis Disease (MLND) in Ethiopia

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Abstract

Maize plants displaying severe mosaic, chlorosis and necrosis were observed in five provinces of Ethiopia. ELISA test from symptomatic samples revealed the presence of maize chlorotic mottle virus (MCMV) and sugarcane mosaic virus (SCMV), two components of maize lethal necrosis disease. In 2014 and 2015, surveys conducted in Oromia, SNNP, Benishangul Gumuz, Amahara and Tigray regions showed that the two viruses were present in a significant number of plants. Mixed infections were detected and associated with severe severity and yield losses. The report of maize lethal necrosis disease in Ethiopia is of high level concern for producers in the country because of the potential of an epidemic that can devastate maize seed and grain production. According to this survey report MLND severity in SNNP was the highest with a mean rate of 3.5, followed by Oromia, Benishangul Gumuz, Amahara and Tigray with mean severities 3.2, 2.5, 2 and 1.5, respectively. It is estimated that severely affected areas can experience a massive yield loss of over 95 % and this will affect total maize yield produced in the country. Thus it is recommended that farmers should avoid growing maize in consecutive seasons, continuous/stagger cropping, movement of affected crop debris from one region to another; practice crop rotation and intercropping with non-cereal crops, rouging out affected maize debris and application of insecticides to curb the impact of the disease and improve maize yield.

Keywords: Maize Lethal Necrosis, MCMV, SCMV, Distribution, Prevalence, Ethiopia.

Introduction

Maize (*Zea mays* L.) is critical for food security in sub-Saharan Africa (SSA); eastern and southern Africa use 85 % of the maize produced as food, while Africa as a whole uses 95 % as food (Shiferaw *et al.* 2011). In Ethiopia it is one of the most important strategic crops and ranks second to teff in area coverage and first in total production. According to Abate *et al.* (2015), the national average maize yield in 2014 was 3.4 t ha^{-1}. The low yield is attributed to a combination of constraints among which diseases play a major role. Worldwide, more than 50 viruses have been reported on maize causing an array of symptoms in single or mixed infections (Lapierre and Signoret 2004; Redinbaugh and Zambrano 2014). Of these, at least a dozen viruses have been considered important in terms of prevalence and economic losses in several maize producing areas (Redinbaugh and Pratt

2008). Maize lethal necrosis disease (MLND) is one of the most devastating viral diseases of maize in eastern and central Africa (Mahuku *et al.* 2015). It was first observed in Kansas and Nebraska (Niblett and Claflin 1978; Doupnik 1979; Uyemoto *et al.* 1980), where the disorder was caused by a mixed infection of maize chlorotic mottle virus (MCMV, Tombusviridae) and either wheat streak mosaic virus (WSMV) or maize dwarf mosaic virus (MDMV), both belonging to the family Potyviridae. Symptoms include mosaic, chlorosis and eventually necrosis, resulting in either plant stunting or death (Niblett and Claflin 1978; Uyemoto *et al.* 1980; 1981). During the past few years, MLND has been reported in China, Kenya and other African countries as a result of a synergistic interaction between MCMV and Sugarcane mosaic virus (SCMV, Potyviridae) (Xie *et al.* 2011; Wangai *et al.* 2012; Adams *et al.* 2014). However, the outbreak of MLND in East Africa is becoming a serious challenge to maize production and poses a big threat (potential yield losses 30-100 %) to food security for the majority in this region (Wangai *et al.* 2012).

In Ethiopia, four types of viruses were reported to infect maize. These are: maize streak virus (MSV), sugarcane mosaic potyvirus (SCMV), maize dwarf mosaic potyvirus (MDMV) and maize mottle chlorotic stunt virus (MMCSV) (Tewabech *et al.* 2002). Maize streak virus is the most dominant viral disease of maize while the remaining are minor, with no significant economic impact on maize production. However, during the last two years severe virus like symptoms were reported in Oromia and Southern Nations, Nationalities and People's (SNNP) Regions, the two major maize producing regions of the country. In July 2014, cases of maize infection by Maize Lethal Necrotic Disease was réported from eastern rift valley regions of Ethiopia and confirmed by ELISA test. Currently, MLND infection has spread to enormous areas of the country. Both MCMV and SCMV are transmitted through mechanical means and are known to be seed transmitted. In addition to this, MCMV can be experimentally transmitted by thrips and beetles while SCMV is vectored by aphids (Cabanas *et al.* 2013). The disease has now gained the momentum to spreading to other parts of the country where maize crop is grown because of insufficient knowledge on how to contain its dissemination and manage the disease. It has therefore created major concern in Ethiopia because of the impact it might have on maize production in the country.

