Background on MLN (Maize Lethal Necrosis)

(history, causal pathogens, transmission, disease symptoms, yield losses)

Workshop on Survey and Sampling for MLN Pathogens; March 3-4, 2016; CIMMYT, Harare

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Distribution of MCMV and MLN

Maize lethal necrosis has occurred fairly rarely and sporadically around the world over the past 40 years.

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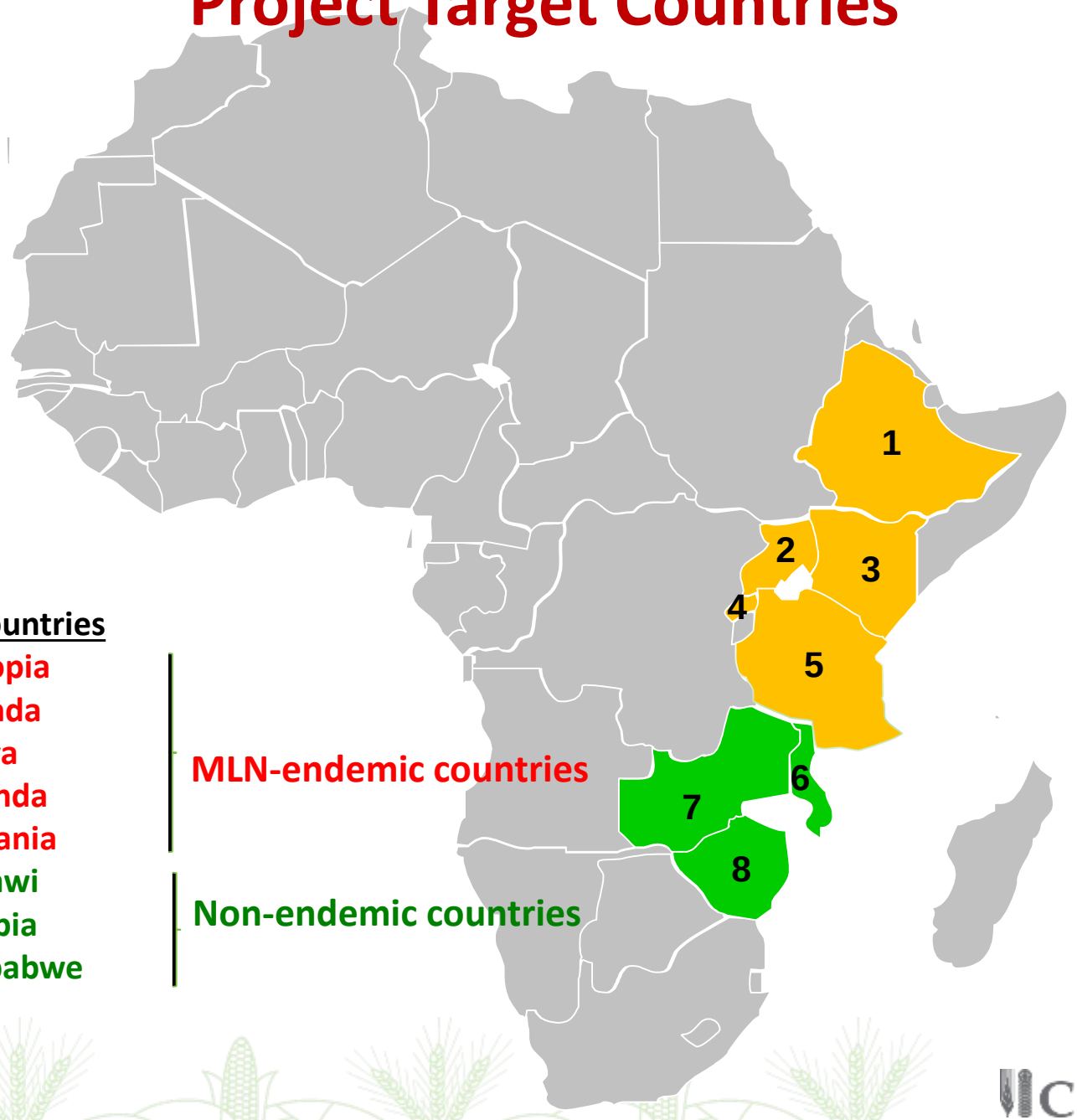
Slide from Peg Redinbaugh

Project Target Countries



Target countries

1. Ethiopia
2. Uganda
3. Kenya
4. Rwanda
5. Tanzania
6. Malawi
7. Zambia
8. Zimbabwe

MLN-endemic countries

Non-endemic countries

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Global occurrence of MCMV/MLN

