





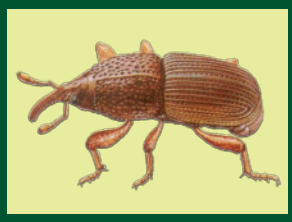

Mass Rearing of Stem Borers, Maize Weevil, and Larger Grain Borer Insect Pests of Maize

Tadele Tefera, Stephen Mugo,
Regina Tende and Paddy Likhayo

CIMMYT®

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Tadele Tefera[1*], Stephen Mugo[1],

Regina Tende[2] and Paddy Likhayo[2]

[1]International Maize and Wheat Improvement Center (CIMMYT), Nairobi, Kenya
[2]Kenya Agricultural Research Institute (KARI)
[*]Corresponding Author: Tel: +254 20 722 4602; e-mail: t.tefera@cgiar.org

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Acronyms and abbreviations

CIMMYT	International Maize and Wheat Improvement Center
DFID	Department for International Development
HPR	Host plant resistance
IRMA	Insect Resistant Maize for Africa project
KARI	Kenya Agricultural Research Institute
LGB	Larger grain borer
NARL	National Agricultural Research Laboratory
masl	Meters above sea level
MW	Maize weevil

1 Introduction

Maize (*Zea mays* L.) is important for agriculture and livelihoods in eastern and southern Africa as it is the major staple food. However, maize yield in Africa is very low, 1.5 t/ha, against a global average of 4.9 t/ha. Constraints to maize production include both abiotic and biotic factors. Among the biotic constraints in maize production, are insect pests in the field and in storage. The most economically important insect pests of maize in Africa include stem borer in the field, and both the maize weevil (MW) and larger grain borer (LGB) in storage (post-harvest pests). Maize plants are less able to tolerate stem borer attack than sorghum and pearl millet plants because they do not produce tillers, and the effect on grain yield is therefore greater. Colonization of the plant by borers, severity of infestation and damage strongly depend on the cropping system, soil fertility, and environmental conditions, which affect the nutritional status of the plant. Stem borer damage is aggravated by the poor nutritional status of the plant. Studies on several stem borers species in Africa showed that an increase in nitrogen is related to higher pest loads and tunnel damage. However, soil nutrient levels, such as nitrogen, also greatly influenced the plant's tolerance to stem borer attack. This is due to an increase in plant vigor, which is reflected in lower yield losses (Setamu et al. 1995). Damage caused by stem borers can average 20 to 40%, which means between two to four bags of maize are lost out of every 10 that could be harvested (De Groote et al. 2003).

Several control measures are currently used, but each comes with its own challenges and limitations. Due to high of costs and labor, farmers are often resigned to using no control measures at all. Four commonly used general approaches to insect control include: chemical control using insecticides; biological control, which involves identification and introduction of natural enemies of the pests into an area; cultural control using a broad range of field and crop management techniques; and finally, host plant resistance (HPR), in which the plant has in-built control mechanisms, which confers its own resistance to the insects.

Chemical control is the most effective and commonly used method at the farm level. Different insecticides have been recommended for control of maize pests. However, they are not accessible to small scale farmers. Host plant resistance using conventional breeding to control maize pests is a technology embedded in the seed, and is, therefore, the most convenient method for use by subsistence farmers in integrated pest management. It is also environmentally safe. However, this method requires a large number of insects for screening.

A stem borer mass rearing facility was established at the Kenya Agricultural Research Institute (KARI)-Katumani in 1999, with the support from the Rockefeller Foundation and the International Maize and Wheat Improvement Center (CIMMYT), through the Insect Resistant Maize for Africa (IRMA) project. The main purpose for the mass rearing facility is to supply stem borers for use in maize resistance screening research activities at KARI and CIMMYT field trials.

Five stem borer species are found in the Kenyan maize growing fields. These are spotted stem borer (*Chilo partellus* Swinhoe, Lepidoptera: Pyralidae), the *African stem borer* (*Busseola fusca* Fuller, Lepidoptera: Noctuidae), *Coastal stem borer* (*Chilo orichalcocillielus* Strand, Lepidoptera: Crambidae), *Pink stem borer* (*Sesamia calamistis* Hampson, Lepidoptera: Noctuidae), and *Sugarcane borer* (*Eldana saccharina* Walker, Lepidoptera: Pyralidae) (Overholt et al. 2001). The stem borer species are reared in the insectary, with the bulk production being for *C. partellus* and *B. fusca*, which are of greater economic importance (Songa et al. 2001a, Songa et al. 2002b, Songa et al. 2004). The facility is also used for training both students and staff from Kenya and other countries involved in the current IRMA III (conventional) project activities.

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 (*Prostephanus truncatus* Horn, Coleoptera, Bostrichidae) Research Project, coordinated from KARI–National Agricultural Research Laboratory (NARL). The laboratory had two small rooms: one used for rearing stored product insect

pests, and the other as a store. When the project ended in 1994, the facility was handed over to KARI - Katumani but KARI - NARL post-harvest section continued maintaining an office and carrying out research work at the facility.

Maize varietal screening for resistance to storage insect pests maize weevil (*Sitophilus zeamais* Motschuksky, Coleoptera: Curculionidae), and lager grain borer work started in 2001. During the first phase of IRMA I Project (1999–2003) CIMMYT renovated two rooms, stocked glass jars, installed light tables and four 210 liter capacity plastic drums for fumigation. CIMMYT has continued to use the facility and has expanded it to its current capacity of one rearing room, screening room of 10,000 jars, and cob screening room.

CIMMYT and KARI have embarked on developing maize resistant to stem borers, maize weevil and the larger grain borer for eastern and southern Africa (Mugo et al. 2009). Variety screening studies require a consistent supply of large quantities of these pests at specified time periods. Stem borers are reared on an artificial diet in order to have dependable large and continuous supplies of insects for screening plant materials. The stem borer and post-harvest rearing facility has a significant impact on research focused at managing post-harvest insects and maize stem borer populations through host plant resistance. The rearing facility supports national and international research projects aimed at developing maize varieties that can be incorporated in integrated pest management of stem borers and post-harvest pests of maize.

This manual will describe the methods used to rear stem borers, maize weevil and larger grain borer in the facilities at KARI - Katumani and KARI - Kiboko, both in Kenya.

2 Maize Stem Borers

2.1 Biology

In *C. partellus*, the eggs are flattened, scale-like, and ovoid, and are laid in overlapping clusters (Figure 1). The moth prefers smooth surfaces of the plant for oviposition, lays most of its eggs on the lower surfaces of the leaves and

upper part of mid-ribs. Maize plants at whorl stages (7–8 weeks after seedling emergence) are preferred for oviposition than an older plant. Incubation of eggs takes about 5–7 days, depending on prevailing environmental conditions. Upon hatching, the neonate larvae migrate from the oviposition site into the whorls, where they establish and feed on the tender young leaves. Third instar larvae migrate from the whorl to bore into the stems. On older plants, the young larvae may feed on leaf collar tissue, before boring into the stem. Fully grown larvae feeding in the stem prepare for their exit as moths by cutting a circular exit hole in the stalk rind just before pupation. Pupation takes place within the stem and the papal period takes 7–10 days depending on temperature (Kfir et al. 2002; Ofomata et al. 2000; Muhammad and Underwood 2004).