Despite the importance of maize and its widespread production and consumption, recent reports indicated drastic reductions of expected maize yield (BNMRC 2016). Evidence from preliminary reports indicated that most farmers had little knowledge on MLND and its control mechanisms. Further evidence showed that, there was limited research on maize viral diseases and more particularly in maize lethal necrosis disease. The present study therefore was conducted to determine the prevalence, distribution and impact of MLND in Ethiopia.

Materials and Methods

Field Observation and Data Collection Procedures

National survey was carried out by multidisciplinary team across all maize growing areas of Ethiopia during 2014 main season and 2015 off- & main-seasons. Five regions (Oromia, SNNP, Amahara, Benishangul Gum and Tigray), twenty zones and forty one districts were assessed in the survey. Zones and Districts were selected purposively. The sites were selected in consultation with the respective research centers at each agro-ecology and staff of the regional and zonal bureaus of agriculture and district agricultural development offices in each region. Multi stage sampling strategy was employed. Major maize growing zones were targeted for this study. Locations in each district, fields in each locality and maize plants in every field were assessed after random selection. Random selection of the maize plants was done by moving in a zigzag ways to make the assessment representative of the field.

Plants were inspected visually and types of symptoms were recorded. Disease incidence was scored as percentage of infected plants out of total plants in 4 X 4 m^2 area. Disease severity was estimated using a 1-5 scale where, 1= No MLND symptoms, 2 = Fine chlorotic streaks on lower leaves, 3 = Chlorotic mottling throughout plant, 4 = Excessive chlorotic mottling and dead heart, 5 = Complete plant necrosis) (CIMMYT 2013). Additional data including: GPS co-ordinates, field size, crop growth stage, variety, MLN disease history, field history, weeds with disease symptoms, fertilizer usage and rate and sowing date were taken. Yield reduction was estimated based on projection of visual observation of crop, status of disease severity, growth stage and possibility of crop ever reaching maturity. The type, infestation level and general status of insect vectors like aphids, thrips and stem borers were recorded. Samples of diseased maize plants & suspected alternate hosts were collected for further laboratory identification. The samples were diagnosed by Enzyme-linked-Immune-sorbent-Assay (ELISA) test method following standard protocols described in DSMZ manual for DAS-ELISA kit and lateral fellow assay (LFA).

Sampling and Questionnaires Administering Procedures

A simple random sampling technique was used to get one hundred and sixty four (164) respondents who were maize growers. Since farmers affected by maize lethal necrosis disease were unknown, the researcher decided to use Snowball sampling technique to identify them. Once the first respondent was identified by the researcher, it became easier to locate the next and the subsequent respondents. The respondents directed where to go next as they knew the other growers in their community. The study applied fishers' formula to yield a representative sample size (Mugenda and Mugenda 2003). Data collection questionnaire was used as research instrument. Questionnaires administered by a researcher were used to collect data from respondents on maize lethal necrosis disease and maize yields. The research instrument content was shared with experts for their necessary input and approval before embarking on data collection. Respondents were informed of the purpose of the study and the need to respond honestly. This was to ensure that the data collected is reliable and non-biased. Data were analyzed using Microsoft excel and to test the influence among independent and dependent variables, Statistical Package for Social Science (SPSS) (version 20) software was used.

Results

Proportion of farmers affected by MLND

Of the 164 respondent farmers, 154 (93.8 %) were affected by maize lethal necrosis in 2014 and 2015 cropping seasons; whereas only 10 (6.2 %) of the respondent indicated that they were not affected by MLND. These showed that MLND affected almost all maize farmers of the surveyed areas.

