



Methods of Screening Maize for Resistance to Stem Borers and Post-harvest Insect Pests

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Front cover photo:

Top: Maize ears damaged by maize weevil and LGB, showing dust (flour) production on the floor (taken at Kiboko Post-harvest laboratory, ear screening room);

Bottom: Leaf damage and stem tunneling, resulting from feeding by stem borers.

Back cover photo:

Upper left to right: Maize tassel damaged by stem borers, and grain infested by the maize weevil in a jar;

Lower left to right: Population of LGB separated from an infested grain, and an ear damaged by the LGB.

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Acronyms and abbreviations

CIMMYT	International Maize and Wheat Improvement Center
DfID	Department for International Development
ESA	Eastern and southern Africa
GLS	Gray leaf spot
GMP	Global Maize Program
HPR	Host plant resistance
IRMA	Insect Resistant Maize for Africa project
KARI	Kenya Agricultural Research Institute
LGB	Larger grain borer
MSV	Maize streak virus
MW	Maize weevil
NARL	National Agricultural Research Laboratory
OPV	Open-pollinated variety
RF	Rockefeller Foundation

1. Introduction

Maize is a major staple food crop in Africa. However, maize yields in Africa are highly affected by an array of biotic and abiotic stresses. Insect pests, both in the field and in storage, are among the most economically important biotic constraints in maize production and storage. The most economically important insect pests of maize in Africa include stem borers in the field, and maize weevil (MW) and the larger grain borer (LGB) in storage (post-harvest pests). As they do not produce viable tillers, maize plants are less able to tolerate stem borer attack than sorghum and pearl millet plants. The effect on kernel yields is therefore greater. Colonization of the plant by stem borers, severity of infestation and damage strongly depend on the rainfall received, cropping system and soil fertility, which affects the nutritional status of the plant. Stem borer damage is aggravated by drought and other stresses and the poor nutritional status of the plant. Studies on several stem borers in Africa showed that an increase in nitrogen is related to higher pest loads and tunnel damage (Haile and Hofsvang, 2001). However, soil nutrient levels, such as nitrogen, greatly influenced the plant's tolerance to stem borer attack as well. This is due to an increase in plant vigor, which is reflected in lower yield losses (Jiang and Cheng, 2003). Yield losses caused by stem borers damage can average 20–40% in Africa, which means from two to four measures of maize are lost out of every 10 that could be harvested (De Groot *et al.*, 2003).

Currently, there are a number of control measures against the pre- and post-harvest insect pests, but each comes with its own problems or limitations. Farmers often resign themselves to using no control measures at all as cost of buying chemical insecticides is high. Essentially, there are four general approaches to insect control: chemical control, by which we mean the application of insecticides; biological control, which means identifying and introducing natural enemies of the pests into an area; crop management, which includes a broad range of field and crop management techniques; and finally, host plant resistance (HPR), by which the plant offers its own resistance to the insects.

Chemical control is the most commonly used and most effective at farm level. Different insecticides have been recommended for the control of maize pests although these are not accessible to small-scale farmers. An alternative strategy is

to use HPR. HPR uses conventional plant breeding to impart resistance to storage insect pests and is, therefore, the easiest control method for subsistence farmers, as well as the most environmentally safe. However, this method requires large numbers of insects and maize genotypes for screening.

A stem borer mass rearing facility was established at the Kenya Agricultural Research Institute (KARI), Katumani in 1999, with support from the Rockefeller Foundation (RF) and the International Maize and Wheat Improvement Center (CIMMYT) (Songa *et al.*, 2004; Tefera *et al.*, 2010). The main purpose of the mass rearing facility is to supply stem borers for use in maize resistance screening research activities at KARI and CIMMYT laboratory and field trials. The five Lepidopteran stem borers, namely the spotted stem borer (*Chilo partellus* Swinhoe), African stem borer (*Busseola fusca* Fuller), coastal stem borer (*Chilo orichalcocillielus* Strand), pink stem borer (*Sesamia calamistis* Hampton), and sugarcane borer (*Eldana saccharina* Walker), are reared in the insectary, with the bulk production being for *C. partellus* (Figure 1) and *B. fusca* (Figure 2), which are of greater economic importance (Tefera *et al.*, 2010; Tende *et al.*, 2010; Songa *et al.*, 2004). The facility is also used for training students and staff from Kenya and other countries involved in the current Insect Resistant Maize for Africa (IRMA-III Conventional) project activities.



Figure 1. Larvae (left) and adult (right) *C. partellus*

(source:[http://www.cd3wd.com/cd3wd_40/Biovision/export/default\\$ct\\$127\\$scrops.html](http://www.cd3wd.com/cd3wd_40/Biovision/export/defaultct127$scrops.html)).



Figure 2. Larvae (left) and adult (right) *B. fusca*

(source: <http://www.infonet-biovision.org/default/ct/102/pests>)

The Kiboko Post-harvest Laboratory was built in 1990 with funds from the Department for International Development (DfID) of the British Government. This was an outreach laboratory of the Kenya/UK Larger Grain Borer Research Project, coordinated from KARI – NARL (National Agricultural Research Laboratory). The laboratory had two small rooms: one used for rearing insect pests for stored products, and the other as a store. When the project ended in 1994, the facility was handed over to KARI - Katumani but the KARI - NARL post-harvest section maintained its office and research work at the facility.

Maize varietal screening for resistance to storage insect pests—maize weevil (*Sitophilus zeamais* Motsch.), and larger grain borer (*Prostephanus truncatus* Horn) (Figure 3) began in 2001. The first phase of the KARI-CIMMYT - IRMA I Project (1999–2003) rehabilitated the two rooms, stocked glass jars, installed light tables and some 210-liter capacity plastic drums for fumigation. CIMMYT and KARI have continued to use the facility and expanded it to its current capacity that can screen maize kernels in about 10,000 jars in a cycle of three months.

