Breeding for Insect Resistance

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Outline

• Insect pests globally and in SSA
• Management of stemborer pests
• Fall armyworm: a new maize pest in Africa
• Use of Bt maize for stemborer and FAW Control
• Management of postharvest pests
• Concluding remarks
Insect pests globally and in SSA
Lost to stem borers = >10%
Lost to post harvest pests = >15%
Total = >25%
Insect pest problem:

- Part of agricultural ecosystems
- Affects 46% of global maize growing area
- Insect pests causes 25% world maize loss annually
  - Field pests causes 14.5% world loss annually
  - Storage pests causes 10% world loss annually
- 52 million MT of grains valued at $5.7 billion
- 60% of maize loss is in the tropics
- US$550 M worth of insecticide used annually to control insect pests in crops
# Insect pests in maize production and storage in various agro-ecologies

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<tr>
<th>Insects</th>
<th>Upper wet MA</th>
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<th>Dry MA</th>
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</table>

- TZ: Highlands, Africa
- LLT: Africa
- LA: of LA
- Arid environs
- Minimium tillage
- C.
- Humid environ.
Importance of stress factors/breeding goals in various maize mega-environments in Sub-Saharan Africa.

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<th>Africa</th>
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<th>Diseases</th>
<th>Pests</th>
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Fall Armyworm (*Spodoptera frugiperda*): A new pest in Africa
Management of stemborer pests in maize
Important of stemborer species in SSA

*Busseola fusca*  *Sesamia calamistis*  *Chilo partellus*  *Eldana saccharina*

*Window*  *Dead heart*  *Stem tunneling*  *Stem breakage*  *Mycotoxin contamination*
**Chilo partellus** = the spotted stem borer

- **Nature of damage**
  - Cause rows of oval perforations in leaf whorl
  - Damage the growing point: dead-heart
  - Severe in the lowlands

**Geographical distribution**
- Introduced to Africa
- Australia, Africa, Southeast Asia
Life cycle of *Chilo partellus*

- **Eggs are laid** in clusters on the ventral surface of the leaves near the mid ribs. Each female lays ~225 eggs.
- **L.P.** 2-5 days

- **Egg batch**

- **Larvae**
  - Larva passes 5-6 instars, the larva yellowish brown with reddish brown head and prothoracic shield and measures 25mm long with series of black dots.
  - L.P. 28-35 days

- **TLC.** 30-40 days

- **Pupae**
  - It pupate inside the stem in a small chamber.
  - L.P. 8-15 days
*Busseola fusca* = African maize stem borer

**Nature of damage**
- The larvae migrate to the leaf whorl for feeding
- Causes dead-hearts
- 2\(^{nd}\) generation larvae feed on tassels, ears, stems

**Description**
- Larvae grayish body
- Adult is dark brown moth

**Geographical distribution**
- Sub-Saharan Africa, 500-2500m
Life cycle of *B. fusca*
Sesamia calamistis = The pink stem borer

Nature of damage
• Similar to C. partellus and B. fusca

Description
• Pinkish larvae, 3-4 cm long
• Adult is light brown

Distribution
• Warmer coastal areas of Africa
Some wild habitat stemborers

- Busseola segeta
- Busseola phaia
- Sesamia oriaula
- Scimesa piscator
- Sesamia poaphaga
- Sesamia pennisetii
- Sesamia sp5
- Manga melanodonta
- Manga nubifera
## Yield loss in maize due to stemborers

<table>
<thead>
<tr>
<th>Country</th>
<th>Species</th>
<th>Yield loss (%)</th>
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<td>South Africa</td>
<td><em>B. fusca</em></td>
<td>10-100</td>
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<tr>
<td>South Africa</td>
<td><em>C. partellus</em></td>
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<td>Lesotho</td>
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<td>Kenya and Tanzania</td>
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<td>Kenya and Tanzania</td>
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<td><em>E. saccharina</em></td>
<td>30-50</td>
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</table>
How to Control stemborers?

1. Chemical control
   - Broad-spectrum insecticides
   - Bio-pesticide

2. Cultural control

3. **Host plant resistance**

4. Biological control using
   - Parasitoids
   - Predators
   - Pathogens
Control methods for stem borers

• Stem borers are difficult to control, largely because of the nocturnal habits of the adult moth and the cryptic behavior of the immature stages (Seshu Reddy and Sum 1992).

