# Maize Lethal Necrosis (MLN):

## A Technical Manual for Disease Management

**Editor**
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CIMMYT MR
International Maize and Wheat Improvement Center

CGIAR RESEARCH PROGRAM ON Maize

In collaboration with international and national research and development partners

## Chapter 8

# MLN Pathogen-free Commercial Seed Production: Standard Operating Procedures

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## 1. Need for MLN/MCMV-free Commercial Seed Production and Deployment

The preceding chapters have shown the extent to which viruses that cause MLN can be transmitted through contaminated maize seeds, thereby contributing to the spread of MLN disease within and across countries. Formal and informal seed movement locally and across borders plays a significant role in the spread of transboundary diseases like MLN. Therefore, diagnostic tests for MLN viruses at various stages during seed production and before shipment of seeds is crucial.

Quality seed production leading to regulator's approval as certified seed is key for effective management of MCMV/MLN. Even if a 0.01% infection in a seed field of about 55,000 plants per hectare is missed, this would mean about 5-6 infected plants, which then become sources for further transmission by insect-vectors. If this infected seed production field is harvested, and the seeds find their way to the farmers' fields, in the presence of vectors like thrips and aphids, not only the entire field but also the village itself will be at the risk of devastation by MLN (especially when the farmers plant MLN-susceptible varieties). Therefore, MLN threshold levels in commercial maize seed production fields during field inspections should be close to zero (≤1% infection) from the perspective of infected plants. Fields with higher infection levels should NOT be certified for sale of seed to the farmers. At the seed level, the stringency level should be even higher: zero tolerance for any contaminated seed.

Seed producers/growers, therefore, need to maintain high levels of quality crop management during commercial maize seed production, beginning from field practices to processing. This also calls for the importance of harmonization of seed laws across borders, riding on regional platforms such as EAC, COMESA, SADC, etc. to ensure that the threshold/tolerance levels for MLN in seed fields are the same for ensuring free movement of seed without the risks of MLN spread.

During the early years of MLN outbreak in eastern Africa, most of the local/regional seed companies in the MLN-prevalent countries lacked necessary knowledge of the disease and its transmission, as well as protocols to produce MLN pathogen-free clean seed. Recognizing this critical gap, the African Agricultural Technology Foundation (AATF), the Alliance for a Green Revolution in Africa (AGRA) and CIMMYT, under the USAID-funded MLN Diagnostics and Management Project (2015-2019), worked intensively to: (i) support the commercial seed sector in Kenya, Uganda, Tanzania, Rwanda and Ethiopia in the production of MCMV/MLN-free seed throughout the breeder-foundation-certified seed value chain, and (ii) promote the use of certified clean seeds by farmers in respective countries.

## 2. A Harmonized MLN-free Commercial Seed Production Checklist

To ensure MCMV/MLN-free commercial seed production and access to the certified seeds by end users, there was a need of developing country-specific harmonized checklists for MLN control and management (Annex 1). We, therefore, focused on the development of harmonized MLN management checklists, with proven standard operating procedures (SOPs) for production of MCMV/MLN-free commercial maize seed by the seed sector in MLN-prevalent countries. The SOPs were adapted from the protocols successfully used by major maize seed companies in the USA which produce large quantities of maize seed in MCMV-prevalent areas, especially Hawaii. These protocols were appropriately refined and customized to suit respective eastern Africa countries', including targeted agro-ecologies, existing seed laws, governance and local agricultural pest management practices. The SOPs are expected to guide breeders and seed producers/growers for production and exchange of MCMV/MLN-free seed.

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Representatives of key seed stakeholders in respective countries in MLN-prevalent countries in eastern Africa were systematically involved in the formulation of the SOPs. The stakeholders included representatives of seed companies, contract seed producers/growers, breeders, plant pathologists, national seed trade associations, NPPOs and the Ministry of Agriculture comprising Crop Protection, Inspectorate and Plant Health departments. Discussions revolved around assessing the practicability of the SOPs with a view of identifying key practices that were adoptable according to the country seed laws and common practices, how best to popularize the SOPs, and how to mitigate possible challenges that could arise during the implementation of the SOPs. After these consultative meetings, official communiques were prepared as agreed by the participants. Harmonised checklist with SOPs (Annex 1) along with an ODK-based survey questionnaire (Annex 2) were formulated and used in five MLN-affected countries (Ethiopia, Kenya, Rwanda, Tanzania and Uganda).