2.2 Distribution

C. partellus is widely distributed in eastern and southern Africa. This pest is not native to Africa, but was accidentally introduced from Asia. It is essentially a hot lowland area pest, seldom found above an altitude of 1500 masl (Ofomata et al. 2000). Since its appearance on the African continent, it has continuously expanded its distribution in the warm, low-altitude regions of eastern and southern Africa;. Currently, it is the most economically important stem borer in many areas (Polaszek 1998).

The African maize stem borer *B. fusca* is indigenous to Africa and is a common pest in many African countries throughout sub-Saharan Africa. Its distribution and pest status varies with the region. In eastern and southern Africa, it is a pest at higher altitudes (above 600 masl), but in Central Africa it occurs from sea level to over 2000 masl, while in West Africa, it is primarily a pest of sorghum in the dry savannah zone (Kfir et al. 2002; Tefera 2004).

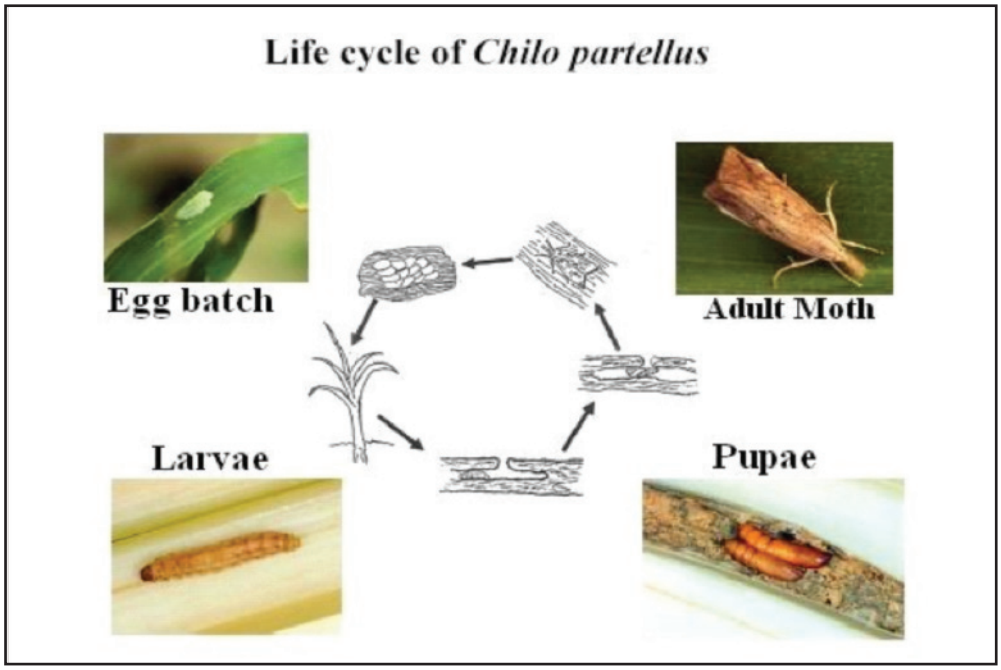

Figure 1. The life cycle of the spotted stem borer.
(Source, http://agritech.tnau.ac.in/crop_protection/crop_prot_crop_insectpest%20_cereals_sorghum%20-%20Copy_clip_image001.jpg)

The larvae are up to 25 mm long when fully grown (Figure 2a), with a prominent reddish-brown head. The body is creamy-white to yellowish-brown, with four purple-brown longitudinal stripes and usually with very conspicuous dark-brown spots along the back, which give them a spotted appearance. Young caterpillars initially feed in the leaf whorl. Older caterpillars tunnel into stems, eating out extensive galleries. In warm conditions, larval development is completed in about 15–20 days. Caterpillars pupate in damaged stems. The pupae have a length of up to 15 mm long, are slender, shiny and light yellow-brown to dark red-brown in color. Adults emerge 5–12 days after pupation.

The adults are relatively small moths with wing lengths ranging from 7–17 mm and a wingspan of 20–25 mm (Figure 2b). The forewings are dull, generally light yellow-brown with some darker scale patterns. The hind wings are white. Adults emerge from pupae in the late afternoon or early evening. They are active at night and rest on plants and plant debris during the day. The moths are seldom seen, unless disturbed.

(a) (b)

Figure 2. The larvae (a) and fully expanded wings of the adult (b) spotted stem borer.

Figure 3. The adult African stem borer.

For *B. fusca*, the eggs are round, flattened and about 1 mm in diameter. The female lays a batch of up to 150 eggs between the leaf sheaths and stalk. The eggs are at first white, but turn darker as they get older. Eggs are laid in a long column stretching up the stem, under a leaf sheath. They hatch after about 10 days. The young larvae are deep purple or black in color. They crawl up the plant into the funnel. During the early stages, the caterpillars feed on the leaves in the funnel of the plant. This results in characteristic lines of holes and "windows". When the attack is severe, the shoot may turn yellow and die. If the plant dies, the caterpillars will move to another plant. If the plant survives, the later stages of the caterpillar will bore into the stem and feed there on the central tissue of the stem. The larval period takes 35 days or more. When fully grown, the caterpillars are up to 40 mm long. They have a pinkish-white color with small black spots along the sides of the body. The mature caterpillar cuts a hole into the stem before pupating within the tunnel. Eventually, the moth will

use this hole to emerge. The pupa is brown and about 25 mm long. The pupal stage of the first generation will last about 2 weeks. Before the crop ripens, there are usually two generations. Some of the second generation eggs may be laid on the cob. The caterpillars will feed on the cob, but usually move into the stem when fully grown. Before pupating, they will go into a long diapause which lasts until the next rains. After this, they prepare a pupal chamber in the stem, in which to pupate. The adult is a pale brown nocturnal moth with a wingspan of 35¬40 mm (Figure 3) (Kfir 2002; Polaszek 1998).

