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Maize Lethal Necrosis Disease (MLND) – A Review

Temesgen Deressa* Girma Demissie
National Maize Research centre; Ethiopian Institute of Agricultural Research (EIAR), P.O.Box 03, Bako, Ethiopia

Maize lethal necrosis is a serious disease of maize in Africa from its first appearance in Kenya. In Ethiopia the disease was observed during in the previous assessment and it was confirmed by ELISA test. The disease has now gained the momentum in spreading to many countries of East African where maize crop is grown simply because of insufficient knowledge on how to manage the disease. It has therefore raised a major concern in Eastern Africa communities because of the effect associated. The disease is naturally known to affect varieties of maize resulting in chlorotic mottling of the leaves, severe stunting and necrosis which as a result 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. Maize Lethal Necrosis disease is caused by double infection with Maize chlorotic mottle virus (MCMV) and any of the cereal viruses in Potyviridae group; Sugarcane mosaic virus (SCMV), Maize dwarf mosaic virus (MDMV) or Wheat streak mosaic virus (WSMV). Highly affected areas may experience a massive yield loss of over 95% and this will affect total maize yield produced in the region. Evidence from previous studies indicated that most farmers had little knowledge on MLND and its control mechanisms. Further evidence showed that, there was limited research on crop diseases and more particularly in maize lethal necrosis disease. This paper deals with detailed review on the salient sequence of events associated with the occurrence of MLND, the present understanding on vector control-crop management experiences and findings all over the world that could potentially used to prevent spread of MLND in Ethiopia.

Background

Maize lethal necrosis was first identified in the USA in 1976 (Niblett and Caflin, 1978). MCMV is a new virus for Africa; it had not been reported previously in Kenya, but was first identified in Peru in 1973 (Castillo and Hebert 1974) and subsequently reported in the USA, parts of Latin America, and China (Niblett and Claflin 1978; Uyemoto 1983; Xie *et al*. 2011). Wangai *et al*. (2012a) reported MCMV and MLN in Kenya for the first time. A serious new disease of maize appeared in the farmers' fields in eastern Africa in 2011. Called maize lethal necrosis (MLN; or corn lethal necrosis, CLN), it can devastate maize crops. Infection rates and damage can be very high, seriously affecting yields and sometimes causing complete loss of the crop. In general the spread of Maize Lethal Necrosis (MLN) in the maize growing regions of Eastern Africa has intensified since the first outbreak was reported in September 2011 in Kenya.

The disease is difficult to control for two reasons:

1. It is caused by a combination of two or more viruses that are difficult to differentiate individually based on visual symptoms.
2. The insects that transmit the disease-causing viruses may be carried by wind over long distances.

In Africa, the disease was first reported in Kenya, (South Rift Valley - Bomet and Naivasha districts) in September 2011 although its extent at that point suggested that the disease has been present for some time. According to the Kenyan Ministry of Agriculture, two percent of the maize harvest was affected in 2012. MLND has also spread rapidly into Tanzania, Uganda, Rwanda and South Sudan in the meantime. There is a preliminary report of the disease in Uganda (IPPC, 2014). In Ethiopia the disease was first observed in the Rift Valley. After the disease was identified by ELISA test in June 2014 agricultural experts have been travelling to Kenya to assess the damage done by the disease and to learn more about it. In Ethiopia, although its first epidemics was reported in Central rift valley in 2014 main season (Mahuku et al. 2015), MLND attack has now been confirmed in all major maize growing areas of the country. Predictions made using genetic algorithm model estimated that Ethiopia has the potential to lose its entire maize area (E Isabirye and Rwomushana, 2016). A series of meetings and training have also been held to tackle the disease. Extension workers are now being sent out to tell farmers to use certain chemicals to eliminate pests that might spread the problem.

National and global research and extension organizations, laboratories, and seed companies are working together to control the spread of the disease and to develop and deploy disease-resistant maize varieties for the farmers as soon as possible.

Hosts/Species Affected

The experimental host range is restricted to the Poaceae with maize as the main natural host (Gordon et al., 1984). The following species have been infected by mechanical inoculation: *Bromus* spp., *Digitaria sanguinalis, Eragrostis trichodes, Hordeum* spp., *Panicum* spp., *Setaria* spp., *Sorghum* spp. and *Triticum aestivum* (Castillo and Hebert, 1974; Niblett and Claflin, 1978; Bockelman et al., 1982) and *Zea mays* subsp. *mays* and *mexicana* (Castillo and Hebert, 1974). The Kansas serotype 1 also infected *Zea mays* subsp. *parviglumis* and *Zea luxurians* (Nault *et al*., 1982).

When and how are maize plants infected?