• IPM is based on information of insect population dynamics, pest monitoring and integration of several compatible control measures.

• In Africa, a combination of cultural, chemical and biological control of maize stem borer measures are practiced. Various control techniques have been tried, some with partial success, but all have limitations and none has provided a complete solution.
Chemical control for stemborers

- Chemical control using synthetic contact insecticides provides only protection against early attacks but not against borers feeding inside the ear.
- It requires pest monitoring and training of farmers but in most countries appropriate training capacities do not exist.
- Inappropriate use of chemicals may affect human health and interfere with natural control by predators and parasitoids leading to outbreaks of secondary pests.
  - Borers are difficult to control with insecticides
  - Stem borers are cryptic feeders—late instars (3rd instars)
  - Early (1st, 2nd) larval instars—leaf whorl feeders
Cultural Control for stemborers

- Manipulation of the environment in such a way as to render it unfavorable to the pest (the crop and the land)
  - Crop manipulation (intercropping, destruction of residues, planting dates)
  - Land manipulation (crop rotation, tillage)
- Most relevant and feasible pest control for small holder subsistence African farmers
Pros and cons of cultural control for stemborers

Pros
- Readily available to the farmers
- Don’t entail extra investment in equipment to apply
- Little adverse effect on the environment

Cons
- Labor intensive
- Knowledge of biology & ecology of the pest
- Requires cooperation among farmers
 Habitat management strategy

- **Intercropping**
- Single row, double row, strip intercropping:
  - Maize-legume
  - Reduced infestation of stem borers
    - Maize-cowpea
    - Maize-common bean
    - Maize-legume trees
- Maize-cereals
- intensified infestation of stem borers
  - Maize-sorghum
  - Maize-millet
Habitat management

- Utilization of biodiversity for management of cereal stem borers
- Benefits from grasses and legumes in cultivation of maize
- Combined use of trap and repellent plants
- Called the **Push-Pull** strategy
Plants used in the push-pull system

Trap plants = Pull (attractants)
Repellent plants = Push

Legume silver leaf (*Desmodium uncinatum*)
Napier grass (*Pennisetum purpureum*)

Challenges:
- seeds availability
- adoptability and land shortage
Bio-control: Fungi for stem borers control

- Entomopathogenic fungi produce toxins
- *Metarhizium anisopliae* and *Beauveria bassiana*
- Collection and characterization of isolates
Bio-control for Stemborers

- Classical BC has been attempted against the invasive *Chilo partellus* using the parasitoid *Cotesia flavipes*.

- Redistribution of parasitoids – i.e. the geographic expansion of a parasitoid species or population beyond their native range – has been attempted against *B. fusca* introducing Kenyan populations of *C. sesamiae* in western Africa and the West African egg parasitoid *Telenomus isis* into Kenya.

- However, in most cases BC achieves only partial control of the pest and has to be combined with other control techniques.
Some parasitoids of stem borers

*Trichogramma bournieri*

*Telenomus sp.*

*Pediobus fuvus*

*Cotesia flavipes*
Host plant resistance for Stemborers control

- HPR maize breeding programs by CIMMYT and IITA have focused on lepidopteran and coleopteran pests.
- Antibiosis from hydroxamic acids and flavonoid glycosides in maize has been key for managing pests.
- Because many traits related to maize resistance to insects are multigenic incorporating them into breeding populations has been difficult.
- However, the use of marker-assisted selection can facilitate breeding once genes for these traits are identified.
- One option to enhance maize HPR and transgenic insect-resistance includes efforts to combine natural traits with transgenic traits for maximum effectiveness.
Breeding for insect Resistance

1. Has lagged behind disease resistance
2. Requires knowledge on the biology of maize and the pest
3. Dependent upon:
   • The insect pest,
   • Efficient insect rearing technique,
   • Efficient artificial infestation of maize plants,
   • Genetic techniques, and
   • Plant breeding techniques
Genetic control of resistance to insect pests

Stem borers

- Resistance to stem borers is available
- Resistance is controlled mainly by many nuclear genes (polygenic) genes with additive effects