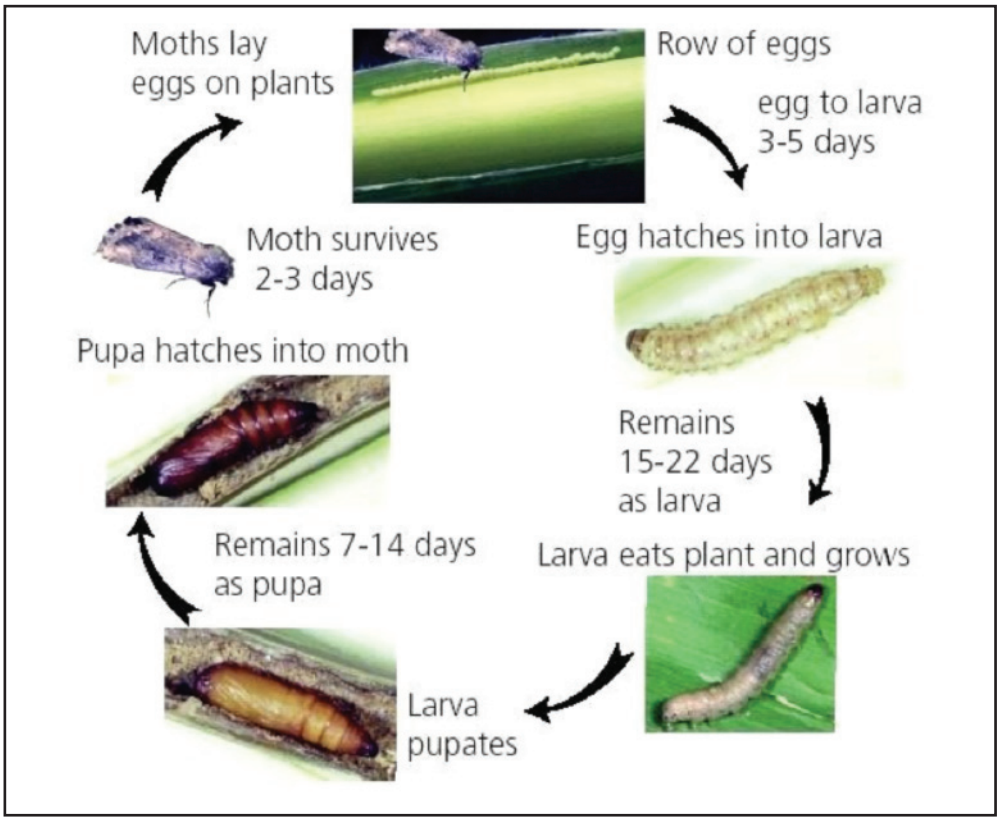

Figure 4. The life cycle of the African stem borer.
(Source: http://www.push-pull.net/images/Busseola%20fusca.jpg)

2.3 Damage and loss

C. partellus injury to maize includes leaf feeding (Figure 5a) and subsequent development of "deadhearts" (death of the central growing tip), and tunneling (Figure 5b) within the stalk which results in disruption of the flow of nutrients to the ear (Kfir et al. 2002; Polaszek 1998). The lesions are formed by the

scraping of the epidermis and parenchyma on one side of the leaf, often leaving the other side intact and transparent. When the leaves unfold, the lesions are seen as small holes or windows on the leaves. In some cases, the larva bores right through the perpendicular axis of some of the leaves in the inner whorl and when these unfold, the lesions appear as an array of holes of similar size and shape. Foliar damage caused by the first and second instar larvae results in the reduction of the total leaf area and a depression in the photosynthetic capacity of the plant. The third instar larvae bore into the stem or feed on the developing tassel. Stem tunneling is caused by third to sixth instar larvae. These bore into the stem and chew their way through it, destroying the central pith and conducting tissues, thus causing reduction in nutrient uptake, stem breakage, and infection by secondary microorganisms (Polaszek 1998).

The amount of yield loss depends on the severity of leaf damage and stem tunneling. Yield loss estimates vary greatly depending upon the country, season, maize variety, and fertilization (Overholt et al. 1996; Kfir et al. 2002; De Groote et al. 2002). However, in East Africa, yield was reduced by 15–45% (Seshu Reddy and Sum 1992), while in South Africa, yield losses in maize exceeded 50% (Kfir et al. 2002). More recently, following an extensive survey of farmer fields in Kenya, with and without insecticidal control, De Groote et al. (2003) found that all stem borer species caused average annual losses of 13.5%, valued at US$ 80 million.

In *B. fusca*, damage is caused by the caterpillars, which first feed on young leaves (Figure 5a), but soon enter into the stems. During the early stage of crop growth, the caterpillars may kill the growing points of the plant, causing what is known as dead heart (this makes the youngest leaves vulnerable—they can be easily pulled off). At a later stage of growth, they make extensive tunnels inside the stem (Figure 5b), disrupting the flow of nutrients to the grain. Tunneling weakens the stem so that it breaks and falls over (Polaszek 1998; Adugna and Trod 2001). In older plants, the first generation caterpillars bore (Figure 5c) in the main stem, but later, some of the second generation bore into the maize cobs.

(a)

(b)

(c)

(d)

Figure 5. Damage symptoms of stem borer (a) leaf damage, (b) stem tunneling, (c) stem boring, and (d) cob damage.

3 Mass Rearing of Maize Stem Borers

3.1 Colony establishment

A large founder colony of at least 500 population collected over a wide area is used. This will ensure genetic diversity, and help establish a pathogen- and parasite-free colony. Genetic decay has been found to be higher when small initial populations are used to initiate the colony. However, the field collected insects should pass through strict quarantine protocols before they are transferred to the insectary (Polaszek 1998).

3.2 Colony maintenance

Colony maintenance involves periodic gene infusion done by mating the laboratory colony and the wild population. It is used to avoid genetic decay,

maintain heterozygosity among insect populations and to preserve the original wild behavior of the insects. At the KARI-Katumani insectary, stem borer larvae and pupae are periodically collected from infested maize stubbles and stalks. The plant materials are dissected with a knife to obtain larvae and pupae. The field collected insects are reared in isolation for F1 to avoid any contamination. Parasitized, diseased and deformed insects are discarded. The second generation is allowed to cross-breed with the laboratory colony (Onyango et al 1994).

3.3 Rearing facilities

The main components of the stem borer mass rearing facility include laboratory space, equipment, diet, consumables and personnel.

Laboratory space

The stem borers' developmental stages (egg, larva, pupa and adult) differ in their environmental requirements and management, thus, require establishing separate rooms. The stem borer mass rearing requires several rooms based on the capacity and objectives of the insectary. These are 1) diet preparation and infestation room, 2) larvae development room, 3) pupae harvesting room, and 4) moth emergence and oviposition room. Other facilities include a washing room, a store and an office. The rearing laboratory is a new habitat for insects, and should therefore have environmental conditions conducive to their development and effective field performance. The rooms should be kept free from disease, parasites and predators. At the KARI-Katumani insectary, stem borer larvae are reared successfully under controlled environmental conditions (28 ± 2°C; 65 ± 5% RH; L12:D12 photoperiod).