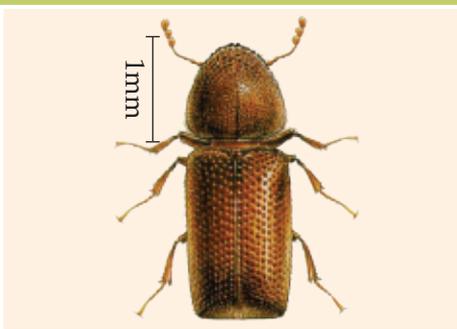


Figure 3. Adult LGB (left) and MW (right).

Source: http://www.fao.org/inpho/content/compand/text/ch23_04.htm

CIMMYT and eight NARs partners (Kenya, Ethiopia, Uganda, Tanzania, Malawi, Zambia, Zimbabwe and Mozambique) have embarked on developing maize germplasm resistant to stem borers and maize resistant to both the maize weevil and LGB for eastern and southern Africa (CIMMYT, 2009). Variety screening studies require a consistent supply of large quantities of insects at specified time periods. Mass rearing of stem borers on an artificial diet offers the most preferred and dependable method of obtaining large and continuous supplies of insects for evaluating plant materials. The stem borer and post-harvest insect-pest rearing facilities have a significant impact on research focusing on breeding for resistance to maize stem borers and post-harvest insects through HPR. The rearing facilities also support national and international research projects aimed at managing maize stem borers and post-harvest insect pests of maize.

Mass rearing of stem borers and post-harvest insect pests of maize was presented in a previous publication (Tefera *et al.*, 2010). This manual will describe the methods used to screen maize genotypes against the stem borers, maize weevil and larger grain borer.

2. Host plant resistance to insect pests in maize

Host plant resistance (HPR) is defined as the collective heritable characteristics by which a plant species can reduce the possibility of successful use of the plant as a host by an insect species (Beck 1965). HPR is available to farmers encapsulated in the seed, which ensures that after purchasing the seed, farmers need not invest in any more inputs to control stem borers and post-harvest pests of maize. In this way, stem borer and post-harvest resistant maize reduces yield losses from stem borers and post-harvest pests, as well as reduces or eliminates the expense of insecticides and their associated health risks (Mugo *et al.* 2001).

HPR to stem borers and post-harvest insect pests is a genetically controlled trait that manifests itself as antibiosis and antixenosis; however, it can also be manifested for stem borers as tolerance but not for post-harvest insect pests. "Antibiosis" is where the biology of the pest is adversely affected after feeding on the plant or the seed. "Antixenosis" or non-preference is where the plant and the seed are not desirable as

a host and the stem borer and post-harvest pests seek alternative hosts. “Tolerance” refers to a situation where the plant is able to withstand or recover from stem borer damage. “Resistance” may be controlled by different allelochemicals that kill or impair the growth of the pest. For instance, phenolic acids have been studied extensively as biochemical components correlated with resistance to the maize weevil through mechanical resistance (cell wall bound hydroxycinnamic acids) and antibiosis (phenolic acids amides) in the pericarp and aleurone layer (Garcia-Lara *et al.* 2004). Morphological factors, including increased leaf fiber, silica, surface wax and high hemicelluloses content have been identified as resistance mechanisms against stem borers (Bergvinson *et al.* 1995).

3. Breeding for resistance to maize insect pests in Africa

Despite the heavy losses caused by stem borers and storage pests in Africa, only CIMMYT’s Global Maize Program (GMP) includes breeding for resistance through the Insect Resistant Maize for Africa (IRMA) Project in collaboration with national partners (Mugo *et al.* 2008, CIMMYT 2011). This is attributed to the genetic and logistical challenges posed by screening and selecting for insect resistance. Pest resistance in maize is usually inherited in a polygenic or quantitative manner, with some influence of environmental factors; therefore, breeding for pest resistance is a time- and research-intensive endeavor. The recent identification of sources of resistance to important insect pests of maize and their incorporation into a limited number of adapted materials could be useful in setting up successful impact-oriented insect resistant breeding programs in eastern and southern Africa (CIMMYT, 2010).

Significant breeding efforts have been made at CIMMYT to incorporate the complex traits into elite maize varieties that are acceptable to African farmers. These efforts have most recently resulted in the development and release of open-pollinated varieties (OPVs) and hybrids in Kenya by the national partners (Mugo *et al.* 2001, Mugo *et al.* 2003, and Mugo *et al.* 2008, CIMMYT 2011). In 2006–2007, three maize OPVs and three maize hybrids with conventional stem borer resistance, and six post-harvest insect pest and stem borer resistant maize hybrids were released in Kenya. In addition, regional and international collaborators in China, Indonesia,

Mali, Nigeria, the Philippines, Peru, Thailand and Vietnam requested and received seeds, in 2006 and 2007, and collaborators in Ethiopia, Uganda, Tanzania, Malawi, Zambia, Zimbabwe and Mozambique are currently testing experimental stem borer and post-harvest insect-pests resistant maize germplasm for evaluation and use in their breeding programs (CIMMYT 2011). Vietnam in particular, identified CIMMYT insect resistant inbred MIRT4AmF101 as combining well with Vietnamese commercial inbred (Vietnam Country Report, RETA No. 6208, 2007). Even though the germplasm does not show extreme resistance to stem borers, it significantly reduces borer damage.