Country	MCMV / MLN	Year	Reference
Peru	MLN	1973	Castillo and Hebertt (1974)
USA	CLN	1976	Niblett and Cafflin (1976)
Argentina	CLN	1982	Teyssandier et al. (1982)
Mexico	MCMV/MLN	1987	Delgadillo and Gaytan (1987)
Thailand	MCMV	1983	Cited in Uyemoto (1983)
Brazil	MCMV	1983	Cited in Uyemoto (1983)
China	MLN	2011	Xie et al. (2011)
Kenya	MLN	2012	Wangai et al. (2012)
Tanzania	MLN	2012	CIMMYT Task Force Report to Ministry of Agriculture, Tanzania
Uganda	MLN	2012	Godfrey Asea's presentation in MLN Workshop (Nairobi; Feb 12-13, 2013)
Rwanda	MCMV	2013	Claver Ngabiyasonga's presentation during MLN Training Workshop (Nairobi; July 1, 2013)
Ethiopia	MLN	2015	George Mahuku et.al., December 2015, Volume 99, Number 12 Page 1870 http://dx.doi.org/10.1094/PDIS-04-15-0373-PDN

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Emergence of MLN in eastern Africa

- **September 2011:** Disease first reported in the lower parts of Longisa division of **Bomet District** of Kenya.
- **February 2012**: Noticed in Bomet Central division, spreading into neighboring Chepalungu, Narok North, Narok South and **Naivasha Districts of Kenya.**
- **April 2012**: Disease further spread to Sotik,, Koinon, Transmara, Rumuruti, Kisii, Bureti, Kericho, Mathira East, Imenti South and **Embu Districts** of Kenya
- **In Tanzania**: Lake zone (Mwanza and Musoma), Manyara, Arusha and Moshi

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MLN is a viral disease caused by combined infection of maize with Maize Chlorotic Mottle Virus (MCMV) and any of the Potyviruses infecting cereals, especially Sugarcane Mosaic Virus (SCMV)

The disease was first reported in Africa, particularly in Kenya in Sept 2011, and since then reported in Uganda, Tanzania, Rwanda, D.R. Congo, and Ethiopia.