Equipment

Installation of appropriate equipment in the rooms in relation to function saves time, increases efficiency, and enhances safety. Depending on the availability of resources and scale of operation, several pieces of equipment can be installed (Table 1). This list is not complete but it is sufficient to run the existing rearing activities at Katumani.

Table 1. List of equipment and their function at the KARI-Katumani insectary.

	Equipment	Function
1	Sterilizer	Sterilize diets, metal and glassware
2	Microscopy	Identification of microorganisms
3	Balance	Weigh diet ingredients, larva and pupa
4	Distiller	Removes impurities from water
5	Oven	Cooking diets, boiling water
6	Fridge	Keep diets fresh for a short while
7	Fume hood	Protects laboratory workers from fumes and potentially dangerous chemicals
8	Leaf grinder	To ground maize/sorghum leaf into powder
9	Shelves	Rearing larvae
10	Cages	Adult mating and oviposition
11	Humidifier	Maintain relative humidity
12	Heat fun	Maintain temperature
13	Standby generator	To use in case of power failure

Laboratory consumables

Consumables are products that are recurrently consumed by the insectary (Table 2).

Table 2. List of laboratory consumables and their function at the KARI-Katumani insectary.

	Consumable	Function
1	Stationery	Recording diet, infestation date, collection date, etc
2	Paper wax	Adult female oviposition site
3	Paper towels	Cleaning
4	Detergents	Cleaning and disinfestations
5	Glass wares	Diet preparation

Diet ingredients

The insect diet is a mixture of nutritive substances including carbohydrates, proteins, fat, minerals, and vitamins, each with a specific function in the development of the insect and safe shelf life of the constituted diet (Table 3).

Table 3. List of diet ingredients and their nutritional composition in the Katumani insectary.

	Diet ingredients	Roles/composition
1	Brewers' yeast	Minerals, proteins
2	Common bean powder	Proteins, carbohydrates, low fat, minerals, vitamins
3	Sorghum / maize leaf powder	Natural diet
4	Sorbic acid	Preservative
5	Ascorbic acid	Vitamin C
6	Vitamin E capsules	Vitamin E
7	Methyl hydroxybenzoate	PH balancer, bacteriostatic, preservative
8	Agar powder	Solidify and hold the ingredients together
9	Formaldehyde	Preservative

Personnel

Insectary personnel should be well trained and motivated. Insect rearing is labor-intensive and it is a 7-days-a week job. The minimum personnel requirements are an insectary manager, laboratory technical assistant, and/or laboratory cleaner. This number can vary depending on the scale of operation.

Diet preparation

The diet for stem borer rearing in the KARI-Katumani insectary is adopted from Onyango and Ochieng-Odero (1994) and Songa et al (2004) (Table 5). It is presented in three fractions (Fraction A, Fraction B and Fraction C).

Fraction A

All the powdered ingredients (Figure 6 and 7) from this fraction including sucrose and vitamin E are mixed using a plastic spoon, in a clean container under a fume hood. The distilled water is boiled, cooled to 60°C, and then mixed with the pre-mixed ingredients using a blender for 1 minute. Methyl-P-hydroxybenzoate that has been dissolved in 20 ml of ethanol absolute is then added into the mixture in the blender, and then mixed for a further 2 minutes.

Fraction B

Agar powder is weighed (Figure 8) in a separate container and then added to cold distilled water in a separate saucepan, boiled while stirring periodically, and then cooled to 60°C. Ingredients of Fraction B are added to Fraction A in the blender and then mixed for 3 minutes.

Fraction C

Finally, formaldehyde 40% is added to the ingredients of fraction A and B in the blender and then mixed for 3 minutes.

Table 4. Diet ingredients for rearing stem borers at CIMMYT-KARI, Katumani, Kenya.

	Ingredient	*C. partellus*	*B. fusca*
		Quantity (g or ml) per 3 liter diet	Quantity (g or ml) per 3 liter diet
	Fraction A		
1	Maize leaf powder	75.6	79.8
2	Common bean powder	265.2	264.9
3	Brewer's yeast	68.1	67.8
4	Ascorbic acid	7.5	7.5
5	Sorbic acid	3.9	3.9
6	Methyl-p-hydroxybenzoate	6.0	6.0
7	Vitamin E capsules (200 iu)	6.3	6.3
8	Sucrose	105.9	105.9
9	Distilled water	1209.3	1209.3
	Fraction B		
1	Agar (Tech No. 3)	37.8	37.8
2	Distilled water	1209.3	1209.3
	Fraction C		
1	Formaldehyde 40%	6.0	6.0

3.4 Diet infestation

The surface of the diet in each jar is first punctured in several places using a sterilized plastic rod to facilitate larval penetration. Diet infestation is usually done using surface sterilized black-head eggs or neonate larvae from pre-sterilized eggs. In *C. partellus*, 50 egg masses or neonates are introduced into each jar. After infestation, the mouth of the jar is covered with a paper towel and then covered with a screw cap that is ventilated with very fine wire mesh to prevent the larvae escape. The paper towel is meant to absorb excess moisture within the rearing jar during larval development. In the case of *B. fusca*, neonate larvae are introduced singly into heat sterilized glass vials containing the diet, using sterile camel hair brush no. 1. The vials are closed with tight fitting cotton-wool plugs and arranged in the metal trays in batches of 100 vials per tray.

The jars and vials containing the larvae are kept on shelves (Figure 9) in larval rearing room at controlled environmental conditions (28 ± 2°C; 65 ± 5% RH; L12:D12 photoperiod). The larvae are allowed to feed undisturbed until pupation.

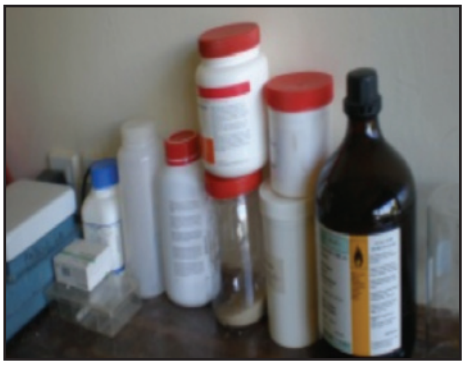

Figure 6. Bottles with diet ingredients.

Figure 7. Bottles with bean and maize leaf powders.

Figure 8. Balance to weigh diet ingredients.

3.5 Management of larvae and pupae

Larval and pupal monitoring should take place daily in such a way that diets contaminated with fungus or insects such as ants or mites are discarded immediately. Close monitoring for pupal harvesting starts 30 days after diet infestation. All containers are checked daily after this date in order to avoid moth emergence within the rearing jars.

Pupal harvesting is done at once when most (50%) of the larvae have pupated. The larvae that would not have pupated by this time are kept in sterilized plastic jars containing clean moist paper towels until they pupate. The diet from each jar is emptied onto a clean tray, and the pupae are sorted and transferred into a plastic container lined with tissue paper. The pupae are cleaned with a gentle spray of distilled water. They are then placed on tissue paper to drain excess moisture. The pupae are transferred to clean petri dishes (9 cm in diameter) lined with moist tissue paper.