Losses caused by post-harvest insect pests are most likely higher than those caused by stem borers, but the development of maize cultivars resistant to storage pests initially seemed illusionary. Even though resistance found in some local varieties was found to be heritable, breeding progress was slow. This situation changed dramatically with the identification of a few Caribbean germplasm bank accessions (CubaGuard) at CIMMYT that were collected in areas known to be hot spots for LGB in the Americas, where LGB originated, and which showed significant levels of resistance, but with poor agronomic characteristics (Kumar, 2002). Resistant lines were developed from a cross between “CubaGuard” and “Kilima”. Kilima is a Tanzanian OPV which has resistance to LGB (Derera et al., 1999). The “CubaGuard” (accessions Cuba 89, 90 and 106) was formed at CIMMYT in 1993 from the seed regeneration nursery of Caribbean land races that had undergone more than four cycles of selection and inbreeding under infestation with LGB. Lines developed from this effort were crossed into lines from Kenya and Zimbabwe to elevate the level of LGB resistance in African germplasm. The resultant hybrids were evaluated across various agro-ecologies in the maize barns in Kenya, and also screened for resistance in the laboratory. The hybrids showed superiority in kernel yield, with an average of more than 6t/ha compared to less than 3.2t/ha for the commercial varieties (Figure 4). Moreover, they showed an increased resistance to maize weevils with 6% weight loss compared to 20% of the checks. The hybrids also showed an increased resistance to the LGB with 20% weight loss compared to 42% of the checks (Figure 5). Based on these assessments, the hybrid varieties reduced post-harvest losses by 50%. As such, the development and identification of resistance to LGB and maize weevil were a breakthrough in the conventional breeding of insect resistance in the second phase of the IRMA Project (IRMA-II) (Mugo *et al.*, 2008).



Figure 4. Maize ears from three LGB resistant hybrids (top row) and four commercial checks (bottom row) after artificial infestation by *P. truncatus* (after three months of storage).

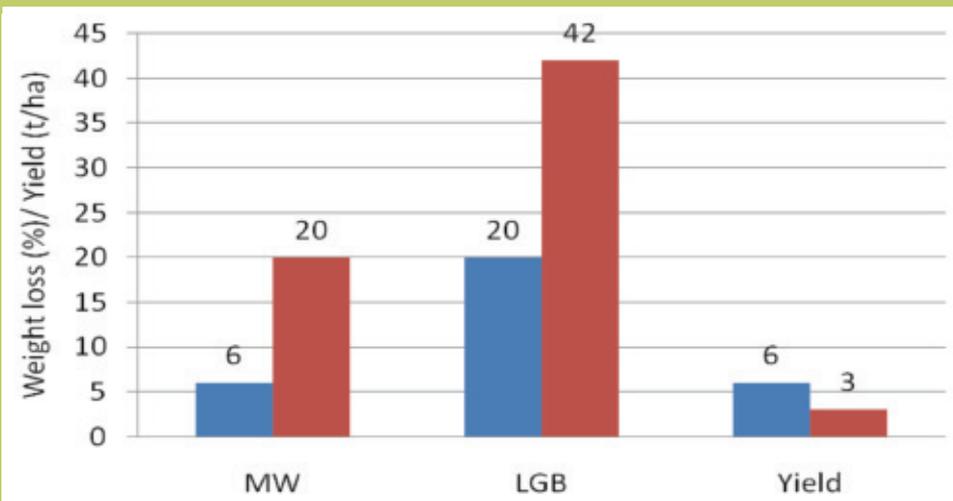


Figure 5. Yield and percentage weight loss caused by the maize weevil (MW) and by LGB to six resistant three-way cross hybrids (blue bar) compared to six commercial checks (red bar).

4. Screening maize germplasm for resistance to stem borers

4.1. Maize

For an effective comparison among the materials to be screened, it is essential to create the conditions necessary for normal plant growth. Maize plants that are stressed, for instance, because of abiotic (drought, soil fertility), and biotic factors (weed, diseases) are abnormally susceptible to stem borers and will make the result unreliable. Resistance expression may also be masked by variation in plant vigor due to differences in maturity or heterosis. Therefore, early, medium and late maturity materials and those of low and high altitude need to be evaluated separately. Maize hybrids and inbred lines must also be compared separately to avoid masking of the resistance potential by differences in plant vigor. The inclusion of checks (susceptible, resistant, and commercial) is also advisable. The growth stage at which resistance is best distinguished is selected for comparison among the test entries.

4.2. Stem borers

For an artificial infestation, the laboratory-reared stem borers should be active, survive under field conditions and cause damage to the host plants. Infestation should be done using insects of the same age group (black head egg) or first or second instar larvae. It is advisable to infest plants with the insects early in the morning (between 7 and 9 am) or late afternoon (after 4 pm). This is to avoid exposing the neonate larvae to harsh sunny conditions which could lead to desiccation in larvae before they are conditioned to the climate and the host. The level of insect pressure applied to each test plant is also critical. The appropriate insect pressure is defined as the number of insects required to have consistent responses from the susceptible check.

4.3. Screening methods and rating

The stem borer maize screening requires an adequate amount of test seed and/or plants, test insects (from mass rearing), and a method to evaluate the levels of resistance among the test entries. Ratings used in maize stem borers screening are mostly based on degree of plant damage, but in some cases, insect numbers are also used. To compare the responses of the entries, a numerical rating system such as 1–5 or 1–9 (with 1 being the highest resistance), should be used. The most commonly used stem borer leaf damage assessment is the 1–9 visual rating scale. Based on the visual rating, the test entries can be categorized into one of the following groups (Table 1).

Table 1. Stem borer leaf damage assessment using the 1-9 visual rating scale.

Scale (1-9)	Description	Resistance reaction
1	No visible leaf feeding damage	Highly resistant
2	Few pin holes on older leaves.	Resistant
3	Several shot-holes injury on a few leaves.	Resistant
4	Several shot-hole injuries common on several leaves or small lesions.	Moderately resistant
5	Elongated lesions (> 2 cm long) on a few leaves.	Moderately resistant
6	Elongated lesions on several leaves.	Susceptible
7	Several leaves with elongated lesions or tattering.	Susceptible
8	Most leaves with elongated lesions or severe tattering.	Highly susceptible
9	Plant dying as a result of foliar damage.	Highly susceptible

The screening method selected should give distinctly different reactions between susceptible and resistant entries. When these reactions are distinct, moderate resistance can also be detected. Plant reaction and subsequent damage rating depend on the number of insects per plant, plant vigor, plant age, and environmental factors such as temperature and humidity. The insect population selected should also have clearly separate reactions for the susceptible and resistant entries. When the insect populations are too high, all entries may appear susceptible. On the other hand, when it is too low, all the entries may appear resistant. Plants that lack vigor because of nutrient deficiencies or other factors, and plants that are extremely young may also be wrongly rated as susceptible although under optimal conditions they may be resistant.