Incidence, severity and distribution of MLND causing viruses in Ethiopia

A high incidence (50-80 %) and severity (1-4 on 1-5 scale) of MLND were found in symptomatic randomly selected plants at South Tigray, Raya Azabo district (Table 1). In general, MCMV incidence was lower than SCMV incidence and similar to the rate of co-infection with MCMV and SCMV. Likewise a high incidence (55 %) and severity (2.5) of MLND were found in symptomatic randomly selected plants at Awi zone, Ankasha Guagusa district (Table 1). Whereas the lowest incidence (<20 %) and severity rate (not more than one) were observed at South Gonder, West Gojam and East Gojam zones of Amahara region (Table 1).

In Oromia and SNNP regions up to 100 % incidence and severity rate of 5 were observed (Table 1). Among six surveyed zones in Oromia region East Shoa and Jimma were highly affected zones with highest incidence (20-100 %) and severity rate (2.5), while the remaining zones were moderately affected by the disease (Table 1). According to the villages' development agent (DA) the disease appeared recently but it has devastated many fields in a short period. He added that decaying, bad smelling, yellowing, wilting and finally drying of leaves was observed. Those plants affected after silking had problem of seed setting. Severely affected plants matured and dried more than one month earlier than that of the previous years. Our survey revealed that severely affected leaves and cobs showing mosaic, chlorosis and necrosis in highly affected grain and seed production fields (Fig. 1). High population of vectors such as aphids, thrips and beetles were observed and these indicated the presence and/or spread of the disease.

Among ten zones surveyed in SNNP region, except in Gurage zone MLND infections were observed in all nine zones. Wolita, Gamo Gofa and Sidama zones were highly affected by the disease with an average incidence of 90, 80 and 70 % and severity rate of 3.5, 3.5 and 4.5, respectively. The disease incidence and severity in the remaining zones were moderate. In Benshangul Gumuz, Kamashi zone the disease incidence and severity in the observed fields were estimated to be more than 40 % and rating scale of 2, respectively (Table 1). Moreover, ear rotting and shriveling of seeds were observed. Some vectors like aphids and beetles were also observed which indicates the presence and potential spread of the disease to the neighboring fields. The current survey work showed that MLND distribution has been confirmed in all assessed regions in the country. Currently MLND is the number one threat to maize production and seed system in Ethiopia.

Table 1. Maize Lethal Necrosis Disease (MLND) situation in Ethiopia in 2014 and 2015

Region	Zone	No. of Fields	Incidence (%)	Severity (1-5 scale)	Estimated Yield Loss (%)
Tigray	South Tigray	5	50 – 80	1 - 4	0 - 70
Amhara	South Gondar	1	20	1	0
	Awi	1	55	2.5	35
	West Gojjam	1	10	1	0
	East Gojjam	1	15	1	0
Oromia	East Shewa	8	20 - 100	2 - 4	20 -100
	West Shewa	5	0 - 60	0 - 2	0 - 80
	Jimma	6	10 - 100	2 - 5	15 - 100
	West Arsi	5	5-60	0-2	0-80
	Illu Aba Bora	2	0-50	0-2	0-30
	East Wollega	3	20-60	2-2.5	20-60
SNNP	Sidama	2	40-100	4-5	40-100
	Gurage	2	0	0	0
	Sulthe	5	0-100	0-5	0-5
	Alaba	2	50-60	2-2.5	40-60
	Hadiya	2	80-100	2-5	40-65
	Wolita	5	80-100	2-5	75-100
	Gamo Gofa	3	60-100	2-5	40-100
	South Omo	3	10-80	2-3	10-70
	Dawro	4	5-60	2-2.5	5-50
	Konta	1	55	2.5	45-50
Benshangul Gumuz	Kamashi	9	40-100	2-4	40-60

In symptomatic plants collected from across the country, 52 %, 16 % and 43 % of the samples were infected with MCMV+SCMV, MCMV only and SCMV only, respectively (Table 2). Nearly all of the SCMV infected plants were co-infected with MCMV. Samples from asymptomatic plants did not test positive for any of the viruses. Field monitoring revealed a strong association between co-infected plants and severe symptoms including leaf necrosis. Besides the above result, ninety five (95) extra samples collected from suspected areas were tasted for MCMV by ELISA and the result revealed that all of them were positive for MCMV (Data not shown). The positive test result for MCMV is sufficient evidence for the existence of MLND.