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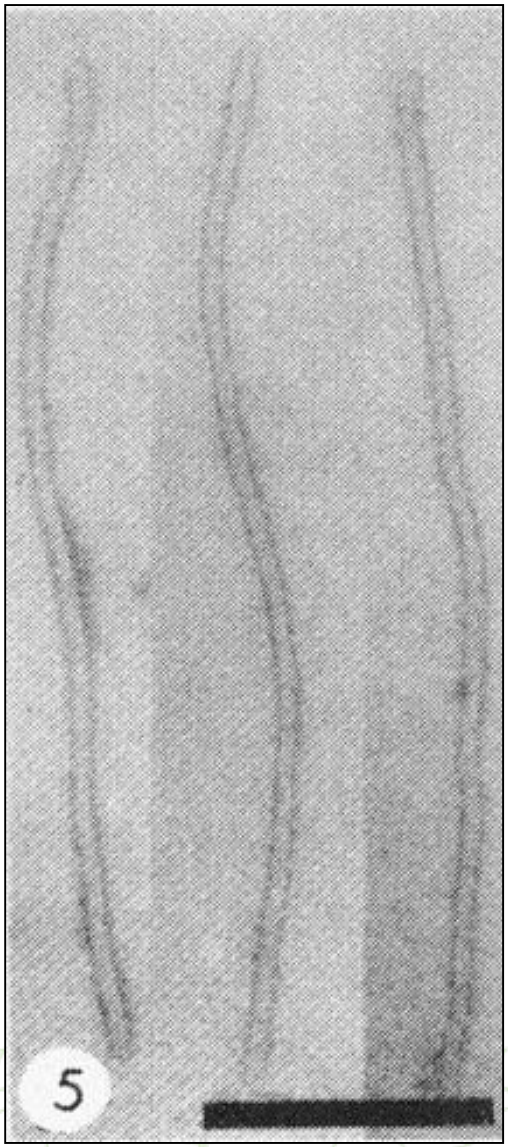
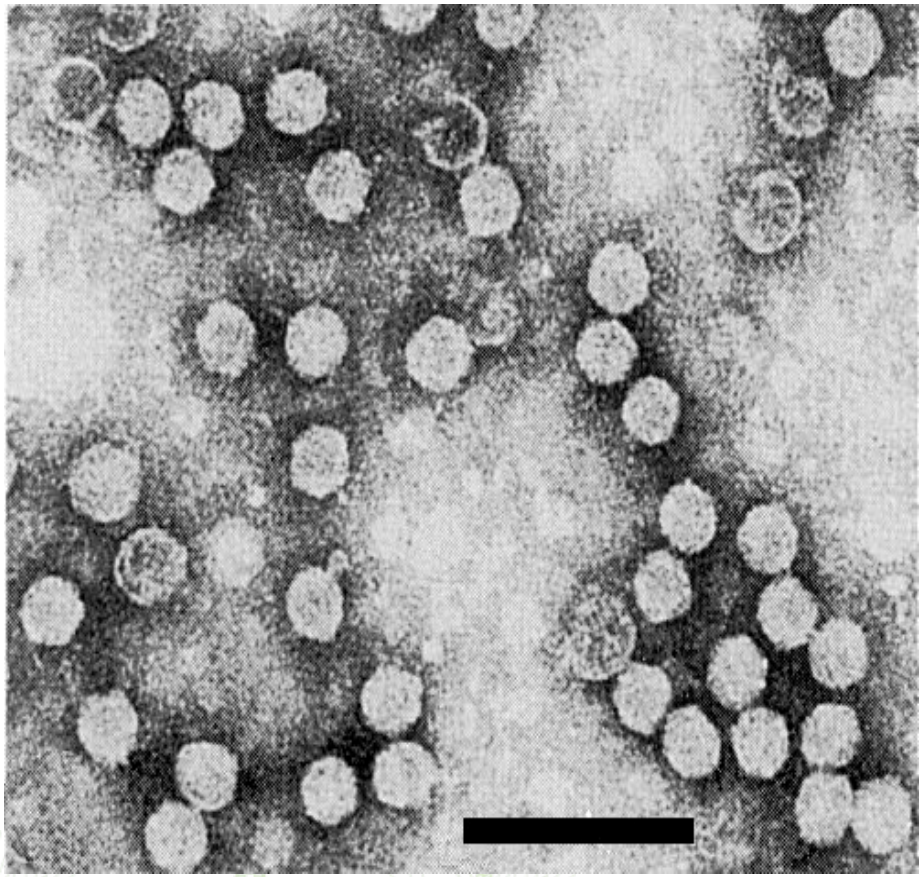
Sugarcane mosaic virus (SCMV)



Maize chlorotic mottle virus (MCMV)



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Disease Symptoms

Symptoms of the disease

- Symptoms observed vary widely depending on;

  -Germplasm

  -Time of infection

  -Prevailing environmental conditions

  -Ratios of the viruses infecting the plant

- The symptoms can easily be confused with drought , micro- nutrient deficiency or stalk borer infestation

Symptoms in artificially inoculated maize plants in screen house

MCMV

SCMV

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Early Symptoms

Mild mosaic and mottling

Mild mosaic and mottling

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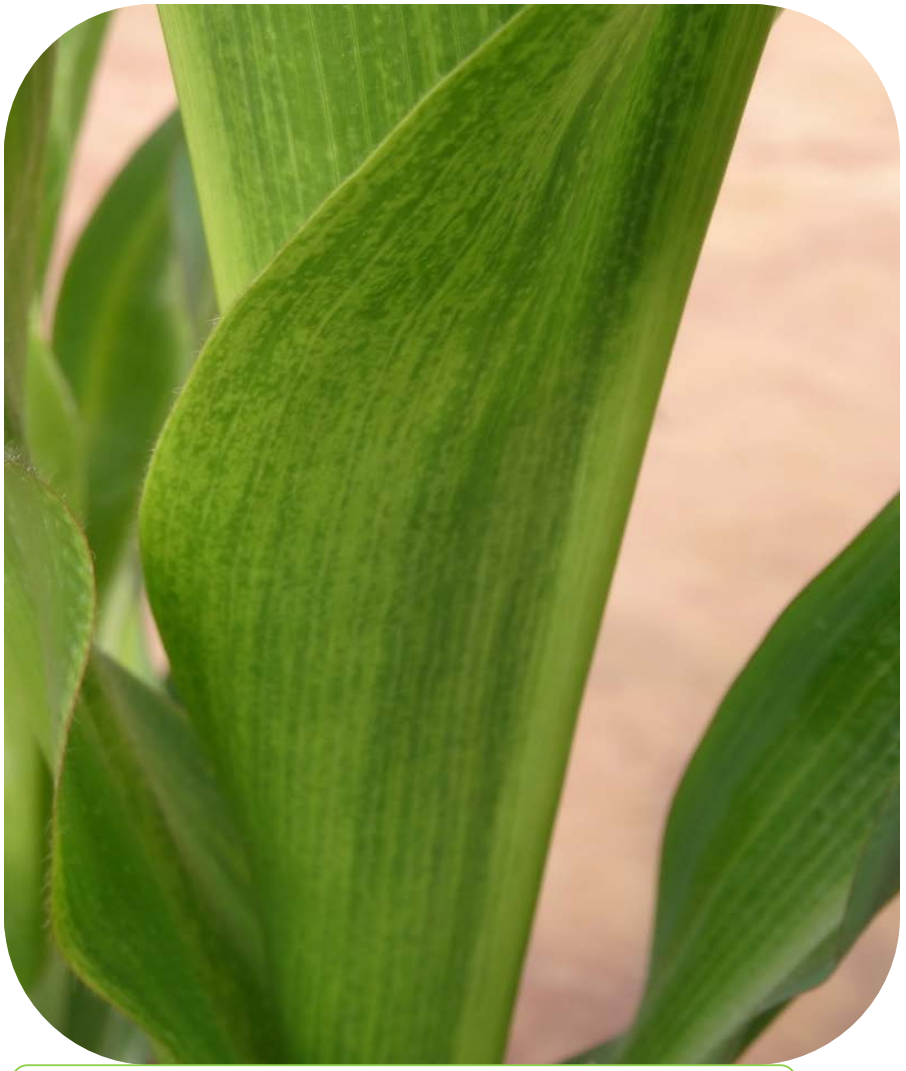
Early MLN Symptoms