# 3. Fostering Adoption of Harmonized Checklist and SOPs for MLN-free Commercial Seed Production

A major strategy that was used to foster adoption of the SOPs was the involvement of key seed stakeholders in respective countries during the development stage. This instilled ownership of the document by the stakeholders especially seed companies and contract growers, and hence quick adoption and use. Buy-in of the National Plant Protection Organizations (NPPOs) in respective countries was also key to include MLN tolerance thresholds in seed certification procedures. Other strategies for the adoption and use of SOPs are listed below.

## 3.1. Training of Seed Companies and their Contract Growers on SOPs

A total of 574 participants from NPPOs and NARS institutions, 544 participants from commercial seed companies, and 2313 small-scale contract seed growers in eastern Africa were trained during 2016-2019 on the SOPs for MLN-free seed production. The course content included on-farm MLN diagnostics, disease scouting, leaf and seed sampling, and testing using immunostrips and ELISA.

Most seed companies conduct their seed production using contract growers. It was therefore important to conduct training of not only seed companies but also their contract growers to assist them in understanding and agreeing on the contents of the harmonized checklists for proper implementation. Special focus was given to practices which required hands-on attention; stakeholders were therefore taken to the maize fields for trainings. Some of these trainings included MLN symptoms identification, field scouting and detection using rapid diagnostic kits among others. Rapid MLN diagnostic kits (MCMV immunostrips) were procured and distributed in small quantities as trial kits to the seed companies following individual trainings on their use to facilitate internal quality assurance and early detection of MLN. Seed companies were also encouraged to make private procurement of the kits for their continued MLN surveillance programs.

## 3.2. Data Collection Tools

Constant follow-up missions with seed companies and their contract growers are key to ascertain the status of MLN in seed production fields, level of adoption and use of the SOPs, and further gauge the effectiveness of the SOPs to seed production. These follow-ups aid in identifying challenges towards adoption and use of the SOPs as well as for devising mitigative actions to ease the use of these SOPs.

Hence, detailed data collection tools were developed and encrypted into the Open Data Kit (ODK) tool (ODK) for real-time monitoring and data collection. From the analysed data, most effective practices as perceived by stakeholders were: a) observing cropping and disease history before planting; b) timely planting (at the onset of rains); c) crop rotation with legume/non-cereal crops; and d) having maize-free windows (2-3 months) in a year. The most challenging practices, on the other hand, were: a) cleaning vehicles and farm equipment before and after use; b) soil testing (sited as expensive); and c) ensuring that the source of basic seed is MLN-free.

## 3.3. Engagement with Farming Communities

The expected output from the MLN-free commercial seed production initiative is to ensure the end-users i.e., farmers have access to clean MLN-free seed for their planting. Farmer education on MLN management practices is important to prevent MLN viruses' re-infection in farmer fields despite having obtained clean seeds. This is also important because informal seed systems are highly prevalent in SSA, even in a crop like maize (McGuire and Sperling, 2016), including includes farmer-saved seeds. Farmer field schools and trainings were held on MLN management practices, with particular emphasis on obtaining and using clean seed, besides

adoption of MLN-tolerant varieties. Where MLN-tolerant varieties were available, small seed packs of these varieties were distributed in farmer forums in partnership with seed companies to achieve rapid adoption through farmer-led demos.

Farmer sensitization was further conducted by the development and dissemination of relevant Information, Education and Communication materials (IECs) on MLN Management (**Annex 3**). The IEC materials were also translated to Swahili (**Annex 4**) and Amharic (**Annex 5**) for better comprehension by stakeholders, including farmers and maize seed growers. Farmer engagements also included visits to extract first-hand testimonies from the farmers on awareness of MLN disease presence, ability to identify MLN symptoms, routine on-farm agronomic management practices, seed sources, etc.

## 4. Feedback on Relevance, Efficacy and Practicability of the Harmonized SOPs towards MLN-free Commercial Seed Production

- Some seed growers had a challenge differentiating MLN symptoms from symptoms of nutrient deficiency and moisture stress. This caused a delay in rogueing suspected plants at the early stages of crop growth.
- There were some challenges with the use of MCMV immunostrip kits as they required to be stored under refrigeration for long term use; this limited the use of the kits by some stakeholders who did not have refrigeration facilities.
- SOPs recommend testing of harvested maize seed samples. This was not practiced by many small seed companies (except in Kenya) as the requirement is not enforced and there are no facilities available for its implementation in some countries.
- Adherence to different practices differed; for instance, most seed companies observed timely planting (95%) and management of weeds in seed fields, scouting, rogueing, and destroying of infected plants (90%); while practices such as establishing field history based on last MLN disease records (35%) were less implemented. Some SOPs were not much practiced, such as ensuring healthy seed from the source, seed dressing with systemic insecticides specific for MLN-transmitting vectors, and MLN testing of the harvested seed (with a few exemptions).