Three petri dishes containing about 100 larvae are put in a metal framed emergence cage (oviposition cage, 45 x 60 x 45 cm), ventilated at the top with fine wire mesh. The emergence cages are kept at room temperature (23–24°C); 12:12 light: dark photoperiod. A relative humidity of 80–90% is maintained in the cage by placing a plastic cup containing water-soaked cotton wool in the cage at all times.

3.6 Management of moths

The oviposition cage is lined with a wax/butter paper at the bottom as an oviposition substrate (Figure 10). For C. *partellus*, the paper is molded to form several pleats, while for *B. fusca*,it is rolled into thin tubes which are closed at each end. These are then suspended through slits at the top of the cage. The moths feed on water from a water-soaked wad of cotton wool in a petri dish placed in each cage. About 100 moths are kept in each oviposition cage. The oviposition cage is checked daily:

1. The fresh wax paper is replaced.
2. Eggs that have been oviposited on wax papers are collected.

3. The water-soaked cotton wool in the petri dish is also replaced.
4. Dead moths are removed from each cage after which the cage is cleaned and disinfested.
5. The live moths are transferred to a freshly prepared cage.

3.7 Management of eggs

Eggs laid on the waxed paper (Figure 9) are transferred to clean plastic containers, which are kept in the oviposition room and allowed to develop for 3–4 days. A RH of 80–90% is maintained in the container, by putting a wide plastic dish with water-soaked cotton wool at the bottom of the container, below the oviposition paper. The eggs are cut off the waxed paper using scissors (50 eggs per batch), their surface sterilized by dipping them in 10% formaldehyde for 15 minutes, rinsing them thoroughly using distilled water and then drying them on filter paper. In about 4–6 days the eggs are developed into a black-head stage which can hatch into the neonate larvae after 1–2 days. The development of the eggs can be arrested by placing them in a refrigerator at 10°C for up to three days without loss in hatchability. Both the black-head stage eggs and the neonate larvae can be used to infest the artificial diet or will be used for field screening of maize genotypes.

Figure 9. Egg batches on the ridges of wax paper.

3.8 Maintaining the quality of insects

Quality is a term relative to the end-user's needs. The ultimate goal of rearing is to obtain insets of acceptable quality. Over the generation period of laboratory rearing, insects in captivity lose their typical behavior. In this regard, the KARI-Katumani insectary strives to produce stem borers that are as competitive as the wild population of the same species. The wild populations are used for relativity studies against the performance of the laboratory-reared insects. The parameters used in determining quality of laboratory-reared stem borers include survival rate, developmental period (egg to adult), deformities, live weight, reproductive capacity (number of eggs laid, hatchability, sex ratio), growth index (the ratio of percent pupation over mean larval development period) and adaptability under field conditions. The quality of the laboratory-reared insect is monitored periodically against the aforementioned quality parameters.

3.9 Managing disease problems in the insectary

'Disease problems' refers to microorganisms responsible for contaminating insect colonies and artificial diets in an insectary (Polaszek 1998; Songa et al 2004). An insect artificial diet is also equally suitable for growth of some microorganisms. Microbial organisms in the artificial diet cause spoilage and alter biological performance of insects. Several species of these organisms including bacteria (*Streptococcus* sp., *Serratia* sp. and *Pseudomonas* sp.), fungi (*Aspergillus* sp., *Rhizopus* sp. *Penecillum* sp.), protozoa (*Nosema* sp.) and viruses are reported to have contaminated insectaries. Most of the microorganisms are not harmful to the insects although they contaminate the artificial diet. However, *S. marcescens* and *Nosema* sp. are pathogenic to insects and may cause an outbreak in an insectary. Field collected insects, the rearing environment and improper handling of the insects and diets during preparation, storage and use are some of the sources of microbial contamination in an insectary. Immediate removal and disposal of contaminated diets and infected insects, proper sterilization of diets, eggs, working area and utilities, good personnel hygiene, and following a recommended safety regulation will minimize microbial contamination in an insectary.

3.10 Human health concerns in the insectary

Moth scales, toxic fumes and microbial contamination are issues of health concern to laboratory workers in the CIMMYT supported KARI-Katumani insectary. Moth scales can cause respiratory problems and allergies. To circumvent this problem, workers are required to wear a face mask at all times. In addition to this, all surfaces in the moth room are cleaned daily using a vacuum cleaner. Toxic fumes from formaldehyde, one of the preservative and sterilizing chemicals used during diet preparation, can be harmful to human health. It is recommended that insectary personnel should wear face masks and work under the fume hood to avoid inhalation of fumes during diet mixing. Some microorganisms such as *Aspergillus* and *Streptococcus* which can contaminate insect diet can also affect human health (Polaszek 1998; Songa et al 2004). Therefore, wearing hand gloves, face masks, and lab coats are recommended to be worn by all personnel dealing with contaminated diets and infected insects.

3.11 Safety regulations

In order to maintain the quality of laboratory-reared insects as well as to avoid any health hazards to insectary workers, it is mandatory to formulate and enforce rules and regulations. The following good practices are followed at the KARI-Katumani insectary:

1. Entry to the insectary is restricted to only the insectary workers.
2. All insectary personnel should maintain high personal hygiene.
3. Laboratory coat, hand gloves and face masks should be worn at all times.
4. Eating, drinking and smoking in the insectary is not permitted.
5. The work area should be cleaned daily with germicides.
6. Contaminated diets (including the insects in it) and diseased insects should be removed and properly disposed of immediately.
7. Insects that have escaped from the rearing jar or cage should be discarded.
8. Undesirable insects (other than the desired stem borer species) should be controlled.

4 Post-Harvest Insect Pests of Maize

4.1 Biology

S. zeamais belongs to order Coleoptera and family Curculionidae. It is a small weevil about 2.4–4.5 mm in length with its head protruded into a snout or a distinct beak or proboscis. At the end of this structure, there is a pair of mandibles or jaws. It is generally reddish brown in color, sometimes dark brown or almost black. The newly emerged weevil is light brown to reddish brown. It has a long and narrow snout, with clubbed and eight-segmented elbowed antennae. It is further identified by the presence of four light reddish-brown or yellowish pale oval spots on the elytra (Khare 1994).

The pre-oviposition period is about three days. It remains fecund throughout its lifetime but the effective egg laying period is 50% of the first 5 weeks of its life span. The female lays up to four eggs in a single maize kernel. The sex ratio of the newly emerged maize weevil is 1:1 and female weevils live longer than male weevils.