Chlorosis and Mottling



Diffuse mottling and chlorosis

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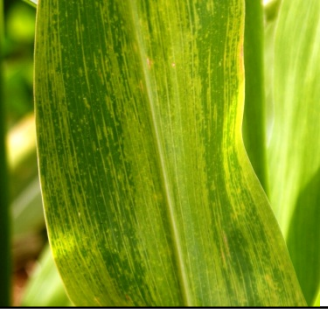
Severe chlorosis and leaf necrosis



Shortened internodes and severe chlorotic mottle

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‘Dead Heart’ symptoms

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Premature drying of the husks

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Poor or no grain filling

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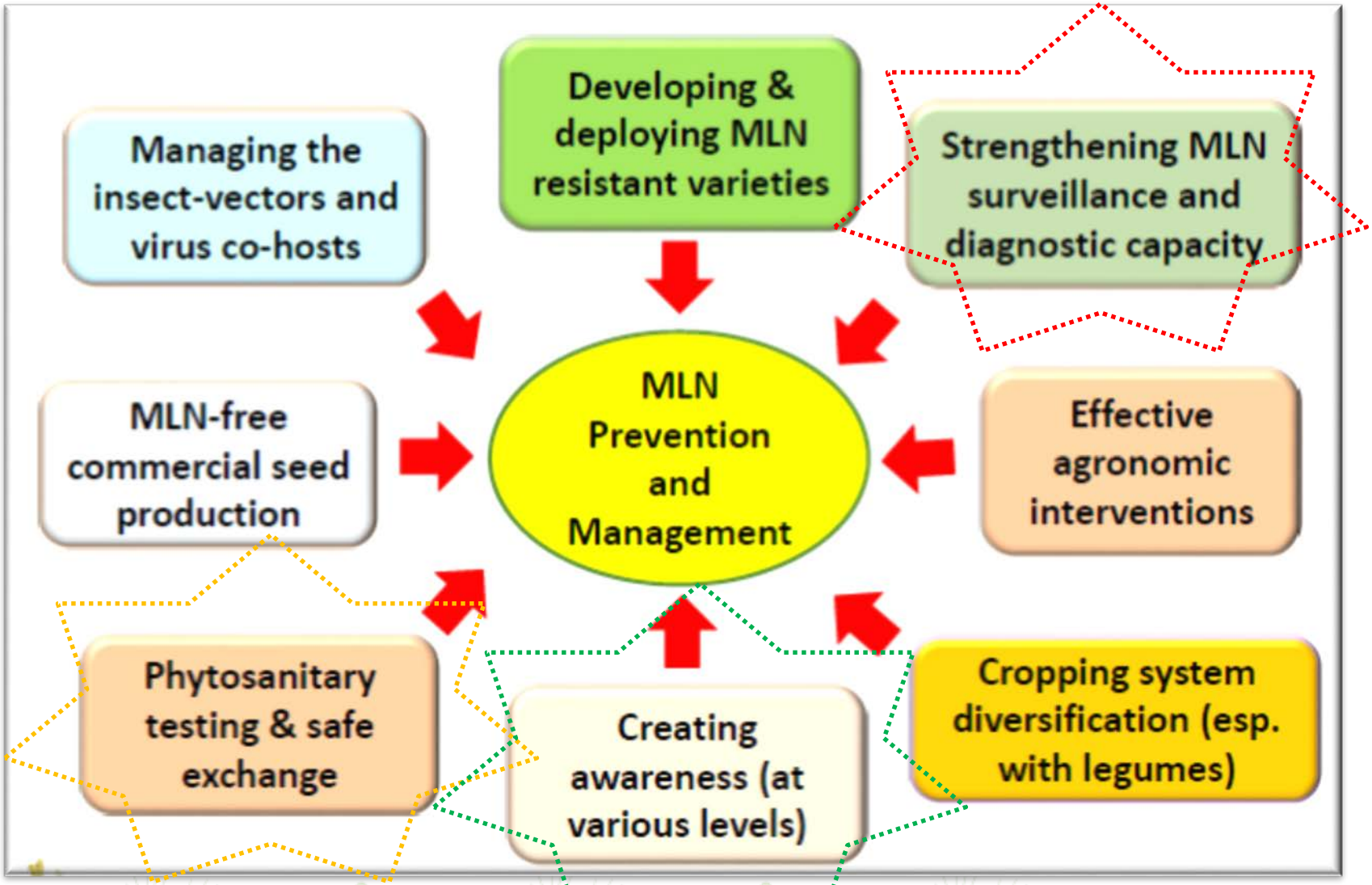
We need to tackle the MLN challenge on diverse fronts