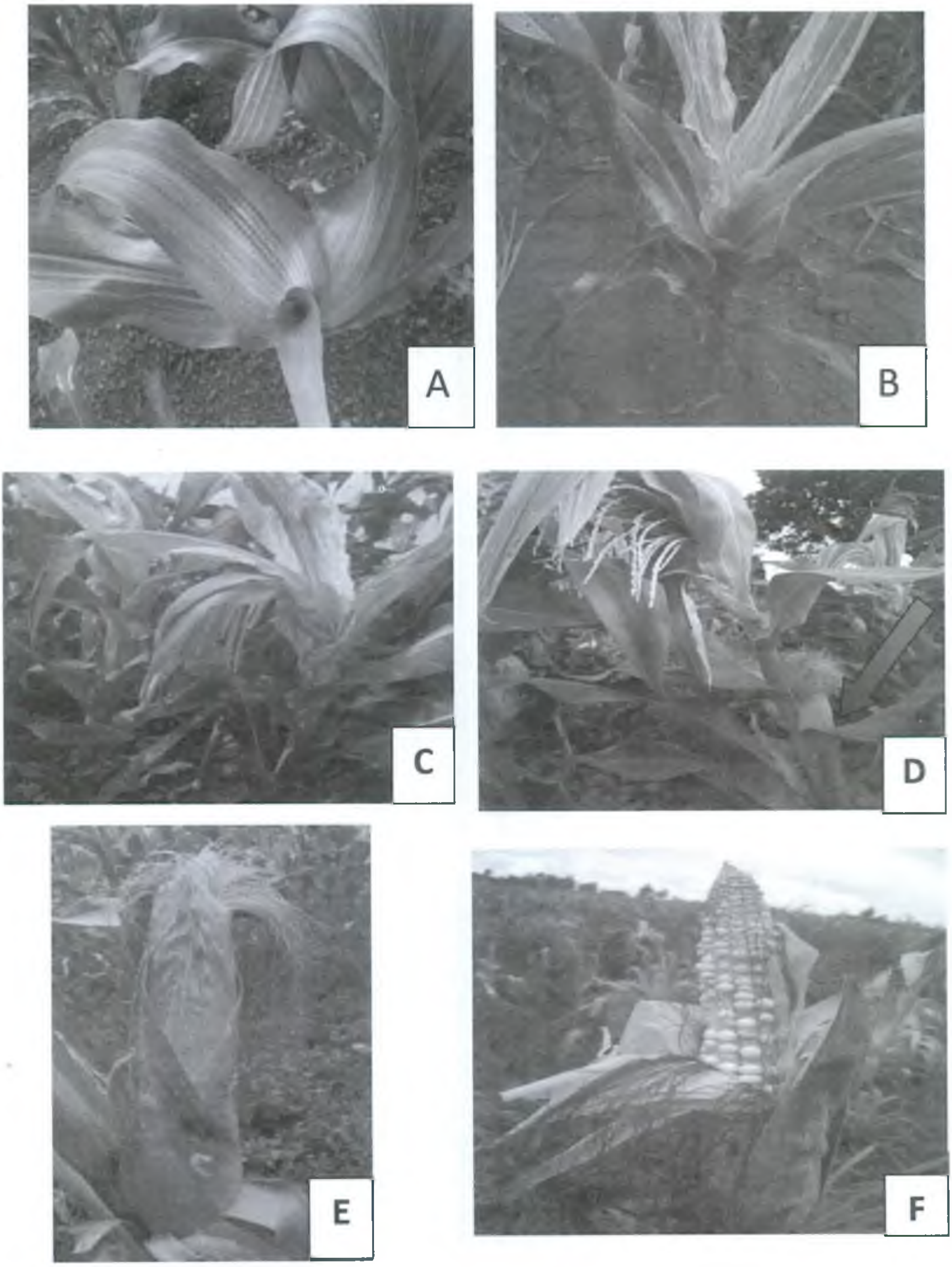

Figure 1. Severe symptoms associated to virus disorders displaying mosaic, chlorosis and necrosis (A= mosaic and chlorosis, B= Necrosis, C= failure to tassel/male sterility, D= mottling on cob at silking stage, E= premature drying of cobs at dough stage, F= Poor grain fill).