4.4. Methods of infesting with stem borers

There are two ways of infesting and screening maize germplasm against stem borers: natural infestation or artificial infestation.

4.4.1. Natural stem borer infestation

Natural infestation is initially conducted by selecting an area with a predictable high infestation level of stem borers, commonly referred to as a “hot spot” area. Natural infestation may be used effectively by adjusting planting dates so that the desired growth stage for infestation coincides with peak periods of high incidence of the pest. Uniform infestations are critical for a successful screening program. However, under natural infestation, it is difficult to achieve such uniformity in the distribution of the infestation, or to control the level of infestation among the screening materials. This is because the insects are prone to escape or there may be excessive infestation. This natural screening method may only work where resources for rearing insects using artificial diets are not available, and if the population pressure of the insect is nearly stable across seasons.

4.4.2. Artificial stem borer infestation

Artificial infestation is the most reliable method of screening maize genotypes against stem borers. In this method, plants are evenly infested with an insect mass produced on natural or artificial diets. Each plant is infested with at least 20 blackhead eggs or neonates at the whorl stage, the 4-week period after seedling emergence. Infestation can be done manually with a camel hair brush or bazooka applicator (Figure 6a). However, the bazooka applicator is recommended for large-scale application. For instance, in CIMMYT, plots of two rows of five meters length are used. The rows are divided into two portions, comprising infested and uninfested plants. The uninfested rows are protected using appropriate insecticide, and used to determine yield and the agronomic performance of the germplasm while the infested rows are used to record damage parameters such as ‘dead-heart’ (Figure 6b) leaf damage (Figure 6c), stem tunneling

(Figure 6d), entry /exit holes (Figure 6e), and tassel damage (Figure 6f). A typical screening trial from CIMMYT field trials at Kiboko (Figure 6g and 6h) is given in detail in Annex 1.



Figure 6a. Bazooka applicator



Figure 6b. Dead heart damage of the infested plant



Figure 6c. Leaf damage



Figure 6d. Stem tunneling



Figure 6e. Entry/ exit holes



Figure 6f. Tassel damage



Figure 6g. Susceptible (left) and resistant (right) inbred lines at Kiboko



Figure 6h. Yield (ears) of susceptible check lines (right) and resistant hybrids (right)

5. Screening Shelled Maize for Resistance to the Larger Grain Borer and Maize Weevil

5.1. Insect pests

A culture of *S. zeamais* and *P. truncatus*, is established to supply similar aged insects for the experiment as detailed in the mass rearing methods (Tefera *et al.*, 2010).

5.2. Maize genotypes

Maize genotypes, including inbred lines, hybrids, and open-pollinated varieties, can be screened (Annex 2 and 3). Freshly harvested seeds of each test entry are procured, cleaned and disinfested by keeping them in a deep freezer at -4 °C for 2–3 weeks or fumigation with phostoxin tablets in a sealed plastic drum for seven days prior to starting the experiment. The moisture content of the seeds should range from 12–13%.

5.3. Screening maize kernels

About 100g of kernels from each of the maize varieties are placed in a 250 cm³ jar with brass screen lids which allow for ventilation and prevent escape of the insects. About 50 newly-emerged unsexed adult insects (sex ratio is assumed 1:1), the maize weevil and LGB, are separately introduced to each jar containing the seeds, and are kept for seven days for oviposition (Derera *et al.*, 2001). Seeds of each variety without the maize weevil or LGB are kept under similar conditions and serve as a control. The treatments are mostly arranged in a completely randomized design with 3–5 replications, kept on laboratory shelves for 90 days at 65 ± 5% RH and 28 ± 2°C. However, the design of the experiment and the number of replications depend on the objectives and the nature of the experiment (Figure 7).



Figure 7. Screening trials in the post-harvest laboratory at Kiboko, Kenya.

5.4. Evaluating kernel damage

Several methods have been described for evaluating weevil and LGB resistance in shelled maize in the laboratory. The screening parameters include determination of kernel weight loss, total insect progeny emerged, percent damaged kernels, percent insect parent mortality (after seven days of introduction), and percent insect progeny mortality, at 90 days post-storage (Tefera *et al.*, 2011). Kernel weight loss has, however, been identified as the best economic indicator.

5.5. Dobie Index of susceptibility

An index of susceptibility, which takes into account both the F1 progeny emergent during the tests, and the average development period of these progeny was developed by Dobie (1974). The Dobie index of susceptibility is based on the assumption that the more the F1 insect progeny and the shorter the duration of development, the more suitable the test entry is as a host. Therefore, the most susceptible test entries have a higher susceptibility index. To calculate an index of susceptibility, the number of F1 insect progeny should be transformed into natural logarithms, and divided by the average development period. This duration is estimated from the middle of the oviposition period to the emergence of 50% of the F1 generation.

Index of susceptibility = $100 \times [\text{Loge} (\text{total number of F1 insect progeny emerged}) / (\text{median development time})]$.

The susceptibility index, ranging from 0 to 11, was used to classify the maize entries; where 0–3 = resistant, 4–7 = moderately resistant, 8–10 = susceptible and 11 = highly susceptible (Dobie, 1974).