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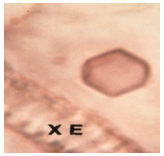

VIRULANT PATHOGEN

VECTOR

SUSCEPTIBLE HOST

DISEASE

CONGENIAL ENVIRONMENT

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MLN Transmission

Can be transmitted through:

1. **Infected seed:** reported transmission rates

- MCMV = 17/42,000 plants (0.04%)
- SCMV = 21/72,897 plants (0.03%)

2. Insects

- Aphids (*Aphis gossypii, Myzus persicae)*
- *Corn Thrips(Frankliniella williamsi)*
- Corn flea beetle (*Chaetocnema pulicaria*)
- Southern corn rootworm (*Diabroticaundecimpunctata*)
- Northern corn rootworm (*D. lonicornis*)
- Western corn rootworm (*D. virgifera*)
- Flea beetle (*Systenafrontalis*)
- Cereal leaf beetle (Oulemamelanopa)

3. Infected soil and plant debris

4. Mechanically

Corn thrips(*Frankliniella williamsi*)



DESCRIPTION OF THE PEST

Thrips are small insects, about 0.04 inch long. Immature thrips are wingless, whitish to yellowish in color, and are most commonly found in whorls, tassels, ears, or on the underside of leaves. Adults emerge continuously throughout the warm months. Adults and immatures may be found in corn at any time during the growing season. Eggs are deposited in plant tissue and hatching occurs in about 5 days during the summer months; the immature stages take about 5 to 7 days to complete development.

DAMAGE

Thrips are most noticeable and of greatest concern at two periods during the corn growing season: on young seedling plants and a2t ear formation. On young seedlings their feeding makes the plants look stunted. A common sign of a heavy thrips infestation is distorted leaves that turn brownish around the edges and cup upward. Usually the plants will grow away from the problem, just as they outgrow severe ragging resulting from wind damage. At ear formation, thrips and thrips injury to developing kernels provides entry for infection by *Fusarium* spp. The actual thrips injury does little damage; however, the ear rot diseases can be devastating.

Foliage-feeding thrips are effective predators on early-season spider mite infestations. Both adult and immature thrips may be found in spider mite colonies feeding on spider mite eggs.

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Corn thrips(*Frankliniella williamsi*)

MANAGEMENT

Treatment is usually not necessary on seedlings because plants recover from thrips injury. Thrips are also beneficial at this time because of their role as mite predators. No threshold has been established for damage from thrips at ear formation.

Biological Control

Minute pirate bugs *(Orius tristicolor)* play a major role in controlling thrips populations.

Cultural Control

Thrips populations tend to build up on weeds. Cultivating nearby weedy areas before corn emerges will reduce the potential of a thrips problem when the weeds begin to dry out. Cultivating weedy areas after corn emergence will increase thrips problems.