Table 2: Laboratory test result for samples collected from four regions (SNNP, Oromia, Amhara and Tigray).

No:	Region	Zone	Total sample tested	Lab Test Result		
				MCMV +SCMV	MCMV only	SCMV only
1	SNNP	Hadiya	19	12	3	4
2	SNNP	Wolayita	24	10	5	5
3	SNNP	Sidama	24	14	3	3
4	Oromia	East shoa	18	4	4	2
5	Oromia	West Arsi	9	1	-	5
6	Oromia	East Arsi	2	1	1	-
7	Oromia	East wollega	20	1	-	15
8	Oromia	Jimma	4	4	-	-
9	Amhara	West Gojjam	41	-	-	6
10	Tigray	North West	14	-	-	2
11	Tigray	South Zone	12	5	-	1
Sub-total			**187**	**52**	**16**	**43**

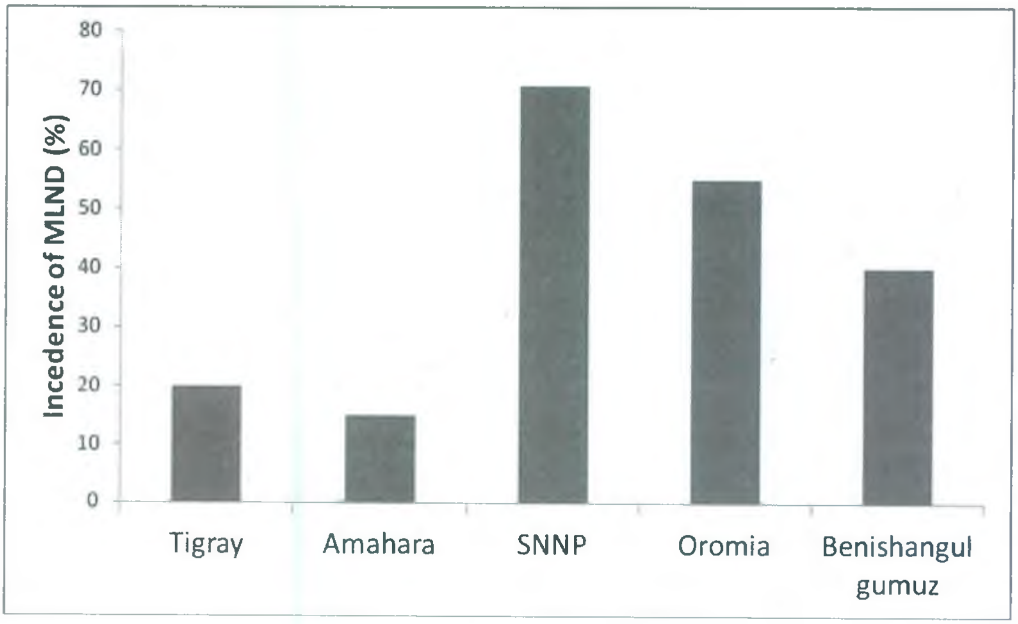

Figure 2. Incidence of MLND in five regions of Ethiopia in 2014 - 2015.

MLND severity analysis by regions

MLND severity in SNNP was the highest with a mean rate of 3.5, followed by Oromia, Benshangul Gumuz, Amahara and Tigray with mean severities of 3.2, 2.5, 2 and 1.5, respectively (Fig. 3).