The susceptibility parameters (number of insects, percent kernel weight loss and percent kernel dust) are integrated to define the resistance/susceptibility responses of the maize entries. Therefore, selection index based on susceptibility parameters are done by summing the ratios between values and overall mean and divided by the number of parameters. The genotypes with selection index values less than 0.8 are regarded as resistant or else susceptible if the selection index is greater than 0.8 (Tefera *et al.*, 2011; Bergvinson *et al.*, 2004).

5.6. Seed (kernel) damage and weight loss

Ninety (90) days after incubation, the glass jars are opened, and the content separated into kernel, insects and dust using 4.7 and 1.0mm sieves (Endecotts Limited, UK) (Figure 8). The number and weight of seed damaged (holed seeds) (Figure 9), and seed undamaged (Figure 10) by the maize weevil and LGB feeding are recorded. Kernel damage is expressed as a proportion of the total number of seeds sampled. The weight of the dust produced (Figure 11) and the number of live and dead insects (Figure 12), are recorded. The dust or flour produced due to insect feeding and the kernels are weighed on a precision electronic scale. Dust weight is expressed as a percentage of the initial kernel weight. Kernel weight loss is determined using (1) the bulk density method (initial kernel weight-final kernel weight, divided by initial weight and multiplied by 100); (2) seed weight loss is determined using the count and weight method of Gwinner *et al.* (1996), expressed below:

Weight loss (%) = $(W_u \times N_d) - (W_d \times N_u) \times 100 / W_u \times (N_d + N_u)$; Where W_u = weight of undamaged seed, N_u = number of undamaged seed, W_d = weight of damaged seed, and N_d = number of damaged seed.

Derera *et al.* (2001) used the following five categories of resistance based on kernel weight loss (Table 2):

Table 2. Five categories of resistance based on kernel weight.

Scale (1-5)	Description	Resistance reaction
1	kernel weight loss $\leq 2\%$, less than the resistant check	Resistant
2	kernel weight loss 2.1-4%, similar to the resistant check	Moderately resistant
3	kernel weight loss 4.1-6%, less than susceptible check.	Moderately susceptible
4	kernel weight loss 6.1-8%, similar to susceptible check	Susceptible
5	kernel weight loss greater than 8.1%, greater than susceptible check)	Highly susceptible



Figure 8. Sieves for separating the kernels, insects and the dust.



Figure 9. Damaged kernels.



Figure 10. Undamaged kernels



Figure 11. Flour production due to insect feeding of the kernels



Figure 12. Insects recovered from the test entry after artificial infestation

5.7. Adult mortality

Mortality is assessed 7–10 days after introduction of the insects. All insects are removed, and both the dead and live insects are counted. The assumption regarding parent adult mortality as an indicator of host resistance is that the insects may die of hunger if the kernels of the test entry is not suitable for feeding. However, this parameter might not be a good indicator of susceptibility, because adult maize weevils and LGB are found to have the capacity to survive without food for several days in a laboratory.

6. Screening dehusked maize ears

At harvest, maize ears which have been fumigated with phostoxin for seven days before use are selected for screening in three ways—in a net bag, in paper bags, and in glass jars. In all cases, the samples are kept undisturbed for 90 days then assessed visually for insect damage on a rating scale of 0–10, where 0 = no damage, and 10 = 100 % insect damage. Therefore, 0–3 = resistant, 4–6 = moderately resistant, 7–10 = susceptible.

6.1. Net bag method

Three clean, uniform dehusked ears, which are free of ear rot, are selected from each plot and placed in a net bag and hung about 2m above the ground on the roof of a barn. Natural infestation is then allowed to take place (Figure 13). At the end of the experiment each ear is visually assessed as described above based on the intensity of damage (Figures 14 and 15).



Figure 13. The net bag method of screening maize entries at the Kiboko Post-harvest Laboratory, Kenya. The photo on the right shows flour (dust) on the floor due to damage from insect feeding.



Figure 14. Slightly damaged ear (example for visual score 1)



Figure 15. Totally damaged ear (example for visual score 10)

6.2. Paper bag method

The dehusked ears are placed in paper bags perforated using a standard paper punch. The paper bags containing the ears are kept on benches and subjected to natural infestation (Figure 16). If natural infestation is not suspected, then laboratory insects are released on the floor of the warehouse to allow the insects to search for their suitable host/ test entries.



Figure 16. A perforated paper bag containing ears of a test entry

6.3. Glass jar method

Three dehusked ears are placed in 2 liters glass jars and 20–25 unsexed, 7-day old adult insects introduced. The jar is then sealed with a vented plastic lid. In all three methods, the samples should be labeled with the date of the experiment set-up, plot number, entry number, and insect species used (Figure 17).



Figure 17. Glass jars containing ears of maize test entries

7. References

- Beck, S. D. (1965). Resistance of plants to insects. *Annual Review of Entomology* 10, 207–232.
- Bergvinson D. J., Hamilton R.I. and Arnason J.T. (1995) Leaf profile of maize resistance factors to European corn borer, *Ostrinia nubilalis*. *J. Chem. Ecol.* 21, 343–354.
- CIMMYT. (2011). Developing Maize Resistant to Stem Borer and Storage Insect Pests for Eastern and Southern Africa - IRMA III Conventional (2009–2013). Progress report submitted to the Syngenta Foundation for Sustainable Agriculture. IRMA III CONV Document No. 5. Nairobi, Kenya: CIMMYT.
- CIMMYT. (2010). Development of maize resistant to stem borers and maize resistant to maize weevil and to larger grain borer for eastern and southern Africa (IRMA-III Conventional). CIMMYT: Nairobi.
- De Groote, H., Overholt, W., Ouma, J.O. and Mugo, S. (2003). Assessing the potential impact of Bt maize in Kenya using a GIS model. Presented at the International Agricultural Economics Conference. Durban, August 2003.
- Derera, J., Giga, P.D. and Pixley, K. (1999) Resistance of maize to the maize weevil: II. Non-preference. *African Crop Science Journal* 9, 441–450.
- Dobie, P. (1974) The susceptibility of different types of maize to post-harvest infestation by *Sitophilus zeamais* and *Sitotroga cerealella* and the importance of this factor at the small-scale farm level. Centro International de Mejoramiento de Maiz y Trigo (CIMMT), Condes, Mexico (pp. 98–113).
- Garcia-Lara, S., Bergvinson, D.J., Burt, A.J., Ramputh, A.I., Pontones, D.M.D. and Arnason, J.T. (2004). The role of pericarp cell wall components in maize weevil resistance. *Crop Science* 44, 1546–1552.
- Haile, H., and Hofsvang, T. (2001). Effect of sowing dates and fertilizer on the severity of stem borer (*Busseola fusca* Fuller, Lepidoptera: Noctuidae) on sorghum in Eritrea. *International Journal of Pest Management* 47, 259–264.
- Jiang, M. X. and Cheng, J.A. (2003). Interactions between the striped stem borer *Chilo suppressalis* (Walk.) (Lep., Pyralidae) larvae and rice plants in response to nitrogen fertilization. *Journal of Pest Science* 76, 124–128.