## 5. Summary of Steps towards MLN/MCMV-free Commercial Seed Production and Lessons Learnt

- A questionnaire for data collection was developed (**Annex 2**), especially targeting seed company personnel and breeders.
- The data collection tool was customized to the ODK app.
- In-country consultants were engaged to individually visit seed companies and/or contract growers to collect information related to MLN disease incidence, severity, production site history, scouting and vector management as well as adoption and use of the MLN management SOPs. The consultants were identified on the basis of understanding the MLN terrain and geographical locations of the seed companies and were engaged in the consultative meetings.
- The field officers were instrumental in identifying the MLN-affected regions in each country, identifying the seed companies/contract growers/breeders etc. operating in each of the targeted countries, and in determining the capacity gaps.
- Rapid diagnostic kits (MCMV immunostrips) were piloted and promoted with selected seed companies and NARS institutions for MLN field testing in order to strengthen internal quality control systems for MLN management. This activity was augmented by on-site training and distribution of 50 MCMV immunostrip testing kits to each of targeted beneficiary institutions.

## 6. Conclusions

While detection of MLN-causing viruses on contaminated seed may not necessarily lead to transmission of the disease to the next generation, from the phytosanitary perspective, it is important to evaluate the presence of MCMV and SCMV in commercial seed lots, especially those meant for exportation to countries where MLN/MCMV is not reported. In practice, keeping a commercial seed production field completely free from the MLN-causing viruses in areas where the disease is widely prevalent requires significant efforts and resources,

but is important for protecting the food security, income, and livelihoods of the resource-poor smallholder farmers (Prasanna et al., 2020). More than 30 seed companies are presently implementing the MLN-free seed production checklist on a voluntary basis in Kenya, Uganda, Rwanda, Tanzania and Ethiopia, and there is scope to further scaling up this to ensure that MLN does not spread to other countries in Africa through commercial seed trade.

## 7. References


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# Annex 1. Harmonized MLN Management Checklist & SOPs (Kenya, as an example).

| HARMONIZED MLN MANAGEMENT CHECKLIST & SOPs (KENYA) | | | | | |
|---|---|---|---|---|---|
| No. | TASK | START DATE | DUE DATE | % COMPLETE | NOTES |
| 1. | Monitor crop disease history of seed production fields to enable adequate control plans | | | | |
| 2. | Maintain adequate levels of soil fertility based on soil tests to ensure healthy crops | | | | |
| 3. | Timely planting to facilitate disease escape and eliminate disease incidence due to late planted crop | | | | |
| 4. | Use of disease free seed stocks in subsequent seed production | | | | |
| 5. | Clean farming equipment to remove contaminated soil debris and minimize spread of disease from one field to another | | | | |
| 6. | Eliminate grasses and other weeds from fields and plot borders to remove vector hosting plants | | | | |
| 7. | Monitor and control insect vector population through tested spraying regimes | | | | |
| 8. | Scout for viral symptoms to ensure early detection and control | | | | |
| 9. | Rogue symptomatic plants and burn/bury to minimize spread of disease | | | | |
| 10. | Sample suspect plants for diagnostic testing within the internal quality control to confirm disease presence | | | | |
| 11. | Post-harvest cob selection and seed testing for MLN causing viruses | | | | |
| 12. | Seed treatment using systemic insecticides to ensure early control of the disease | | | | |
| 13. | Crop rotations with non-cereal crops for 1- 3 seasons depending on disease history | | | | |
| 14. | Closed maize season where appropriate | | | | |

**Name:** ..... **Position:** .....

**Organization:** ..... **Signature:** .....

Ethiopia: Harmonized MLN Management Checklist & SOPs
Tanzania: Harmonized MLN Management Checklist & SOPs
Uganda & Rwanda: Harmonized MLN Management Checklist & SOPs