Functional Modalities of Insect Resistance

1. Non-preference (Antixenosis)
   - Non Suitable of the maize plant as a host
2. Antibiosis
   - Adverse affects on the pest
3. Tolerance
   - The reaction of the plant to insect attack
Status of Breeding Programs for Insect in SSA

Few maize improvement programs include breeding for host plant resistance

1. Genetic challenges posed by the screening and selection for resistance
   - Polygenic control and quantitatively inheritance

2. Logistical challenges in screening and selection
   - Expensive insect rearing

Farmers rely on other non-genetic methods: Chemical, cultural, biological or no control
SBR Variety Testing Protocol

- Alpha lattice designs were used
- Artificial infestation and natural infestation are used in different trials
- In both cases each and every plot is divided into two:
  a) infested area (artificial or natural);
  b) protected area
- Data is collected separately for infested area and for protected area
- Collected data included:
  - Dead hearts
  - Stem borer leaf damage rating
  - Number of exit holes on stems/stalks
  - Number and cumulative length of stem borer tunnels after splitting of stems
  - Standard agronomic trial data
Measured Traits for Stem Borer Resistance

1. Loss of photosynthetic leaf area
   ● Leaf area damage score
2. Dead hearts
   ● Simple counts
3. Lodging from damaged stems
   ● Stem lodging
   ● Number of exit holes / Cumulative tunnel length
   ● Tunnel length: plant height ratio
4. Increased ear rots & mycotoxins
   ● Number of rotten ears - with borer damage
5. Reduced grain yields
   ● Grain yield
   ● Grain yield loss = Protected Yield–Infested yield
Field Infestation: Stem Borers (Larvae)
Maize Leaf Damage

Dead Hearts and Stem Damage
Tunnel Length of Insect Resistant Maize
Stem Borer Resistant and Checks
## SBR Multi locations Results

<table>
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<tr>
<th>Entry</th>
<th>Pedigree</th>
<th>BLUE Yield (t/ha)</th>
<th>Yield Rank</th>
<th>Days to 50% Anthesis (days)</th>
<th>No. Exit Holes (#)</th>
<th>Stem Borer Damage (1-9)</th>
<th>Tunnel Length (cm)</th>
<th>Tunnel Length/Plant Height (#)</th>
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**nlocs**: 16

**Grand_Mean**: 4.80

**LSD**: 0.52

**CV %**: 5

**Heritability**: 0.43
Conclusions

• Stem borers are economically the most important pest. There are a number of control measures against stem borers, but each comes with its own limitations.

• For reasons of costs and availability of pest control methods, farmers often use no control measures at all.

• HPR, the resistance is embedded in the seed, and is, therefore, the easiest control method for subsistence farmers.

• However, HPR, needs investment in germplasm development, establishing insect mass rearing and seed multiplication.
Fall armyworm: A new maize pest in Africa
Fall Armyworm (*Spodoptera frugiperda*) life cycle

- **Eggs**: 2–3 days
- **Larva**: 14 days
- **Pupa**: 8–9 days
- **Adults**: 3–4 days

Female and male adults are shown, with the female marked by the symbol 🌹 and the male by the symbol 🌹.
**FAW identification**

- **Male moth with conspicuous white spot on tip of forewing**
- **Fall armyworm**

- **Kidney-shaped mark on forewing**
- **African armyworm**

- **White inverted Y-shaped suture on front part of the head**
- **White invertebrate**

- **4 distinct tubercles on dorsal side of the last segment arranged in a square pattern**
- **4 distinct tubercles**
The Pest (*Spodoptera frugiperda*)

- Wide host range (>80 plant species) but with major preference for maize
- Short life cycle: 1-2 months (depending on weather)
- Rapid proliferation (>1000 eggs per female)
- Strong migratory capacity of moths: 500 km before oviposition; with suitable wind >1000 km
Rapid Spread of Fall Armyworm in Africa

Source: Erik Stokstad, Science, 5th May, 2017
The Status

• **29 countries in Africa** confirmed the pest incidence (compared to 12 in April 2017).

• **A further 8 countries** have conducted or are presently conducting surveys, and either strongly suspect its presence or are awaiting official confirmation.