The adult maize weevil may remain inside the kernel for some time after eclosion but eventually emerges by chewing its way out. After emergence from the pupae, the adult eats through the outer layer of the grain leaving a roughly circular hole approximately 1.5 mm in diameter (Kranz et al. 1997). The weevils use their elongated snouts, which have jaws for boring into the grain, while the females use their snouts for digging a shallow hole into which they lay eggs. Due to the higher fecundity of females, if the weevils are not controlled their rate of increase is extremely high. However, the weevils do not breed well at temperatures below 20°C or above 32°C, or in food with moisture content below 11%. Mating does not take place before the adults are three days old (Walgenbach and Burkholder 1987). S. zeamais completes development from egg to adult in 31–64 days at 30°C on maize with 13% moisture content. Median development time is 42 days. The actual development period depends on the type and quality of grain being infested. The adult weevil feeds and lives for up to four to five months, and from several months to a year (Kranz et al., 1997; Appert 1987).

The egg is white in color and oval in shape. A fully-grown larva is white in color and is about 4 mm in length (Hill 1983). Eggs are deposited singly in narrow cavities chewed in the kernels and covered with a gelatinous material that quickly hardens to form a protective plug. Upon hatching, the larvae burrow into the grain and form a winding tunnel that increases in size as it grows (Cotton 1956). The larva molts four times before pupation takes place. Larval development and pupation take place inside a single grain and larval damage is thus hidden from visual inspection. Pupation occurs at the end of the tunnel in a cell formed of frass, borings and larval excretions (Abraham 1991). The female weevil produces 300 to 400 eggs over a period of 4–5 weeks and after six days, at 25°C, these eggs hatch into tiny grubs, which stay and feed inside the grain (Hill 1983).

Various factors may influence the number of generations and the life span of adults. Adams (1976), Dobie and Kilminster (1978) and Gomez et al. (1983) have reported that both diet and varietal differences within cereals can affect developmental time and reproductive capacity of *Sitophilus* species. Development periods are extremely protracted at low temperatures (e.g. 98 days at 180C and 70% RH) (Darling 1951).

(a) (b)

Figure 10. The adult maize weevil (a) and grain damage (b).

The larger grain borer, *Prostephanu truncatus* (Horn) (Coleoptera: Bostrichidae), is native to meso-America (Hodges 1994) where it has long been recognized as a destructive pest of maize stored 'on the cob'. It belongs to the family Bostrichidae, members of which are known as powder post or false powder

post beetles (Booth et al. 1990). Bostrichidae mainly live on felled and/or dried wood, but green timber is also attacked by some species, and they are widely recognized as pests of timber (Tooke and Scott 1994). The deflexed head, strong mandibles, and cylindrical body shape of *P. truncatus* are typical features of xylophagous insects. The larger pronotum protects the head during tunneling and provides strong support for the mandibular muscles (Li 1988). *P. truncatus* has remarkable ability to tunnel through hard materials, for instance, adults have been found to penetrate plastic, 35 mm thick (Li 1988). Such mandibular strength can, however, only be applied if the beetle is able to get sufficient leverage (such as between two kernels on maize cob), since it has difficulties when attacking a smooth surface.

The body length of the adult ranges from 2–3.5 mm in length and 1–1.5 mm in width (Figure 11a), with a sex ratio is 1:1 (Birkinshaw 1998). *P. truncatus* is capable of flying. It was estimated that an adult can fly 25 km in 45 hours (Pike 1993). Flight activity is initiated by a reduction in food quality and seasonality in tree growth. Dispersing *P. truncatus* can be captured in flight traps baited with the insects' male-produced aggregation pheromone (Dendy et al 1989).

Host finding is done through pheromone and volatile compounds emitted from the maize grain. Since males produce pheromones at the highest rate when they are on a suitable substrate and are not in the presence of females (Scholz et al 1998), the detection of a pheromone may be a powerful, positive signal for dispersing individuals. It is possible, however, that other volatiles and chemical cues play a role with respect to finding new food sources.

P. truncatus reproduces on maize grain and ears (Figure 11b), dry cassava and other stored commodities. The eggs are laid in small clutches in tunnels, and the egg clutch is usually protected by tightly packed frass when reared on loose maize grain. The female lays on average 5–8 eggs in each oviposition chamber, the chamber being half as long as the insect's body and slightly wider than its abdomen (Bell and Watters 1982). It has a lifetime fecundity of 300 eggs when reared on yellow maize. Fecundity and survival reduce when very hard maize varieties are used (Li 1988). *P. truncatus* undergoes three larval instar

stages and the average larval period is 16 days. The last larval instar makes a pupal case from frass bound with secretions from the larvae within the grain or surrounding flour. The larvae can be differentiated from other stored product insect larvae by their C-shaped body and head retracted into the prothorax. The developmental time from egg to adult at 70% RH ranged from 25 days at 32°C to 167 days at 18°C.

(a)

(b)

Figure 11. The adult larger grain borer and cob damage.

4.2 Economic importance

The maize weevil is an important pest of stored maize in the tropics, particularly when maize is stored on-farm, with no control of moisture content, and without chemical protectants. Grain weight loss of 12–20% caused by the weevil is common, and up to 80% loss may occur for untreated maize grain stored in traditional structures in tropical countries (Boxall 2002). Weevil damage results directly in lost food (reduced grain weight), and also may reduce future maize production for farmers who plant saved grain as seed, a practice that accounts for about 70% of all maize planted in eastern and southern Africa (Boxall 2002). There is also a health risk associated with consumption of weevil-infested maize grain, as such grain has been reported to have higher levels of *Aspergillus flavus* contamination than non-infested maize kernels.

The larger grain borer was accidentally introduced from Central America into Tanzania in the late 1970s, and spread to other countries in the region. In West Africa it was first found in Togo in the early 1980s. It has now spread to at least 18 African countries becoming the most invasive destructive pest of stored maize in Eastern, Central, Southern and Western Africa. Up to date it has been reported in Benin, Burkina Faso, Burundi, Ghana, Guinea Conakry, Kenya, Malawi, Mozambique, Namibia, Niger, Nigeria, Rwanda, South Africa, Tanzania, Togo, Uganda, and Zambia. In some of these countries, it has become a serious pest of stored maize and dried cassava.

The larger grain borer is a serious pest of stored maize, and will attack maize on the cob, both before and after harvest. Adults bore into the maize husks, cobs or grain, making neat round holes and tunneling extensively, producing large quantities of grain dust as they tunnel. The adults prefer grain on the cob to shelled grain, thus damage on unshelled maize is greater than that on loose, shelled maize.