# Annex 2. ODK Survey Questionnaire for Recording MLN Status, Creating Awareness, and Ascertaining Uptake of MLN Management SOPs and Use of Rapid Diagnostic Kit

1. Name and Location of the Seed Company/ Breeder:

Name of respondent:

Country:

2. Have you experienced MLN infection in your production/ breeding fields? (Y/N) (F*or those who answer NO here please let them proceed with Q5 to Q7 for those who answer YES they go on with 3 and 4 then proceed from 8*)
3. If [Yes], when was the last time you experienced the MLN pandemic?

| Season (Short/Long) | Year |
|---|---|
| | |

4. Do you have exposure to MLN SOP? (Y/N)

   List of SOPs for verification
5. If Q2 is [No], what are your thoughts with regards to the relevance of SOPs in management of MLN (*Alex here please least al SOPs against a relevant and irrelevant checklist*)
6. Which of these SOPs have been included in your quality management system as seed producers? (H*ere list all the SOPs against Y/N*)
7. From your knowledge could you pick the top 4 most effective sops in the control of MLN? (*Alex, only allow for 4 responses here for all the SOPs listed*)
8. If Q4 is [Yes], where was your source of SOP?
   - ☐ AATF/AGRA
   - ☐ Self-generated
   - ☐ Ministry of Agriculture
   - ☐ Other source? Specify
9. How severe was the disease (Scale of 1-5)?

   *1 – Not Severe; *5 – Extremely Severe
10. Did you experience any yield loss caused by the disease? (Y/N)
11. How severe was the yield reduction? (100%, 50%, 30%, 20%, 10%, <10%)
12. Specify the location of the disease (country, County, region, district, location/village)
13. Did the disease cause abandonment of any breeding, testing or seed production sites? [Yes/No]; If [Yes], where_____?
14. If Q10 is [Yes], how was the infected material destroyed? [1=Burning, 2=Burying,3=Fed to livestock, 4=used as grain]
15. Do you have any management practice for the disease? (Y/N)

    If [Yes]: List them *(Tick against SOPs documented at number 4).*
16. What are your top 4 most effective MLN Management practices?

    If [No] in Q15: Why?
17. How often do you monitor your seed production field for MLN symptoms and vectors? *The frequency should be per season (Short and Long seasons)*
18. Have you in the past sampled any suspected MLN plants in your fields for diagnosis? (Y/N)
19. If [Yes], what technique was used to confirm samples for MLN viruses? (PCR, Rapid kits, LAMP, sent to external Lab, I do not know)

20. If Q19 is [sent to external lab] please give the name of the lab?.....
21. Have you used the MLN Rapid Diagnostic Kits? (Y/N)

    If [No] in Q21: Provide a reason:
22. Do you routinely use MLN Rapid Diagnostic Kits? (Y/N)