Corn flea beetle (*Chaetocnema pulicaria*

Adult

The corn flea beetle is a tiny pinhead-sized [1.6 mm) in length black insect with thickened hind legs that enable it to leap considerable distances when disturbed. Flea beetle eggs are deposited on weeds, corn, other cultivated crops, or in the soil near the host plant. Larvae hatch, feed on the roots of plants, pupate in the soil and emerge as adults

Damage

Badly damage plants appear frosted. The corn flea beetle feeds on corn leaves by stripping away the top layer of plant tissue. This feeding leaves gray to brown lines or "tracks" etched on the leaf surface. Heavily infested plants may appear gray as their leaves shrivel and die.

Southern corn rootworm
(*Diabroticaundecimpunctata*)

larvae go through three instars, or developmental stages, that each lasts seven to ten days.
The first instar is less than 1/8 inch in length. By the third instar larva can measure up to 1/2 inch in length.
After the larvae have completed the three developmental stages (instars), they will pupate in the soil. The pupal stage is a dormant stage when no feeding takes place. During this stage the larva is developing into an adult.

Flea beetle (*Systenafrontalis*)



Adult

Shiny black adults are nearly twice as long as With a hands lens and good light, you can see the reddish head. Antennae are light colored near the head and dark near the tips. The enlarged femur on the hind leg allows adults

Eggs

Pale yellow eggs are laid singly in the soil.

Larvae

Creamy white larvae have a brown head capsule, three pairs of jointed legs and can get to just under a ½" at maturity. The last body segment has a fleshy upward projection that has hairs on the tip

Cereal leaf beetle (*Oulemamelanopa*)

Adult cereal leaf beetles are about 5-mm long and have metallic bluish-black heads and elytraEggs are about 1-mm.
Eggs darken and turn black as hatching approaches. Larvae are yellowish-orange, but this color is usually obscured by a layer of feces and mucus, giving them a shiny appearance.
The larval stage passes in about two weeks. Mature larvae burrow into the ground to pupate. Adults emerge about two weeks later, and will feed in small-grain and corn fields for a short time before remaining inactive for most of the summer.
Adult feeding in small grains and corn has not been shown to be economically significant and targeting adults

Corn Leaf Aphid
Rhopalosiphum maidis

Heavily infested corn leaves may wilt, curl, and show yellow patches of discoloration. When tassels and silks are covered with honeydew, the pollination process may be disrupted.

The corn leaf aphid is a blue-green or gray, soft-bodied, spherical insect about the size of a pinhead (1.6 mm) in length].
It has approximately 9 generations per year.
Female corn leaf aphids do not lay eggs, as do most other insects, but give birth to living young.
These young, called nymphs, resemble the adults except that they are smaller and are sexually immature.
Adults and nymphs can often be found clustered within the whorls or upper parts of corn plants over isolated or wide areas of a field.

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Controlling the Spread and Impact of MLN in SSA

A project funded by USAID

- 4 years (Oct 2015 – Sept 2019)
- CIMMYT, NPPOs in ESA, AGRA & AATF, in active interface with the commercial seed sector

Objectives:

1. Prevention of spread of MCMV through seed from the endemic to non-endemic areas/countries in SSA
2. Supporting the commercial seed sector in MLN-endemic countries in producing MCMV-free commercial seed, and promoting the use of clean seed by the farmers

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Effective MLN surveillance and monitoring using harmonized protocols

- Maintaining effective surveillance programs in farmers' fields as well as seed production fields in MLN endemic countries
- Maintaining effective surveillance programs at point of grain entries in non-endemic countries
- Preventing the spread of the MLN pathogens, especially MCMV from MLN-endemic to non-endemic countries

Affordable, reliable and user-friendly diagnostics options for MLN

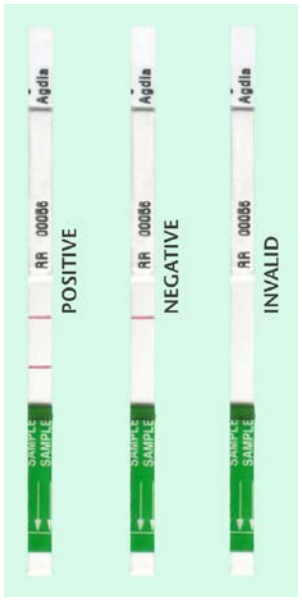
- User-friendly, simple-to-use, cost-effective,robust, and rapid as well as a long shelf life and appropriate specificity
- Besides seed growers and producers, rapid and easy-to-use pathogen MLN pathogen detection methods are also needed by regulatory agencies, exporters, importers, and extension agents.
- Sampling schemes and economic thresholds need to be established to effectively minimize MLN pathogen spread without negatively impacting maize trade.