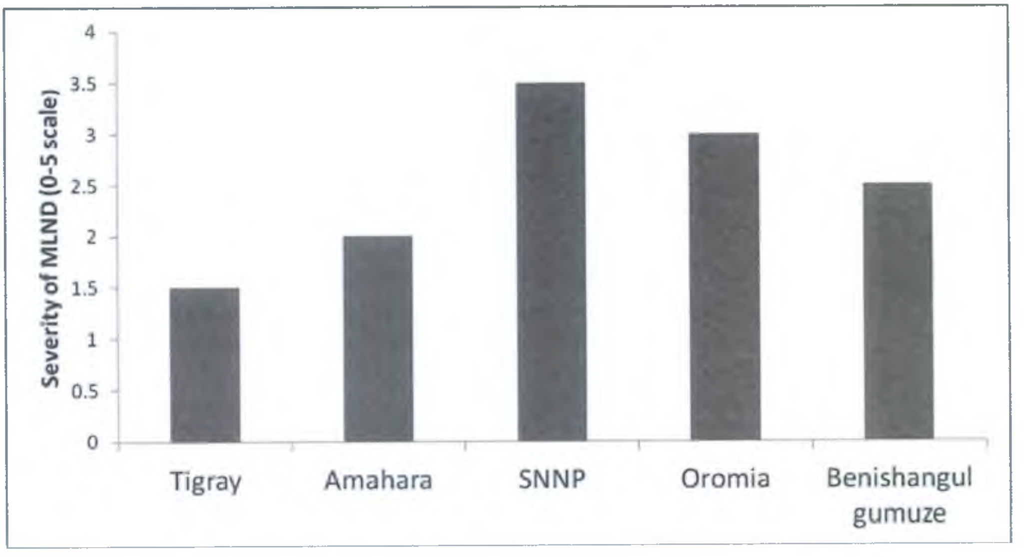

Figure 3. Severity of MLND in five regions of Ethiopia in 2014 - 2015.

Estimated yield loss in five regions

Areas affected constitute substantial maize production acreage, and given huge estimated loss up to100 % on yield becoming a food security issue. The highest yield loss was recorded in SNNP followed by Oromia, Benishangul Gumuz, Tigray and Amahara regions (Fig. 4). The average yield reduction due to MLND in SNNP, Oromia, Benshangul Gumuz, Tigray and Amahara was 65, 50, 45, 25 and 15 %, respectively.

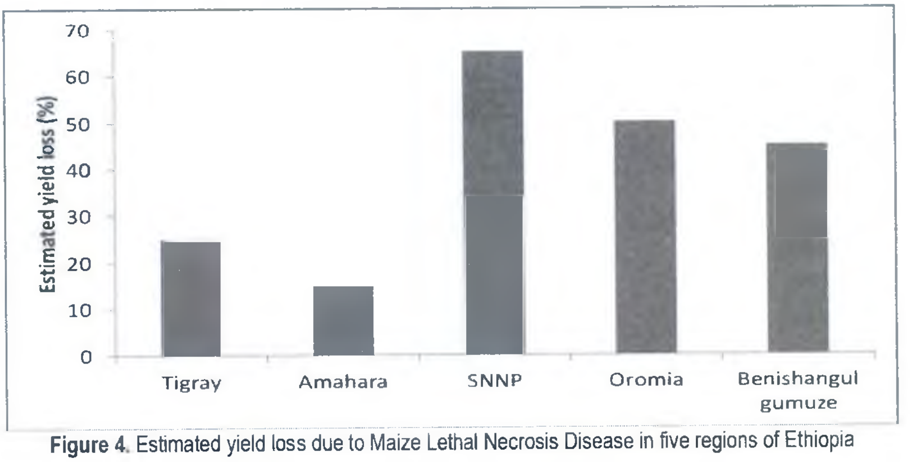

Figure 4. Estimated yield loss due to Maize Lethal Necrosis Disease in five regions of Ethiopia

Prevalence of MLND in 2013-2015

Majority of the respondents indicated that there was no symptom of MLND in their farms in 2011 and 2012. In 2013, some respondents indicated that there were incidences of maize lethal necrosis symptoms leading to 15.12 % of crops affected. The findings further showed that the prevalence increased to 65.1 % and 80.7 % in 2014 and 2015, respectively (Fig. 5). According to the field observations and respondents all commercial hybrids and OPVs are equally affected by MLND except slight tolerance of BH661 at few areas.