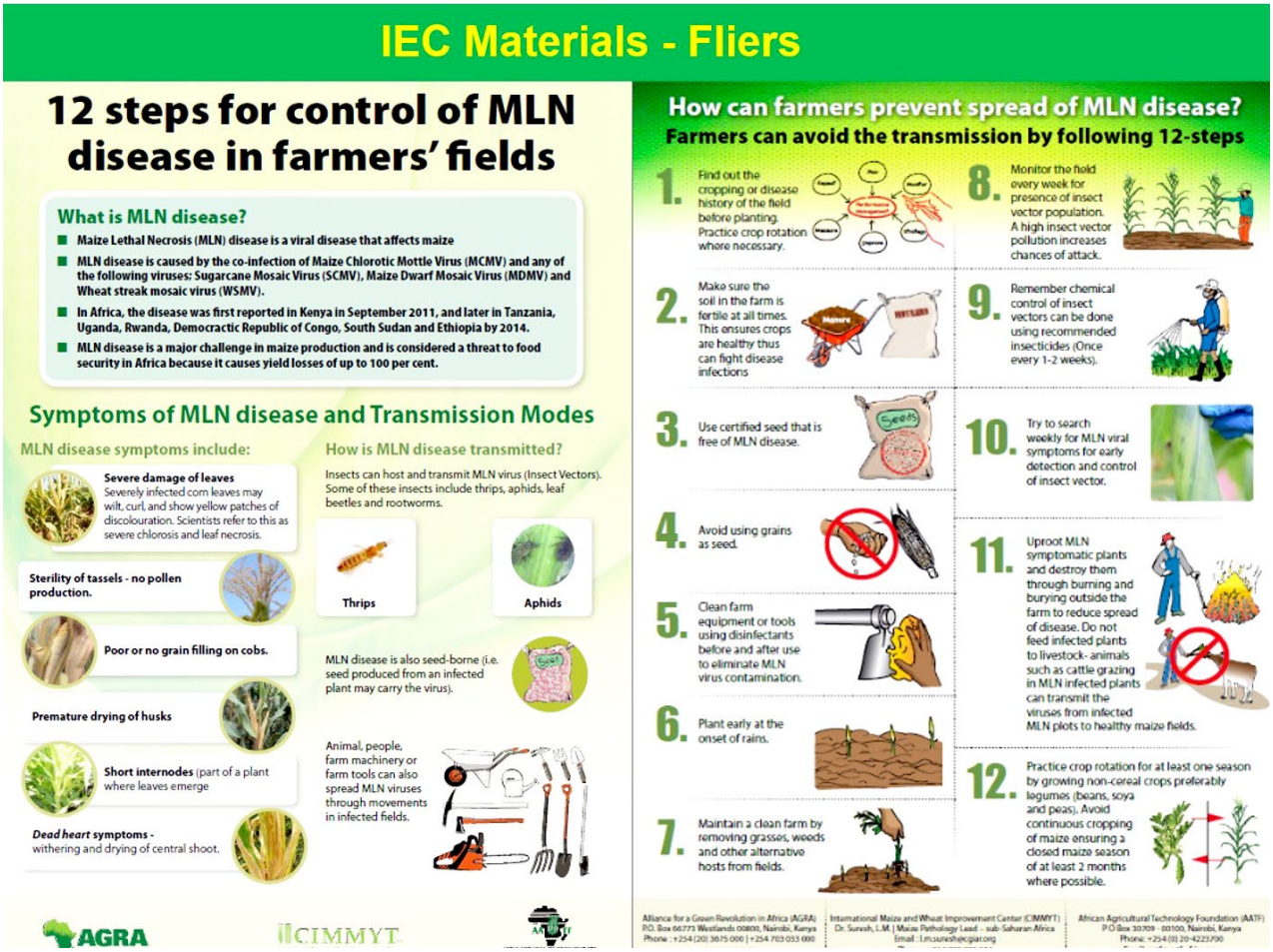
    If [Yes] in Q20 what is the frequency? *(Number of times per season)*
23. Provide a reason for this rate of frequency:
24. How would you rate efficiency of the RPD kits? (Not effective, Effective, Very effective) (Explain)
25. Have you attended a training on MLN diagnostic Kits (Y/N)?

    If [Yes], when and where?

    How many times in the last 5 years?
26. Number of Kits provided by AGRA/AATF
27. How long has your company participated in seed production and commercialization?
28. How long has your company been involved in MLN disease management?
29. Approximately how much is spent on chemicals for controlling MLN insect vector population?
30. What is the total area of land that you use for maize seed production? (in ha)
31. Out of this area what proportion has been affected by MLN disease? (in %)
32. Are your neighbor fields (farmers' fields) infected by MLN disease (Y/N)?

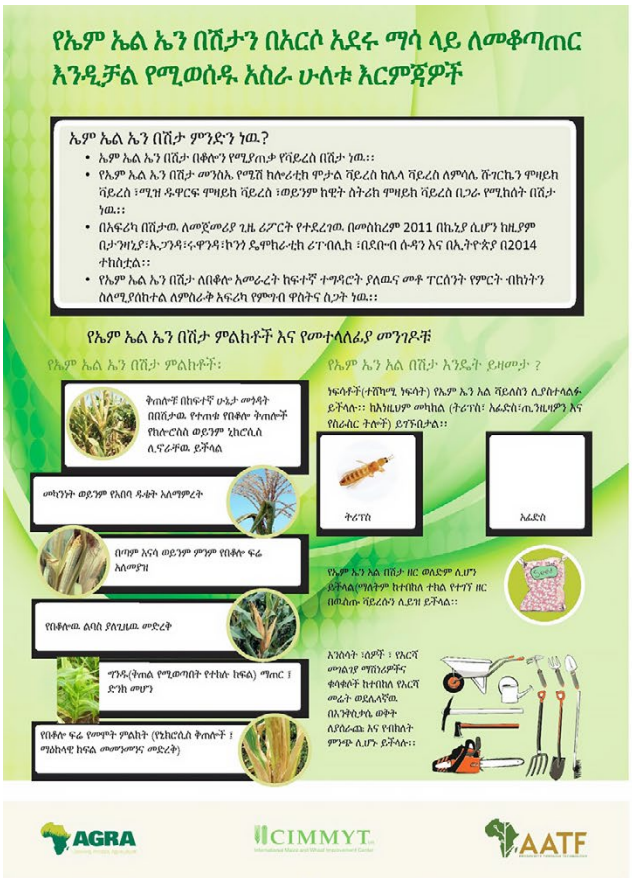
    If [Yes], what measures are used by farmers in controlling it?
33. Do you import any seeds (Y/N)?
34. If q.33 is Yes, where do you import the seeds (maize) from?
35. If q.33 is Yes, what measure do you put in place to ensure you import disease free seed?