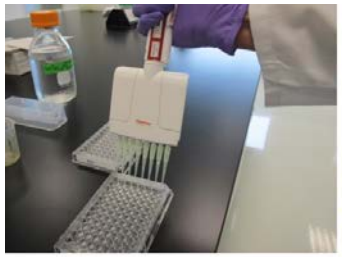
Comparison of MLN Pathogen Diagnostic Techniques

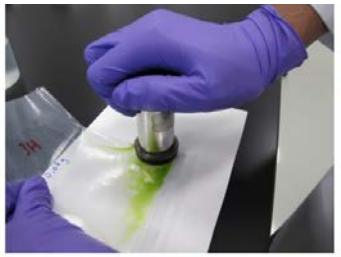
TECHNIQUE	ASSESMENT	REPRODUCIBILITY	TIME	LABOR	COST/SAMPLE
ELISA	VIRAL ANTIGEN	GOOD	HOURS	MODERATE	MODERATE-HIGH
IMMUNOSTRIPS	VIRAL ANTIGEN	GOOD	LESS THAN 30 MINUTES	MODERATE	MODERATE-HIGH
Loop mediated isothermal amplification (LAMP)	VIRAL GENE	EXCELLENT	HOURS	LOW, EXPERIENCED	MODERATE-HIGH
PCR	VIRAL GENE	EXCELLENT	HOURS	HIGH, EXPERIENCED	HIGH

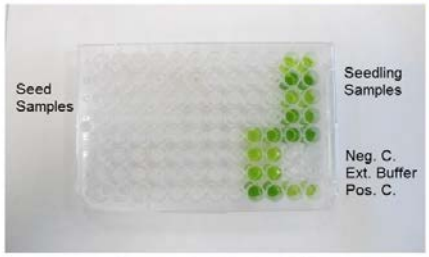
MLN Diagnostics

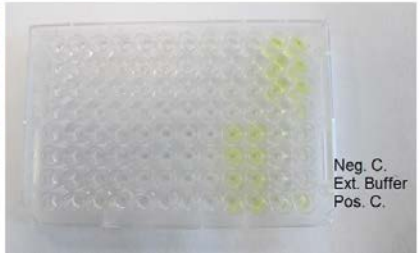
- Immunostrips can be used for surveillance of MCMV/SCMV in the field, followed by confirmation tests using ELISA.
- ELISA tests are more applicable when testing large number of samples
- RT-PCR necessary for research on virus and vector diversity











Effective Detection of MLN Pathogens in Seed Lots

ELISA

- Can be a reliable and routine test for testing large amount of seed and for intense seed exchange activities.
- Does not give information on pathogen viability.
- Testing seedlings with ELISA may not be reliable due to the uncertain seed transmission rate, and not due to the sensitivity of the kits.
- 95% confidence level seems to be acceptable for the detection of MCMV/SCMV in seed with current ELISA kits available in the market.

PCR is necessary when:

- Does not give information on pathogen viability.
- Confirmation of a doubtful ELISA result is necessary.
- MLN pathogen is reported for the first time in an area.

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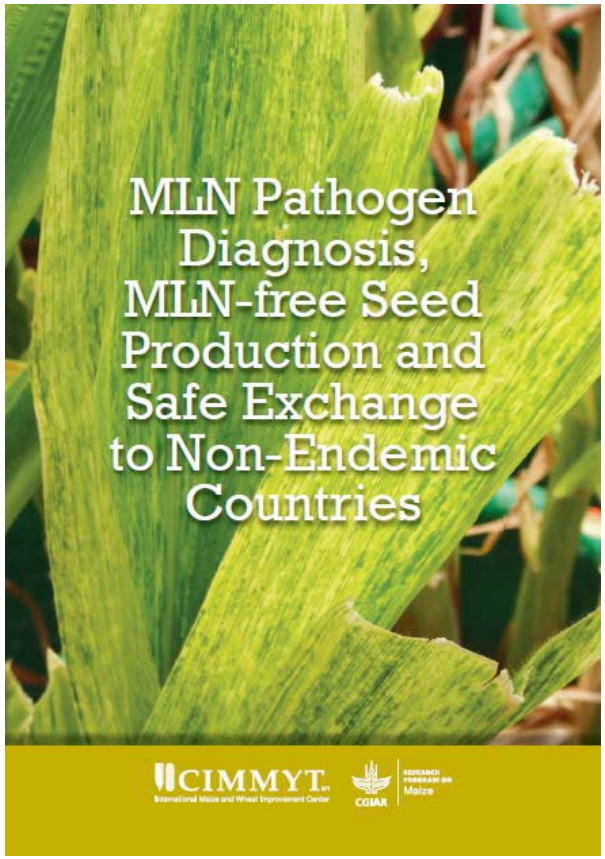
Stepping-up surveillance in MLN-free major maize-growing countries in southern and West Africa