---

Source: CABI Evidence Note (Sept 2017)
The Present and Possible Impact

- More than 1.5 M ha of maize is currently affected in just six countries: Nigeria, Ghana, Kenya, Ethiopia, Zambia, and Zimbabwe.

- The potential impact of FAW on continental wide maize yield lies between 8.3 and 20.6 million tonnes per year of total expected production of 39 m tonnes per year -> losses lying between US$2.5 and US$6.1 billion per year of total expected value of US$11.5 billion per year (CABI Evidence Note, Sept 2017)

- Other impacts:
  - Losses to maize seed production fields
  - Regional and international trade (FAW is a quarantined pest in Europe)
  - Environmental and human health impacts of synthetic/chemical pesticides
The Response

Source: Ernst Neering
FAW management approaches in Africa: no silver bullet!

Integrated Pest management

- Scouting, Surveillance & Early warning
- Environmentally Safer Pesticides
- Biological control
- Cultural & mechanical control
- Host plant resistance
- Policies & Regulation
Establishing small-scale biofactories for regional use of *Trichogramma* spp.

Field release of adults

Field introduction of eggs

Source: Ivan Cruz *et al.*, 2013
Biopesticides: Baculoviruses

*Spodoptera frugiperda* multiple nucleopolyhedrovirus (SfMNPV)

**Advantages:**
- Host specificity,
- Long persistence in the soil,
- Very low non-target risks

IITA has already 4 isolates from Mexico, Nicaragua, USA and Argentina for testing

**Production of own biopesticides at smallholder level**

**Constraints:**
- Mass production costs as commercial product;
- Risks for mutation for in vitro rearing;
- Hygiene requirements in mass rearing facilities;
- Sensitivity to ultraviolet radiation

Source: Georg Goergen (IITA)
Biopesticides: Use of botanicals

Home made pesticide instead of commercial product
Low cost production
Easily applicable at smallholder level
Naturally occurring
Smooth degradation, less environmental pollution

Effectiveness:
Short-lived susceptible to UV degradation
Variable action

Constraints:
• No residual effect;
• Slow effect
• Home production is labour intensive;
• Limitations of the botanical resource;
• Mode of action not always understood

Source: Georg Goergen (IITA)
Host Plant Resistance is a major pillar of IPM.

- Economic, ecological, and environmental benefits
- Seed can combine multiple traits, and is quickly scalable and deployable → in case of native trait resistance, seed costs no more than a normal hybrid
- Compatibility with other direct control tactics
  - Can significantly reduce the insecticide use
  - Not density dependent
- Economic threshold level for insect-resistant varieties could be several fold higher than for susceptible varieties → the elevated economic threshold significantly enhances the effectiveness of other management tactics.
Conventionally-derived Resistance to FAW

CIMMYT, during the 1980s and 1990s, developed an array of populations as well as elite inbred lines (>60 CMLs) with resistance to FAW, derived mainly from the Caribbean maize germplasm.

<table>
<thead>
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<th>FAW-resistant populations developed at CIMMYT</th>
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<tbody>
<tr>
<td>Pop. 304</td>
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<td>Pop. 392</td>
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<tr>
<td>Pop. FAW-CGA</td>
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<td>Pop. FAW-Tuxpeno</td>
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<tr>
<td>Pop. FAW-Non-Tuxpeno</td>
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</table>
Screening of CIMMYT maize inbred lines under FAW infestation in Kakamega

- Susceptible
- Resistant

Susceptible (CML444)
Screening of maize lines under FAW natural infestation at Kiboko (2017)

CML444 (Susceptible)  CKDHL164288 (Putative Resistant)  CKDHL164282 (Susceptible)  CKDHL166062 (Putative Resistant) developed from MBR population
Several elite CIMMYT-derived drought-tolerant lines are highly susceptible to FAW attack. Need for rapid conversion of the FAW-susceptible lines into resistant versions.
Accelerating Development of Improved Maize Hybrids with FAW Resistance and other Adaptive Traits

• Farmers in SSA need a package of traits in the seed – high yield, drought tolerance, disease resistance (e.g., MLN, MSV), FAW resistance...

• Transferring FAW resistance into elite, Africa-adapted tropical/subtropical genetic backgrounds

• Powerful tools for fast-tracking breeding progress → Off-season nurseries, Doubled haploid (DH) technology, molecular markers....