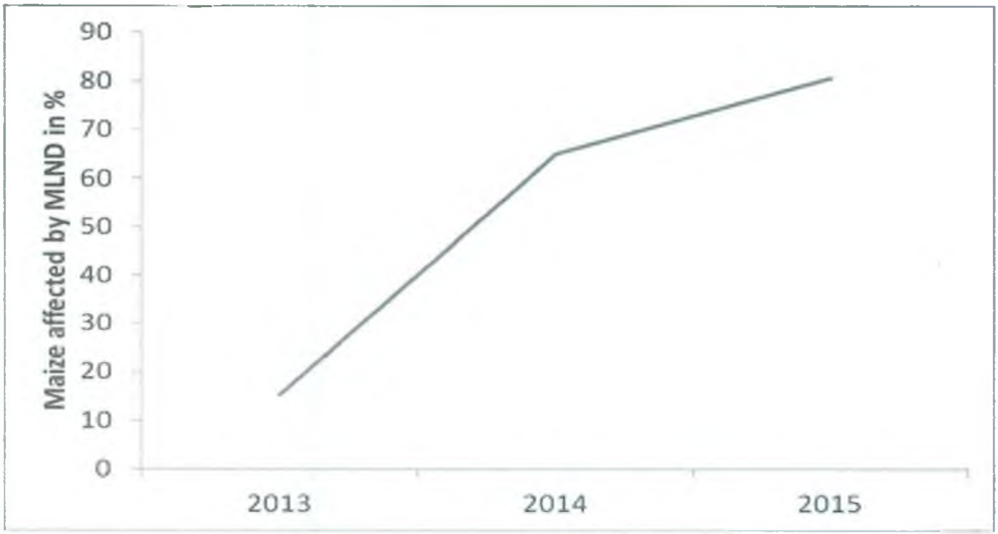

Figure 5. Prevalence rates in percentage of maize crops affected by MLND from 2013-2015.

Discussion

Maize Lethal Necrosis Disease (MLND), caused by co-infection of SCMV and MCMV, is one of the most threatening diseases of maize in countries where the disease has been reported (Carrera-Martinez *et al.* 1989; Jiang *et al.* 1992; Xie *et al.* 2011; Wangai *et al.* 2012; Adams *et al.* 2014). Co-infection of maize by SCMV and MCMV in Ethiopia represents a serious threat to small scale farmers, seed producers and to the local maize industry in general. The Ethiopian government has undertaken several interventions aimed at reducing the impact of the disease such as formation of a task force and monthly technical consultative forum, convening a regional workshop on the management of Maize Lethal Necrosis Disease, surveillance and monitoring of Maize Lethal Necrosis Disease spread, testing of maize genotypes for tolerance and conducting sensitization programmes across the country. The results of this study confirmed the importance of MLND and, for the first time, quantified its incidence, severity and the estimated loss it causes. The study also documented the geographical distribution of the disease. Commercial varieties or hybrids with resistance to MLND are not yet developed in Ethiopia. The spread of the disease on top of its recent outbreaks in Kenya and other parts of Africa have increased the interest of international breeding programs in the development of resistant cultivars (Nelson *et al.* 2011; Redinbaugh and Zambrano 2014). Since the genetics underlying resistance to SCMV has been studied extensively, current efforts are focused in identifying tolerant and resistant sources to MCMV and MLND together (Nelson *et al.* 2011; Redinbaugh and Zambrano 2014; Mahuku *et al.* 2015). Our study showed that all commercial and local maize varieties were more or less affected by the disease and no resistant variety were observed in all the assessed regions.