- Kumar, H. (2002). Resistance in maize to larger grain borer, *Prostephanus truncatus* (Horn) Coleoptera: Bostrichidae). *Journal of Stored Products Research* 38, 267–280.
- Mugo, S., Mulaa, M., Likhayo, P., Wangalachi, A., and Gichuki, S. (2008). Insect Resistant Maize for Africa (IRMA) Project. IRMA Project Document No. 30. CIMMYT: Nairobi.
- Mugo S., Bergvinson, D.J. and Hoisington, D. (2001). Options in Developing Stem borer-Resistant Maize: CIMMYT Approaches and Experiences. *Insect. Sci. Applic.* 21 (4), 409–415.
- Mugo, S., Poland, D., Mulaa, M., Odhiambo, B., and Hoisington, D. (2003). Insect Resistant Maize for Africa (IRMA) Project. IRMA Project Document No. 15. CIMMYT: Nairobi.
- Songa J.M., Bergvinson, D., and Mugo, S. (2004). Mass rearing of maize stem borers *Chilo partellus*, *Busseola fusca*, *Sesamia calamistis*, *Chilo orichacocillielus* and *Eldana saccharina* at KARI, Katumani. Proceedings of the 7th Eastern and Southern Africa Regional Maize Conference. Nairobi, Kenya, 11 - 15 February 2002 (pp. 120–124).
- Tende, R., Tefera, T., and Mugo, S. (2010). Techniques and Procedures in Mass Rearing of Maize Stem Borers at KARI-Katumani. Poster presented at the 12th KARI Biennial Scientific conference November 8-12 2010, KARI Headquarters, Nairobi, Kenya.
- Tefera, T., Mugo, S., Tende, R., and Likhayo, P. (2010). *Mass rearing of stem borers, maize weevil, and larger grain borer insect pests of maize*. CIMMYT: Nairobi (57p).
- Tefera, T., Mugo, S., Likhayo, P., and Beyene, Y. (2011). Resistance of three-way cross experimental maize hybrids to post harvest insect pests, the larger grain borer (*Prostephanus truncatus*) and maize weevil (*Sitophilus zeamais*). *International Journal of Tropical Insect Science* (In Press, doi:10.1017/S1742758411000075).

Annex 1: Instructions for a CIMMYT-Kenya trial involving screening for resistance to maize stem borers

Trial code:	INS-IR-KIB-09B-27
Trial title:	Evaluation of 24 inbred lines for resistance to <i>Chilo partellus</i> at Kiboko
Origin:	Various
No. of entries:	24
Date of planting:	September 1, 2009
Location:	Kiboko
Objectives:	The objective of this trial is to evaluate 24 IRMA III Bt to be converted to Bt MON89034 for resistance to <i>Chilo partellus</i> stem borer at KARI Kiboko. The inbred lines will be artificially infested with 20 first instar larvae of <i>Chilo partellus</i> and will be used for conversion in IRMA III to develop superior insect resistant germplasm.
Design:	The design will be a 6 x 4 alpha-lattice with 3 replications. Appropriate fertilizers and watering will be applied to ensure healthy and vigorous plants.
Treatments:	<p>The plots will be two rows of five meters length planted at 75 cm between rows and 25 cm within rows. Two seeds will be sown per hill and later thinned down to 1 plant per hill.</p> <ol style="list-style-type: none">1. Mark the plots with a string in such a way to have the first 2 and the next 5 hills in each row separated from the other 16 hills in row. You will therefore end up with two nylon strings in the block.2. Infest each of the 5 plants behind the first 2 hills of each row with 20 <i>Chilo partellus</i> neonates at the 4-leaf stage. Protect the remainder of the row with a suitable insecticide on the same day.3. Take leaf damage scores per plant from each of the 10 infested plants 2 weeks after infesting. Use a scale of 1–9 where 1 is the least damage and 9 is where all leaves have borer damage. Repeat after 2 weeks.4. Record number of plants showing dead hearts in the infested area in each plot.

5. Score for leaf disease damage in the un-infested area in each plot when there are differences among genotypes. Start before flowering and score once every 14 days. Use the scale 1 (clean) – 5 (totally diseased) and note the nature of sickness. Score for *Turcicum*, MSV, leaf rust and GLS if they appear.
6. After flowering, measure ear and plant height from all the 10 infested plants and separately from 10 representative plants in the un-infested area of the plot.

Harvesting: Eliminate the first 2 hills of each row one day before harvesting.

1. Harvest the infested and un-infested areas separately. Count the number of plants showing root lodging and those showing stem lodging from each area.
 - a. Count the number of plants and ears harvested from each of the two plot areas
 - b. Estimate the percentage of the kernel lost due to bird damage (If applicable)
 - c. Record shelled kernel weight.
 - d. Take a representative kernel samples for kernel moisture determination from the un-infested area only.
 - e. Measure 100-kernel weight from the un-infested area only.