Maize plants are susceptible to MLN at all stages in their growth, from seedling to maturity. As with all viral diseases in plants, a carrier known as a "vector" transmits the MLN causing viruses from plant to plant and field to field. MCMV is carried by thrips and beetles (Nault *et al.* 1978; Jiang *et al*. 1992) and SCMV by aphids (Brandes 1920; Pemberton and Charpentier 1969). Transmission of MCMV via seed from infected plants is normally very low (0.04%; Jensen *et al*. 1991).

Symptoms

Maize chlorotic mottle virus (MCMV) causes a variety of symptoms in maize depending upon genotype, age of infection and environmental conditions. They range from a relatively mild chlorotic mottle to severe stunting, leaf necrosis, premature plant death, shortened male inflorescences with few spikes, and/or shortened, malformed, partially filled ears (Castillo and Herbert, 1974; Niblett and Caflin, 1978; Uyemoto *et al*., 1981).

When MCMV co-infects maize with a potyvirus, the infected plants in the field show a diverse range of symptoms. Diseased plants develop symptoms characteristic of virus diseases. There is chlorotic mottling of the leaves, usually starting from the base of the young leaves in the whorl and extending upwards toward the leaf tips. The leaves can experience necrosis at the leaf margins that progress to the mid-rib resulting in drying of the whole leaf. If there is necrosis of young leaves in the whorl before expansion, then 'dead heart' symptoms will be visible. Other symptoms include premature aging of the plants and mild to severe leaf mottling. Severely affected plants form small cobs with little or no grain set. The entire crop can frequently be killed before tasseling (Niblett and Claflin, 1978; Uyemoto *et al*., 1980, 1981; Wangai *et al*., 2012a,b).

Biology and Ecology

The disease is caused by a combination of two viruses, *Maize chlorotic mottle virus* (MCMV) and *Sugarcane mosaic virus* (SCMV), a pathogen prevalent in many parts of Kenya affecting cereal crops. The double infection of MCMV and SCMV or any of the cereal viruses in the Potyviridae group (e.g. *Maize dwarf mosaic virus* or *Wheat streak mosaic virus*) gives rise to what is known as maize lethal necrosis disease (MLND), also referred to as corn lethal necrosis disease (CLND) (Niblett and Caflin, 1978; Uyemoto *et al*., 1980, 1981; Wangai *et al.*, 2012a,b).

MCMV is transmitted mechanically and spread by several insect vectors including maize thrips (*Frankliniella williamsi*) (Jiang *et al*., 1990), maize rootworms (*Diabrotica undecimpunctata, Diabrotica longicornis* and *Diabrotica virgifera*), cereal leaf beetles (*Oulema melanopus*), corn flea beetle (*Systena frontalis*) and *Chaetocnema pulicaria* (Nault *et al*., 1978; Jensen, 1985). SCMV is spread by maize aphids (Brandes, 1920). Seed transmission of MCMV has been reported by Jensen *et al*. (1991).

Infection of maize by any of the viruses alone does not cause MLND. Symptoms of MLND are more severe than the additive symptoms of either MCMV or the potyvirus virus alone. The virus complex causes a severe systemic necrosis which culminates in the death of the plant (Niblett and Caflin, 1978; Uyemoto *et al*., 1980, 1981; Wangai *et al*., 2012a). MLND is mainly spread by a vector, transmitting the disease from plant to plant and field to field. The most common vectors are maize thrips, rootworms, leaf beetles and aphids. Hot spots appear to be places where maize is being grown continuously.

Impact of MLND

Maize lethal necrosis disease (MLND) is a serious threat to maize production. In Kansas, crop losses due to MLND have been estimated to be 50-90% (Niblett and Claflin, 1978; Uyemoto *et al*., 1980) depending on the variety of maize and the year. In Peru, losses in floury and sweet maize varieties due to *Maize chlorotic mottle virus* have been reported to average between 10 and 15% (Hebert and Castillo, 1973).

In Kenya, in areas where MLND was very serious, farmers experienced extensive or complete crop loss (Wangai *et al*., 2012a). The infected plants are frequently barren; the ears formed are small, deformed and set little or no seeds, drastically reducing the yield. The areas affected constitute major maize production acreage and given the recorded loss of up to 100%, it has become an important food security issue in Kenya. How severe are farm-level crop losses? Infection rates and damage can be very high, seriously affecting yields and sometimes causing complete loss of the crop (Wangai *et al*., 2012b; Adams *et al*. 2012). Infected plants are frequently barren; ears formed may be small or deformed and set little or no seed.

The impact of the disease can been felt in the whole maize value chain. To help control MLND, the maize seeds have to be dressed with an insecticide in addition to a fungicide seed dressing. Seed producers have incurred an extra cost in the production of seed maize.