When infesting stored maize cobs with the husk intact, the adults frequently begin their attack by boring into the maize cob cores, and eventually gain access to the grain at the apex of the cob by crawling between the cob and husk. They may also bore directly through the husk. This causes considerable losses in stored maize; weight losses as high as 35% have been observed after only 3–6 months storage in East Africa (Hodges et al. 1983; Muhihu and Kibata 1985). The larger grain borer is spread over longer distances almost entirely through the import and export of infested grain. Local dispersal is through the movement of infested maize from surplus to deficit areas, and by flight activity. Although the larger grain borer develops best at high temperatures and relatively high humidity, it tolerates dry conditions, and may develop in grain at as low as 9% moisture content (Haines 1991) in contrast to many other storage pests, which are unable to increase in number under low moisture conditions. For this reason, where infestations of the larger grain borer appear with other storage pests, the LGB is the predominant storage pest under dry conditions.

5 Rearing Post-Harvest Pests of Maize

Rearing post-harvest insect pests of maize for research is an important part of the Global Maize (GMP) Program at CIMMYT. This book aims to provide detailed procedures for culturing the maize weevil *S. zeamais Motsch* and the larger grain borer *P. truncatus*. The procedures described here are those used in the post-harvest insect pest laboratory at KARI-Kiboko, Kenya. The rearing procedures are adopted from CIMMYT (1989), with several modifications to suit the conditions at Kiboko.

5.1 The rearing room

The rearing room should be protected from fluctuations in climatic conditions (temperature and relative humidity). Suitable, constant, environmental conditions should be maintained, for instance, 28 ± 1°C, 65 ± 5°C RH, and 12:12, a light:dark regime. Equipment to maintain these conditions includes humidifiers and heaters. Conditions in the colony room should be monitored daily with calibrated thermometers, and regular readings recorded and kept for future reference. Environmentally conditions for both culturing insects and conducting experiments should be carefully controlled in order to assure reproducibility of results (CIMMYT 1989).

5.2 Rearing containers

The simplest and most convenient container for rearing post-harvest insects is a glass or plastic jar. The capacity of the jars range from 0.25 l to 2 l. However, larger jars are preferable for mass rearing. The colony jars are covered and sealed with a lid consisting of three parts: a piece of filter paper 9 cm in diameter, a circle of 60-mesh brass screen 9 cm in diameter, and a metal ring that screws down onto the mouth of the jar. The brass screen is placed against the inside lip of the metal ring, and the filter papers placed over the screen, facing the inside of the jar. The screen prevents the movements of the adult and larval insects into and out of the colony jar, while the filter paper provides an additional barrier to the movement of small larvae, mites, etc. The entire lid assembly must be tightly secured to the jar.

Each jar should be labeled on the outside, indicating the species it contains, and the date on which the colony was set up. This information can be written directly on the outside surface of the jar using a felt-tip marker with water-soluble ink. This method of labeling is convenient because the labels are easily removed by washing, particularly if the jars are to be reused. However, the simplest way is to use printable label and to affix it on the outer side of the jars.

5.3 Diet preparation

The maize weevil and the larger grain borer are reared on whole maize grain or dehusked cobs. The insects are cultured on maize seed H513, a hybrid mostly grown by farmers in Kenya, but which is susceptible to storage insects at ambient conditions. When the grain is first received or harvested, it must be cleaned and dried before storage or usage. Cleaning could be done by sieving to remove any excess dirt, dust, fine materials, moldy and broken or shriveled kernels. Once the grain is clean and dry, the grains should be either kept in deep freezer at -20 ± 2°C for two weeks in order to disinfest, or fumigated with phostoxin tablets in a plastic drum or barrel for seven days.

For a grain to be used for an insect colony, it should be checked for proper moisture content. The optimum maize grain moisture content for storage insects is 13–14% at a rearing temperature of 28 ± 1°C, 65 ± 5°C RH (Haines 1991). If the moisture content of the whole grain intended for the colony media is not suitable, it must be adjusted—if too high, the grain can be dried by spreading on a clean floor, and blowing air over it. Once the desired moisture level is reached, the grain is returned to the freezer to destroy any possible infestation before it is ready for insect rearing. If the moisture content of the grain is too low, then water must be added. This is done by putting a grain in a container or any mixing device, then gently and evenly adding distilled water over the surface. The formula below gives a guide on the amount of water to add: $Q = A (b – a)/(100 – b)$ where Q = weight of water to be added, A = initial weight of grain, a = initial grain moisture content (wet weight basis) and b = desired final grain moisture content of grain (wt weight basis) (Boxall 1986).

Seal the container and rotate or mix it for 30 minutes. If the moisture is determined to be appropriate, put the grain in storage for future use. If not, then repeat the tempering process or dry as needed.

Four hundred grams of grain (moisture content 11–12%) is placed in one-liter glass jars covered with perforated lids. About 200 unsexed adult insects, either the maize weevil or the larger grain borer, are introduced into the jars. Purchasing grain for insect rearing from a commercial dealer is not appropriate because untreated grain may have been mingled with insecticide-treated grain. It is important to know the storage history of grains if they are not purchased directly from the field. If there is any concern about chemical contamination in a batch of grain, a bioassay should be conducted. To do this, add 100 adult insects of the same species to each of several 250 g samples of the grain in jars. In addition, prepare control samples with grain that is known to be uncontaminated. All the jars should be set up on the same day and checked 3–7 days later to determine mortality. If the suspect grain is contaminated, the numbers of insects surviving in those samples should be significantly lower than those in the control samples.

5.4 Sanitation

Proper sanitation practices are essential to prevent contamination of the colony by unwanted species and the loss or spoilage of colony by diseases. Consequently, once a healthy insect colony has been established, it should be isolated in a designated rearing area. In this area, colony jars should be kept on inverted plastic petri-dishes (9 cm diameter) resting in a shallow tray containing a thin layer of oil, such as mineral or light oil (commonly sold as odina or risella oils). The petri-dishes support the jars above the oil layer, and the oil prevents insects and mites from crawling from one jar to another. As a rule, the jars should never be opened in the colony room, nor should insects be handled in that area. As the colony matures, it is used to inoculate a new colony and then discarded after this. If the old colony is left in the rearing area, it becomes a possible source of mites, psocids, and disease contamination.

As a further precaution against disease, parasites and unwanted insects, stock colony should never be exposed to grain samples and/or insects obtained from a field. Grain samples from the field should be frozen immediately after evaluation, and field insects should not be mixed with the stock colony unless they (field insects) are known to be free of contaminants. Before a field population is admitted to the colony room for rearing, it should be kept in isolation for a few generations, where it can be properly observed for disease and parasites. The work area must be kept free of spilled grain and other debris that might harbor residual insect populations capable of infesting the stock colony.

Any equipment (sieves, trays, brushes) needed to maintain the insect colony must be thoroughly cleaned and disinfested before reuse. Equipment should be used for one species only, and should not be re-used until cleaned and disinfested. All equipment used to handle insects should be washed in hot and soapy water, and placed in an oven at 65°C for 30 minutes. Likewise, all insect colonies (with jars) to be discarded should be placed in an oven at 65°C for at least one hour. Equipment should be stored in a clean, uncontaminated area. After one insect species is handled, work surfaces should be cleaned and/or disinfested before work is begun on the next species. Finally, one must establish a regular schedule for maintenance of the stock colony. This schedule should be timed so that closely related species are not handled on the same day, thereby reducing the risk of cross-contamination.