Country	# of seed companies exporting commercial seed	Countries where seed is exported to	Estimated commercial seed export (in tons)
Zambia	6	DRC, Tanzania, Mozambique, Botswana, Angola, Swaziland, Malawi, Kenya, Rwanda, Ethiopia	3500
Malawi	5	DRC, Tanzania, Mozambique	2000
Zimbabwe	3	Angola, DRC, Tanzania, Mozambique, Malawi	1500

Ensuring MLN-free seed production and exchange

- Immediate steps must be taken to prevent further spread of MLN, especially MCMV, through contaminated seed from the MLN-endemic to non-endemic areas in Africa
- Seed companies should implement **voluntary MCMV control programs** and SOPs to minimize the risk of seed transmission
- Identify and maintain **MLN disease-free seed production zones**
- **Training key staff** on MLN management and diagnostics
- Evaluate the presence of **MCMV in commercial seed lots** meant for exportation to countries where MLN/MCMV is reportedly absent.

Building local capacity for MLN diagnostics and management



A CIMMYT brochure published and disseminated to partners in Africa in May 2015, with **protocols** for:

- MLN-free seed production
- MLN quarantine site in non-endemic countries
- Seed and leaf sampling for MLN pathogen testing
- Detecting MLN-causing viruses

Aerial View of MLN Screening Facility at Naivasha

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MLN Screening Facility established by CIMMYT-KALRO at Naivasha, Kenya

Screening against MLN under artificial inoculation

Season starting	Location	Entries	Total Rows
Nov 2012	Olerai and Sunripe Farm	2,636	5,272
June 2013	Oleria and Marula Farms	8,021	16,042
March 2014	Naivasha Facility	19,539	39,078
Oct 2014	Naivasha Facility	15,322	20,356
Nov 2015	Naivasha Facility	17,000	30,000
	Total	**62,518**	**110,748**

Bad News:
Close to 90% of materials screened are susceptible under artificial inoculation.

Good News:
At least 10% are offering tolerance/resistance to MLN.

Approximately 65% of entries are inbreds

First-generation MLN-tolerant hybrids released and being scaled-up for delivery to farmers in eastern Africa

Country	Variety	Released by	Seed company	Remarks
Uganda	**UH5354**	NARO	NASECO	Released in 2013, and already being commercialized. Over 20 tons of certified seed harvested by Aug 2015.
	UH5358	NARO	To be decided by NARO	Recommended for release in 2015.
Kenya	**H12ML**	KSC	KSC	Released in 2013. KSC will produce basic seed in 2016, and expect to commercialize the hybrid in 2017. Promotion of the hybrid in progress.
	H13ML	KSC	KSC	Bulking of seed in progress; commercialization possible in 2017/2018.
Tanzania	**HB607**	Meru-Agro	Meru-Agro	Released in 2014. The company is planning to produce basic seed in 2016 and commercilize the hybrid in in 2017 .Promotion on progress.

2nd generation MLN Resistant Hybrids (Naivasha; 2016)

15 second-generation CIMMYT-derived MLN resistant hybrids are presently under NPTs in Kenya, Tanzania and Uganda.

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MLN Phenotyping Service to Partners (2015-16)

Source of germplasm	# of germplasm entries	# of rows
NARS in SSA	2568	5144
Private sector	3651	4390
Total	**6,219**	**9,534**

Private Sector

S. No.	Institution
1	SeedCo
2	Western Seed
3	Pannar
4	Monsanto
5	Pioneer
6	NASECO
7	Syngenta
8	Tanseed
9	Gicheha Farm
10	Victoria Seed

Public Sector

S. No.	Institution
1	KALRO-Kenya
2	EIAR-Ethiopia
3	ZARI-Zambia
4	RAB-Rwanda
5	KEPHIS
6	Makerere Univ.
7	NARS-South Sudan

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Thanks to…

- USAID, Bill & Melinda Gates Foundation, SFSA and CRP MAIZE, for supporting the work on MLN
- KALRO, NARES and Private sector partners
- CIMMYT-Africa Team involved in the USAID-MLN Project