Prevention and Control
Seed inspectors can check for MLND in seed farms. A plant health inspectorate organization can test for MCMV in all seed coming into the country including the material for breeding. Domestic regulation can be put in place to prevent the movement of maize products from affected areas to disease-free regions.

The public can be informed about the disease through press releases, posters, brochures, sensitization workshops and radio programmes. Information on the disease could be passed on to the public during field days and Bazaras in churches. Awareness of the disease will help farmers to take it upon themselves to avoid the movement of diseased plant material from one area to another by destroying affected crops, rouging and practicing general field hygiene.

Based on MLND management experiences in the USA, rigorous disease management practices in seed production plots, including use of resistant varieties, controlling weeds/alternate hosts, keeping unnecessary machines/people out of the field, controlling insect-vectors using appropriate insecticide (at weekly intervals), and having adequate isolation from MLN-infected fields, can prevent the spread of the disease. Because individual plants with MCMV or SCMV alone show milder symptoms, seed production fields must be carefully inspected and plants that appear infected removed immediately. The best approach for the management of MLND is to employ integrated pest management practices encompassing cultural control such as closed season, crop rotation and crop diversification, vector control using seed treatment followed by foliar sprays, and host-plant resistance.

Cultural Control and Sanitary Measures
Crop rotation can effectively control MCMV (Uyemoto, 1983). Producers are advised to practice crop rotation for at least two seasons with alternative non-cereal crops such as potatoes, sweet potatoes, cassava, beans, bulb onions, spring onions, vegetables and garlic. Planting different crops each season will diversify farm enterprises. Manure and basal/top dressing fertilizers can be applied to boost plant vigour.

Intercropping maize with beans and other legumes regulates pests (leafhopper, leaf beetles, stalk borer, and fall armyworm) and increases the land utility and abundance of natural enemies. Recommended legumes for intercropping in Kenya are beans, pigeon peas, cowpeas, groundnuts and soybeans. Other crops that have been tried with varying success include potatoes, cassava and pumpkin.

It is necessary to use good field sanitation methods, including weed control measures to eliminate alternate hosts for potential vectors (Wangai *et al*., 2012b). Infected foliar material should be removed from the field to reduce pathogen and vector populations. This material can be fed to livestock, but grain and cobs that are rotten should not be fed to humans or animals. These should be destroyed by burning. Seed should not be recycled; farmers should plant certified seed only.

To create a break in maize planting seasons, plant maize on the onset of the main rainy season and not during the short rain season. This will reduce the population of vectors. Before MCMV had spread to other islands in Hawaii, it had been controlled for several years in the island of Kaua'i. (Nelson *et al*., 2011; Ooka *et al*. 1990).

Chemical Control
Vector control should target soil borne and early season vectors and combine long residual and fast-acting control agents to achieve faster knockdown and longer protection. Imidacloprid is applied as a seed dressing in combination with foliar sprays. In Hawaii producers of maize seed spray regularly after planting to control insects that spread the virus (Nelson *et al*., 2011).

The maize aphid or corn leaf aphid (*Rhopalosiphum maidis*)
It is dark green to bluish-green in colour with black cornicles. Particularly during dry/periods the colonies appear on the inflorescences and young leaves. Feeding by this aphid causes yellow mottling, but this damage is seldom of economic importance. Their role as vector of the sugarcane virus, maize dwarf mosaic virus and maize leaf-fleck virus makes them a pest of considerable importance. This aphid usually attacks maize plants at the end of the mid-whorl stage. Aphid colonies may completely cover emerging tassels, and the surrounding leaves, preventing pollen release. In severe outbreaks the ear shoot is also infested, and seed set may be affected.

Conserve natural enemies. Aphids have a wide range of natural enemies, which normally keep them under control. Use broad-spectrum insecticides like Imidacloprid 70% WG. Recommended Dosages per hectere @ 21 – 25 gm of active ingredients in dilution with 300 – 500 lts of water or as being recommended by the local authorities.

The maize leaf hoppers (*Cicadulina* spp.)
The adults are about 3 mm long, slender and cream to pale yellow green in colour. These leafhoppers have two small black spots between the eyes and brown marks behind the eyes extending along the body. They have

brown lines along the wings. They usually hop away when disturbed. The direct damage cause by maize leafhoppers by sucking plants is insignificant, but the indirect damage is high because they transmit the maize streak virus, a major disease of maize. *Cicadulina mbila* is the most important vector.