It is not clear what factor(s) may have contributed to the sudden surge of MCMV in Ethiopia. Our weak quarantine service, porous border, a combination of continuous use of susceptible hybrids, lack of crop rotation and/or increase of insect populations responsible for virus transmission could be the reason behind this outbreak. SCMV, a potyvirus, is primarily transmitted by several aphid species in a non-persistent manner; whereas for MCMV, at least two insects have been identified as vectors: beetle species of the genus Diabrotica (Nault *et al* 1978) and thrips in the genus Frankiniella (Cabanas *et al.* 2013; Zhao *et al* 2014). In addition, recent studies have demonstrated seed transmission rates of up to 4.8 % for SCMV (Li *et al.* 2007). For MCMV, a report concluded seed transmission rates of up to 0.33 % (Jensen *et al.* 1991). However, recent RT-PCR virus testing on RNA extractions from individual and pooled seeds obtained from MCMV-infected plants in Kenya revealed the presence of the virus in up to 72 % of the seeds (Mahuku *et al.* 2015). Although important, these results did not prove the presence of the virus in the progeny since MLND virus contained on all parts of the seed are not transmitted to the next generation plant except it is borne in the germ. In Ethiopia, however, test of seed samples directly from commercial seed producers and leaf from grow out tests maintained under controlled conditions showed the presence of MLND in 4 (0.02%) seeds out of the tested 26,400 seeds. Taken together, these findings highlight the need to re-assessing the rates of seed contamination and subsequent transmission of MCMV in all hybrids and OPVs commonly grown in Ethiopia. It is well known that even very low rates of seed transmission can have significant epidemiological impacts when an efficient vector is also present (Maule and Wang 1996). Our study revealed that several thrips, aphids and beetles were observed on symptomatic plants. Hence, the identification of the vector(s) responsible for MCMV transmission under field conditions is imperative. A preliminary study using virus-free maize as sentinel plants, under high disease pressure in Ecuador revealed that MCMV and SCMV were transmitted within a week of field exposure. Insect monitoring on newly infected sentinel plants showed high populations of thrips (*Frankliniella* spp.) and aphid species including *Rhopalosiphum maidis* and *Myzus persicae* (Quito-Avila *et al.* 2016).

Conclusion and Recommendations

The disease could become a serious problem and compromise the sustainability of maize production in the country and possibly the region. MLND risk in Ethiopia is high and hence the need for better allocation of resources in management of MLND, with special emphasis on Oromia and SNNP region, which are hotspots as inoculums source for other less affected regions. The rapid spread of MLND in Ethiopia and the potentially enormous threat to food security and trade has aroused the interest of governments, national and global research organizations, and the private sector, culminating in several initiatives. While several short-term interventions have been suggested, the more sustainable long-term solution appears inclined towards the development of MLND resistant/tolerant maize varieties.

The success of this endeavor calls for a facilitative legal and policy environment including explicit governmental support for deployment of modern breeding techniques, including the use of biotechnology. Given the importance of MLND, the damage it does, and its devastating effect on the livelihood of many poor subsistence farmers, urgent action is needed. Since the development of resistant cultivars or hybrids is a long-term alternative for managing the disease, agronomic practices (crop rotation and weed control) combined with effective control of insect vectors should be implemented as the frontline to reduce the impact caused by the disease. The following recommendations are made based on the findings of this study: Farmers should avoid growing maize in consecutive seasons; diversify crops planted and practice crop rotation with non-cereal crops such as beans or other legumes, onions, pumpkins, bananas, potato and sweet potato; Farmers should avoid continuous/stagger cropping and be aware of early planting time to avoid spreading of the MLND; promote good agricultural practices (fertilizer, timely weeding and control of vectors by seed dressing chemicals followed by foliar application) to boost plant vigor and eliminate alternate hosts for MLND vectors; farmers whose fields are affected should get rid of the crop (minimize inoculums buildup). and also movement of affected crop debris or material from one region to another should be stopped to minimize the incidence rates.

Acknowledgments

The authors would like to thank maize growers in all surveyed regions, and the Regional Bureau of Agriculture and Plant Health Clinic for their support during field trips and providing invaluable information. We gratefully acknowledge financial support provided by Association for Strengthening Agricultural Research in Eastern and Central Africa (ASARECA) and the Ethiopian Institute of Agricultural Research (EIAR). We thank Ambo Plant Protection Research Center (APPRC) for analyzing the samples.

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