KALRO-CIMMYT Maize DH Facility at Kiboko, Kenya, with capacity to produce 75,000 DH lines every year
Host Plant Resistance to FAW...

- Massive efforts on HPR needed in maize as well as other major crops affected by FAW in Africa
- Excellent leads to identify and utilize native trait variation in developing elite products with resistance to FAW in CIMMYT maize germplasm
- Intensive efforts required to convert several elite, DT maize lines into FAW-resistant versions to protect the genetic gains made so far in SSA
- Opportunities to fast-track development and deployment of elite FAW-resistant hybrids using molecular markers and DH technology
Effect of Push Pull Technology on FAW

• Ecosystem management approaches can control FAW while enhancing biodiversity

Some Key Messages

- The pest is unfortunately likely to stay for a long time in Africa due to highly conducive conditions.

- Providing the farming communities with the right knowledge of the pest, and when/when not, and how to apply chemical control is URGENT

- We do NOT know everything about the pest and the control measures, especially those relevant for the African context – requires intensive research over the next few years...

- Possible to still move forward with some best-bet management practices, as R4D evolves..
Use of Bt maize for stemborer and FAW Control
Maize with Bt gene 

larvae feeds on Bt maize 

the Bt protein damages the midgut of the larvae 

larvae die within several hours
Performance of Bt, non-Bt and commercial checks

**Yield t ha**

- Bt: c
- Non-Bt: b
- Com Checks: a

40% yield advantage

**Leaf damage (0-9)**

- Bt: a
- Non-Bt: b
- Com Checks: b

>60% leaf damage reduction
Performance of Bt, non-Bt and commercial checks

Holes/plant

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% Stem tunnelling

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Holes

Stem tunnelling
Leaf damage on stack event MON810xMON87460, non-Transgenic isolines and commercial checks maize plants after artificial infestation with 20 neonates of *Chilo partellus* at Kiboko and *Busseola fusca* at Kitale and natural FAW infestation at both locations.
Highly significant differences were observed in both between traited and non-traited material in both infested and control trials for GY.
MON810 Kenya CFT1-3 Executive Summary

- The gene has a positive and significant effect on yield.
- The gene significantly reduced numbers of exit holes and tunnel length after artificial *C. partellus* infestation.
- The gene significantly reduced *B. fusca* larvae survival and leaf area consumed.
The gene has a positive and significant effect on yield across germplasm and trials. The yield increase was 52% on average. The gene significantly reduced numbers of exit holes and tunnel length across germplasm and trials.

<table>
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<tr>
<th>Trait</th>
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<th>Neg Mean</th>
<th>Delta</th>
<th>PER C</th>
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<td>Yield [t/ha]</td>
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<td>-93</td>
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<tr>
<td>Tunnel length [cm]</td>
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<td>3.9</td>
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<td>-95</td>
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</table>
Management of postharvest pests in maize
Important post harvest pests of maize

*Sitophilus zeamais*  *Prostephanus truncatus*  *Sitotroga cerealela*  *Mussidia nigrivenella*

Grain infected with *Aspergillus* spp

Weevil damage

LGB damage on maize
Traditional storage method
Types of losses

- Physical losses
  - Weight loss
  - Opportunity loss
  - Nutritive loss
- Monetary losses
- Quality losses
Estimated value of produced and lost grains annually in SSA

Loss equivalent to:
- Annual caloric value of at least 48M people
- Food aid received for a decade
- 1% reduction PHL, $40M gains
Post Harvest Maize Pests

● Insects are a major cause of maize storage losses in the tropics. They infest and damage grain, resulting in direct and indirect losses of both quality and quantity of the food stored.

● Losses vary according to region, environmental conditions, main storage pest(s), and method of storage.

● Under certain conditions, weight losses of over 30% have been observed after only a few months of maize storage in some African countries.
Control method for postharvest insect pests

- Effective controls of maize storage insect pests rely mainly on pesticide use

- However,
  - Some pesticides (e.g. Actellic) are becoming inefficient. Insect resistance? Or fake products?
  - Application of effective synthetic pesticides pose serious and increasing risks (Health & environmental problems)
  - Farmers need safe alternative methods.
Biological control

• A predator, *Teretrius nigrescens*, of the LGB was released in Africa with some successes in hot-humid areas of West Africa (Borgemeister et al. 1997).