Stem splitting: Split stems from all 10 plants in the infested area of each plot. Count the number of stem borer tunnels, number of visible exit holes and measure the cumulative length of the tunnels in each plant. Attach an additional data sheet for these measurements.

Contacts: For further instructions or questions check with Dr. S. Mugo (+254 20 722 4610, +254 733 720 297, smugo@cgiar.org), Dr. Tadele Tefera (+254 20 722 4602, +254 717 511 972, ttefera@cgiar.org), Mr. H. Karaya (+254 20 722 4609, +254 722 578 082, hkaraya@cgiar.org), or Mr. A. Chavangi (+254 20 722 4607, +254 722 844 352, achavangi@cgiar.org. Fax +254 20-722 4601.

Annex 2. Instructions to screening maize against post-harvest insect pests

Section I: Instructions for planting and trial layout

- 1 Seed is prepared for 3 replications of 2-row plots. There are 84 seeds in each envelope.
- 2 Note that you must supply entries 29 and 30, as these are the local checks. If available, entry 29 should be the one with the highest level of resistance to the resistant to storage pests prevalent in your area. The 2 empty seed envelopes (2 entries x 3 Reps) are marked with colored tags to assist you in locating them, and to remind you that you must supply seed for these envelopes. Note also that the empty envelopes contain a fungicide and pesticide mixture so the seed you provide will carry the same treatment as all entries in the trial.
- 3 You should choose the row length and the planting density to conform to your common practices and recommended plant density for your location. There are enough seeds in each envelope to allow you some flexibility. For example at Embu (optimal rainfall) we will plant 5 m long rows, 0.75 m between rows, 0.25 m between hills, and 2 seeds/hill to be thinned at 1 plants/hill. This will give a population density of about 53,000 plants/ha.
- 4 Experimental design is a lattice (6x5) with 6 plots (12 rows) per block. Note that, although designed as an alpha-lattice, the trial may be analyzed as a randomized complete block design (RCBD) without violating any of the assumptions for the statistical model.
- 5 Please plant a minimum of 2 border rows along each side of the trial. It is also highly recommended that you plant border or guard ranges of at list 4 planting hills (stations) both in front and back of the trial.
- 6 Apply stem borer control in all rows and plants at 6-leaf stage. Repeat at 10-leaf stage.
- 7 Which notes to take for the trial is largely your decision. However, you should include cob damage score and/or cob weight loss as described below.
 - **Plant stand count** for each plot after thinning
 - **Anthesis date:** During flowering, the date when 50% of the plants per plot shed pollen is determined.

- **Silking date:** During flowering, the date when 50% of the plants per plot show silks is determined.
- **Plant height:** The plant height is determined by measuring 10 representative plant from the ground to the first tassel branch.(in case of hybrids)
- **Ear height:** The ear height is determined by measuring 10 representative plant from the ground to the insertion of the top ear.
- **Plant aspect (1–5)** 1 = short plant with uniform and short ear placement; 5 = tall plants with high ear placement.
- **Plant number:** Before harvest, the number of plants is counted after having removed the plants of the first hill on each side of the row.
- **Number of plants with root lodging:** The number of plants with root lodging is counted before harvest.
- **Number of plants with stem lodging:** The number of plants with stem lodging is counted before harvest.
- **Number of ears harvested:** Before harvest, the two plants of the first hill on each side of the plot are eliminated. At harvest, the number of ears per plot are counted. An ear is defined as a cob with at least one-kernel.
- **Ear rot score:** If there is considerable amount of ear rot in the trial, ear rot is scored on a scale from 1 (clean, no rot) to 5 (completely rotten). All ears—including the rotten ones—are kept for measuring field and kernel weight.
- **Ear aspect (1–5),** 1= nice and uniform cobs with the preferred texture in the area; 5= ugly cobs with the undesirable texture in the area.
- **Field weight:** The weight of the ears per plot is taken directly after harvest.
- **Shelled kernel weight:** The weight of the shelled kernel per plot is measured.
- **Kernel moisture:** Kernel moisture is measured.

In addition, score any disease(s) symptoms, which occur at a significant level (score 1 to 5), where 1=none or very few symptoms, 3= intermediate and 5= very susceptible Additional traits of interest (e.g. husk cover, leaf rolling scores, leaf senescence scores etc.) can be recorded.

- 8 Please complete and return to CIMMYT-Nairobi the attached trial information form and return it with the original book (containing original data) as soon after harvest as possible. The information form is essential as it specifies the plot size you used, and describes weather and agronomic conditions that will help interpret the data. Keep a photocopy of these (FieldBook and trial information form) for your use and in case they are lost in the mail.

Section II: Testing for resistance to maize weevil and LGB resistance using natural infestation on ears in the store

The objective of this experiment is to evaluate varieties for resistance to storage pests. You could use your method of infestation and record taking that works for you but the following methodology is suggested to optimize on the number of insects, amount of seeds and labor, as well as adhere to standards for comparison across sites.

1. At harvest, select three representative ears from each plot. Dry all selections to a uniform moisture content.
2. Do not treat the ears with any chemical that control storage pests.
3. Pack the selected ears from each plot into a 2–5 kg mesh bag.
4. Hang the bags from a plank preferably at the wall plate level of a well ventilated kernel store that contains other maize seeds. Guard against damage from rodents
5. Three months from storage, examine each ear and record damage from maize weevils and from LGB on a 1–10 scale (1 = 10% or less of the kernels damaged, 10 = 100% of the kernels damaged).

Section III: Testing for resistance to maize weevil and LGB resistance using artificial infestation on kernels in the laboratory

The objective of this experiment is to evaluate varieties for resistance to storage pests. You could use your method of infestation and record taking that works for you but the following methodology is suggested to optimize on the number of insects, amount of seeds and labor, as well as adhere to standards for comparison across sites.