Control of the maize leafhoppers is difficult since they are very active, remain infectious for a long time and are very quick in transmitting the virus. Plant maize well away from grassland or previously irrigated cereals; in particular, avoid planting downwind of such areas. The numbers of leafhoppers generally increase in irrigated cereals and grasslands or in wild grasses during rainy seasons. Leafhoppers disperse away from these areas when dry. Plant early and if possible planting in an area should be carried out at the same time. Staggered planting of crops will favour multiplication of leafhoppers and increase the risk of virus transmission to later plantings. Keep the fields free from weeds, in particular grasses. Leave a barrier of 10 m of bare ground between maize fields and previously infested crops. This is reported to reduce virus incidence, by restraining movement of leafhoppers. Remove residues of cereal crops since they serve as infection sources. They are attracted to bright green surfaces, so can be caught in sticky green traps. Use Imidacloprid insecticide at recommended dosages per hectare of 21 – 25 gm a.i. in dilution with 300 – 500 lts of water.

Thrips

Thrips are present season long in leafy vegetables, but are usually most abundant during the spring after temperatures begin to increase. They are most important in head, leaf, romaine and baby mix lettuces, cabbage and spinach because of the cosmetic scarring they cause to leaves and contamination of harvested plant parts. Thrips can build up in weedy areas, and other surrounding crops, moving to lettuce in large numbers when host plants begin to dry down. Further, once adults disperse onto plants, they can readily reproduce and rapidly colonize in high numbers. We are uncertain what the developmental rate of thrips is on leafy vegetables and maize, but field observations suggest that they can complete development from egg to adult in less than 3 weeks when temperatures are near 70^0F.

Cultural management has only a limited impact on thrips populations because of their ability to rapidly disperse from native vegetation, weeds and crops. Further, there are few natural enemies that feed on them. Consequently, control with insecticides is often the only viable control alternative. The following points should be considered when attempting to chemically manage thrips populations:

Several products are registered that when used in combination will provide efficacy with limited residual activity (Lannate, Ammo, Othene, Karate, Endosulfan, Imidacloprid and Dimethoate). *Note:* Always consult the products label before applying any insecticide. Frequency of applications will depend on residual of products and immigration of adults from surrounding vegetation. In recent trials, the most efficacious insecticides tested were only able to maintain thrips populations at constant levels, and did not reduce numbers significantly. This should be taken into consideration when determining when to treat. Plant size and temperature may be important factors contributing to the efficacy of these products. The larger the plant, the more difficult it is to obtain good coverage underneath the leaf and near the base of the plant Also, higher temperatures drive thrips development, but may also influence their activity to more readily come in contact with the insecticides.

Host-Plant Resistance

Use of tolerant or resistant varieties ultimately would be the most effective means of managing MLND. Superior resistance to MCMV is widely available in tropical maize seed stocks and provides the best control for this disease. According to Nelson *et al*. (2011), trials performed in Hawaii in 2011 found many tropical inbred lines and varieties to be highly resistant to MCMV. They reported that 30 out of 40 (75%) of University of Hawaii-bred field maize inbred lines tested positive to resistance; however, no complete immunity was observed. Almost all temperate climate inbred lines and hybrids are highly susceptible to the virus (Nelson *et al*., 2011).

The level of MCMV resistance varies widely among pure lines that have been tested in Hawaii, so it is considered a quantitative trait (Nelson *et al*., 2011). Preliminary inheritance studies on the inheritance of traits suggest a polygenic control of the disease, with resistance being partially dominant. This encourages the commercial production of hybrids only if both parents are resistant to the pathogen.

In Kenya, varieties are being screened for resistance/tolerance by KARI and CIMMYT in two sites Naivasha and Bomet. Preliminary data from 43 pre-commercial maize hybrids and seven commercial hybrids at Bomet, Chepkitwal and Naivasha, and of 200 elite inbred lines at Naivasha, during one season of screening under natural disease pressure, suggest that MLN-resistant maize germplasm can be identified and developed quickly. KARI, CIMMYT and other partners will reconfirm the potential resistance of pre-commercial hybrids and inbred lines that show the lowest susceptibility to MLN and work urgently to develop resistant varieties (Makumbi and Wangai, 2013). As MLND is due to the co-infection of two viruses, resistance against any one of the viruses would substantially reduce the damage due to the disease. Results of a trial of elite CIMMYT inbred lines under artificial SCMV inoculation showed several highly-resistant lines (Makumbi and Wangai, 2013).

In the long run, deployment of varieties that are resistant to both MCMV and SCMV will be the best means of managing MLND. Through breeding, both conventional and transgenic maize seeds, resistance to MCMV can be incorporated into the susceptible maize varieties.

Conclusion

The rapid spread of MLN in the Eastern African Region 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 MLN resistant maize varieties. The success of this endeavour calls for a facilitative legal and policy environment including explicit governmental support for deployment of modern breeding techniques, including the use of biotechnology and molecular biology.

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