• Only two populations of *T. nigrescens* from limited geographical and temporal isolation efforts were released separately in Africa (Schneider et al. 2004).

• Additional research needs to be done on the adaptation of the predator to different climatic zones.
Hermetic storage technologies

• The most promising option is the hermetic storage which is promoted by CIMMYT and its partners.
• From the pilot study, metal silo and hermetic bags maintained a low grain damage and loss (< 5%) over 6 months period.
• On station trails were conducted between 2012 and 2013 in the four countries and the resulted confirmed the effectiveness of hermetic storage
• Based on this result there is a need to up scale the technology in maize surplus areas
Metal silos

- A metal silo is of one standard design, cylindrical structure, fabricated by trained local tinsmith from galvanized iron sheet with a top loading inlet and a lateral unloading spout at the bottom and hermetically sealed with rubber band.
Trial Set-up
Results
Conclusion

• Improved hermetic technologies tested (metal silo and super grain bags) can significantly reduce weight loss and grain damage caused by storage pests.

• From the farmers’ side, the technologies are well accepted, however they consider them expensive.

• Need for concerted efforts to ensure that access to these improved hermetic storage technologies is achieved for the farmers.
Development of Post Harvest Pests Resistant Maize

• Developing LGB resistant maize varieties
  o Testing new sources of resistance
  o Developing new sources through crossing germplasm
  o Evaluation of OPVs and hybrids as finished products

• Resistance is based on a genebank accession found to have resistance to LGB.
  o Crosses made to adapted germplasm
  o Inbred lines developed
  o TWCs formed and tested for agronomic performance
  o Screening grain for LGB resistance done at every stage
SPR Variety Testing Protocol

• Alpha lattice designs used
• Standard field agronomic assessments and measurements done
• To evaluate for resistance to storage pests, two different approaches are used; a) choice; b) No choice

SPR Variety Testing Protocol: Choice Method

• At harvest, 3 well filled and representative ears are selected and kept separately for each plot
• All the selected ears are dried to a uniform moisture content (±12.5%) in an artificial drier
• The ears are then packed in 2-5kg mesh bags and hung down from a plank at plate level in a well ventilated shed containing other untreated maize samples.
Free-choice Cob Evaluation for Resistance to the Maize Weevil and to the Larger Grain Borer
90 Days from initial storage date the samples are examined for weevil and LGB damage and scored from 1 (10% damage) to 10 (100% damage).
SPR Variety Testing Protocol: No choice Method

• At harvest, after selecting 3 well filled and representative ears, the remaining clean ears for each plot are shelled in bulk to produce at least 300g of grain for each plot
• 300g of grain are scooped from each plot and dried to a relatively uniform moisture content
• After drying the moisture content for grain from each plot is determined
The jars are incubated in a room with climatic control at 28°C and relative humidity of at least 70% for 90 days.

After 90 days the grain, insects and flour are separated using appropriate sieves.
SPR Variety Testing Protocol: No choice Method

- Samples are separated into components
- Each of the components is weighed and grain weight loss of original sample is also calculated

Collected data included:
- Initial and final grain weights
- Flour weight
- Grain weight loss
- Insects weight
- % Grain damage (derived trait), and
- Standard agronomic trial data
## Hybrid screening SPR Results

<table>
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<th>Entry</th>
<th>Pedigree</th>
<th>BLUE Yield (t/ha)</th>
<th>Yield Rank</th>
<th>AD (days)</th>
<th>% Grain Damage</th>
<th>Dust Weight g</th>
<th>Weight loss %</th>
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Conclusions

1. CIMMYT and IITA have used these methods to develop a good number of inbred lines, open-pollinated varieties, hybrids, and source populations with resistance to stem borers and post harvest pests.

2. This germplasm is available to NARs and seed companies for use:
   - Directly as variety per se
   - As sources of alleles to breed maize for resistance to the major stem borers and to maize weevil and LGB.

3. New populations of stem borer and post harvest resistant germplasm are being developed for major ecologies in ESA.
Thank you for your interest!

“develop crops that can grow in a drought; that can survive in a flood; that can resist pests and disease”
Bill Gates 19 October 2009