1. At harvest, shell the clean ears in each plot after selecting the three representative ears for use in the experiment above.
2. Scoop 300 g of seed from each plot. Dry all seed selections to a uniform moisture content. Determine the dry moisture content of the kernel.
3. Scoop 150 g of the seeds into each of two a 0.5 liter glass jars (one for maize weevil and the other for LGB). Cover each jar with a lid perforated in small enough not to allow the insects escaping from the jar.
4. Infest each of the respective jar with 45 unsexed three-week-old adult LGB or MW, respectively.
5. Incubate the jar in controlled moist environment for 90 days
6. Sieve the products through appropriate screens that separate kernel, insects and flour.
7. Weigh each of the components and calculate weight loss of kernel.
8. Transform data on powder produced and weight loss to arcsine prior to statistical analyses.

All other desired data should be taken as described above.

Section IV: Crop husbandry

- 1 **Thinning:** All trials are planted with 2 seeds per planting station. At about four weeks after planting, all trials are thinned to 1 plant per planting station. If there is one station without a plant, thinning is done so 2 plants are left on each side of the gap. Desirably, the thinning is done before or directly after an irrigation to prevent that plants that became loose through thinning die.
- 2 **Basal fertilization:** Sufficient fertilizer should be applied to avoid any N and/or P deficiency as it will leads to confounding.
- 3 **N topdress:** As required to avoid any nitrogen deficiency
- 4 **Weed control:** Usual measures should be taken to keep the experiments free of weeds.
- 5 **Insecticide application:** 500 ml/ha Regent® should be applied at planting in the open furrow to prevent termite damage during the entire season. The maize crop is periodically controlled for stem borers and other pests, and appropriate measures are taken if any pest becomes a problem.
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4. Put 400 g grain in one-liter glass jars and cover with perforated lids.
5. Release about 200 unsexed adult insects of the two species separately into the glass jars.
6. After 10 days of oviposition, remove all the adult insects.
7. Monitor progeny emergence daily and transfer those emerged on the same day to a fresh grain in glass jars with perforated lids. This will help you to get sufficient number of insects of the same age for subsequent screening experiment.

Grain preparation and artificial infestation

1. At harvest, shell the clean ears from each plot.
2. Dry all the seeds to 12-13% moisture content. Take 100 g seed sample and determine the moisture content.
3. Clean the seeds and disinfest either by fumigating or keeping in a refrigerator as stated above.
4. Put 100 g seeds into each two 250 ml glass jars, one for the LGB and the other for MW. Cover the jars with a perforated lid to allow ventilation but not allow insects to escape.
5. Infest each respective jars with 45 unsexed 7-10 days old adult LGB or MW.
6. Incubate the jars in a controlled condition (65% \pm 5% relative humidity and 280C \pm 20C temperature) for 90 days.
7. Sieve the incubated grain through appropriate screens that separate grain, insects and flour (dust).
8. Count the number of live and dead insects, record dust and grain weight
9. Calculate percent weight loss in one of the two methods as follows:

a)
$$\frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100$$

- b) Take randomly 100 seeds from each jar, divide the seeds into damaged and undamaged, record the weight and the number of the damaged and undamaged seeds. Then calculate weight loss as follow:

$$\text{Weight loss (\%)} = \frac{(\text{Wu} \times \text{Nd}) - (\text{Wd} \times \text{Nu})}{\text{Wu} \times (\text{Nd} + \text{Nu})} \times 100$$

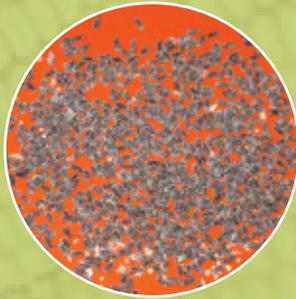
Where, Wu = Weight of undamaged seed, Nu = Number of undamaged seed, Wd = Weight of damaged seed, and Nd = Number of damaged seed.

10. Transform number of insects to log₁₀ and grain weight loss to (arcsine $\sqrt{\text{proportion}}$) before subjecting them to ANOVA.
11. The following five categories of resistance can be used on grain weight losses:
 - Resistant (grain weight loss $\leq 2\%$)
 - Moderately resistant (grain weight loss 2.1% to 4%)
 - Moderately susceptible (grain weight loss (4.1% to 6%)
 - Susceptible (grain weight loss 6.1% to 8%) and
 - Highly susceptible (grain weight loss greater than 8.1%).

II. Procedures in screening unshelled ears

1. At harvest, take three de-husked but unshelled ears per plot, free from insect damages and ear rots.
2. Put the ears a plastic net bag (commonly called onion bag), and hang about 2 m above the ground on a roof of an aerated room.
3. Release a total of 1000 adult insects, 500 individuals from each MW and LGB, on a plastic tray placed on the floor of the room, with the principle that the insects would crawl or fly to search the host (ears) of their choice.
4. Incubate the ears for 90 days.
5. After 90 days, remove the ears, and take a visual score of the damage based on 0-10 scale; where, 0 (no damage or clean ear), 1 (10% ear damage), 2 (20% ear damage), 3 (30% ear damage), 4 (40% ear damage), 5 (50% ear damage), 6 (60% ear damage), 7 (70% ear damage), 8 (80% ear damage), 9 (90% ear damage), and 10 (100% ear damage).
6. Ears with the damage scale of 0-2 rated highly resistant, 3-4 resistant, 5-6 moderately resistant, 7-8 susceptible and 9-10 highly susceptible.

Dear partners, kindly return the raw data to the CIMMYT to the address: Tadele Tefera/ Stephen Mugo, CIMMYT, ICRAF House, UN Avenue, 1041-00621, Gigiri, Nairobi.



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