## Annex 3. MLN Disease Management in Farmers' Fields.



# Annex 4. MLN Disease Management in Maize Fields (in Swahili)

# Annex 5. MLN Disease Management in Maize Fields (in Amharic).




## Annex 6. Twelve Steps for MLN-free Seed Production (in Spanish).

# Doce pasos para tener semilla libre de MLN

**La Necrosis Letal del Maíz o MLN** (por sus siglas en inglés, Maize Lethal Necrosis) es una enfermedad viral que afecta al maíz.

La enfermedad MLN es causada por una co-infección del Virus del Moteado Clorótico del Maíz (MCMV), y cualquiera de los siguientes virus: Virus del Mosaico de la Caña de Azúcar (SCMV), Virus del Mosaico del enanismo del Maíz (MDMV) y Virus del Mosaico Rayado del Trigo (WSMV).

**1** Conocer el historial de cultivos y enfermedades del campo donde de planea sembrar.


**2** Mantener adecuados niveles de fertilidad en el suelo para asegurar cultivos sanos. Hacer análisis de suelo después de un máximo de tres años, para hacer una selección adecuada de fertilizantes.


**3** Usar semilla certificada libre de MLN en la subsecuente multiplicación de semilla.

**4** Limpiar implementos y maquinaria agrícolas para eliminar residuos contaminados con MLN.

**5** Sembrar al inicio de la temporada de lluvias para facilitar el escape de la enfermedad.

**6** Monitorear frecuentemente el campo para detectar el incremento en la población de insectos vectores. Eliminar pastos, malezas y hospedantes alternativos en el campo.

### Síntomas de la MLN y modos de transmisión

- Daño severo de las hojas. Las hojas severamente dañadas pueden marchitarse, torcerse y presentar manchas amarillas y un secado prematuro. Los científicos se refieren a esto como clorosis severa y necrosis foliar.
- Espigas estériles. No hay producción de polen.
- Pobre llenado de grano, o no desarrollo de grano en las mazorcas.
- Secado prematuro de las hojas que cubren las mazorcas.
- Entrenudos cortos (los nudos son donde crecen las hojas).
- Síntomas de corazón muerto (marchitez y secado del brote central o cogollo).

**7** Controlar a los insectos vectores con insecticidas recomendados y un adecuado régimen de aspersión.

**8** Inspeccionar bien el campo para detectar las primeras plantas sintomáticas, arrancar y destruir las plantas enfermas (quemar y enterrar fuera del campo).

**9** Muestrear las plantas que se sospechen tengan MLN para hacer pruebas de detección. Desinfectar el perforador de hojas después de la toma de muestra en cada planta.

**10** Seleccionar mazorcas y hacer pruebas de detección de virus causantes de MLN en semillas post-cosecha. Eliminar (quemar) semilla infectada.


**11** Tratar semilla usando insecticidas sistémicos para asegurar un control temprano de los insectos vectores y subsecuente enfermedad.

**12** Hacer rotación de cultivo al menos por una estación de siembra, con cultivos no cerealeros, de preferencia leguminosas. Permitir un periodo de al menos dos meses sin siembra de maíz.

### ¿Cómo se transmite la MLN?

- Los insectos son vectores y transmiten el virus de la MLN; estos vectores incluyen trips, afidos, escarabajos foliares y gusanos de la raíz.
- La MLN también es transmitida a través de la semilla, es decir, la semilla producida en una planta enferma puede llevar el virus.
- La gente, los animales, la maquinaria e implementos agrícolas, también pueden acarrear el virus a través del movimiento en los campos infectados.

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