5.5 Establishing and maintaining colony

The first step in establishing an insect colony is to obtain adults of the desired species. Keep records of the source of the insect strain and the date that the colony was initiated. The two most common sources of adults are field populations and a colony maintained at an alternative location, from which adults may be obtained upon request. Once adults are acquired, they should be placed in colony jars on the appropriate growth medium and isolated from other colonies and rearing areas. They should remain in isolation until it is reasonably certain that they are free of diseases and parasites. Generally, these problems can be detected by regular visual inspections of larval and adult populations in the new colony.

If colonies are contaminated with mites and psocids, they may be difficult to disinfest. The adults from the colony can be transferred frequently, always destroying the old medium immediately. Lowering the RH makes the colony environment less desirable for mites and psocids. If this procedure doesn't work new breeding stock may be necessary. Appearance of mites and psocids may be an indicator of high moisture in colony because of overcrowding or poor moisture control.

After the adults of a species have been obtained and determined to be disease- and pest-free, the next step is a deliberate and gradual expansion of the colony population. The development of the progeny produced by the original adult population should be closely monitored to anticipate the emergence of the first generation of new adults. When sufficient adults have emerged, these are removed from the original colony jar and used to set up new colony jars. The original colony may not be recorded or it may be retained to allow the emergence of adults for more colony jars. This will depend upon whether one wants to have all the individuals in a colony population at the same stages of development, or to stagger the development of these individuals over specific time intervals. In the latter case, the original colony should be retained so that new adults can be used to set up a second new colony (jars) at designated time intervals. This process can be repeated until the emergence of adults in the original colony has ceased.

In order to expand a colony, it is preferred that the original colony jar provide adults for at least two new colony jars. Likewise, each colony jar of the second generation must be used to set up two or more jars for the third generation, and so on. This expansion process is sustained until the colony reaches the desired proportion, which depends largely on the anticipated needs. The size of colony can be altered by changing either the frequency with which colony jars are set up or the number of jars that are set up each time the colony is handled. Larger jars are desirable for production of large numbers of the maize weevil and the LGB.

To avoid genetic decay in a colony, new cultures collected from a diverse area can be set up using adults from each locality. After rearing each colony for one generation and making sure that they are free of pathogens and parasites, the colonies can then be combined into a single jar, allowing them to mix for few minutes before introducing them into the new oviposition substrate (jars containing maize grains). This prevents selective effects that might result from maintaining separate population lines in separate jars.

A list of equipment used in culturing post-harvest pests of maize at the KARI-Kiboko insectary is given below (Table 5). This list by no means exhaustive; however, it is sufficient to run the existing rearing activities. The equipment includes glass jars (Figure 12), electronic balance (Figure 13) covered with wire mesh, screen mesh (4.7 and 1.0 mm) (Figure 14), seed moisture meter (multi-grain, Seedburo) (Figure 15), oven (Figure 16), refrigerator (Figure 17), glass vial (Figure 18), thermometer (Figure 19), camel hair brush (Figure 20), trays (Figure 21), tweezers (Figure 22), seed fumigation barrel (Figure 23), scissors (Figure 24), net bag (Figure 25), shelves (Figure 26), heat fan (Figure 27), and labels (Figure 28).

Table 5. A list of equipment used in culturing post-harvest pests.

	Equipment	Function
1	Glass jars	Insect culturing and screening shelled maize
2	Electronic balance	Weighing seed and dust
3	Screen meshes	Separating grain, dust and insects
4	Moisture meter	Determine grain moisture content
5	Oven	Sterilizing jars and metal ware
6	Refrigerator	Disinfestations of seed
7	Glass vials	Temporarily storing insects after counting them
8	Thermometer	Temperature recording
9	Camel hair brush	Collecting insects
10	Trays	Working area during counting insects
11	Tweezers	Holding insects
12	Fumigation barrel	Disinfestations of seed
13	Scissors	Cutting
14	Net bag	Incubating unshelled ears
15	Shelves	Incubating jars
16	Heat fun	Heating the room
17	Labels	Tagging the jars or net bag

Figure 12. Glass jars (2 l).

Figure 16. An Oven.

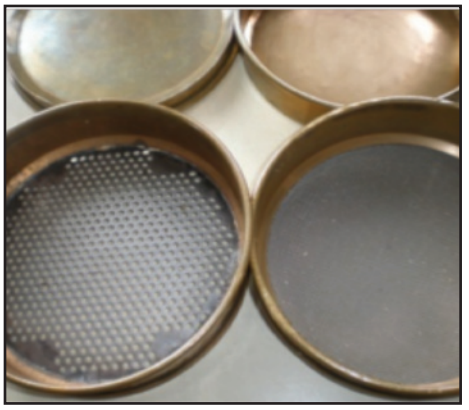

Figure 14. Sieves.

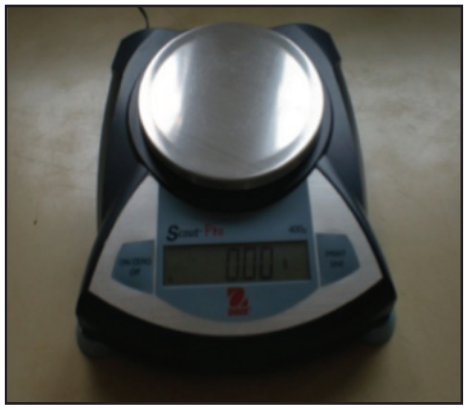

Figure 13. An electronic balance.

Figure 15. A seed moisture meter.

Figure 22. A tweezer to handle insects.

Figure 17. A refrigerator.

Figure 24. Scissors.

Figure 18. A glass vial.

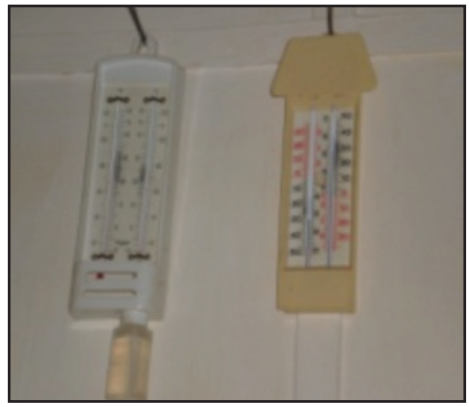

Figure 19. Thermometers.

Figure 20. A Camel hair brush.

Figure 21. A tray with insects.

Figure 23. A seed fumigation barrel.

Figure 27. A heat fan.

Figure 25. Net bags.

Figure 26. Shelves with jars.

Figure 28. Manila tags (label)

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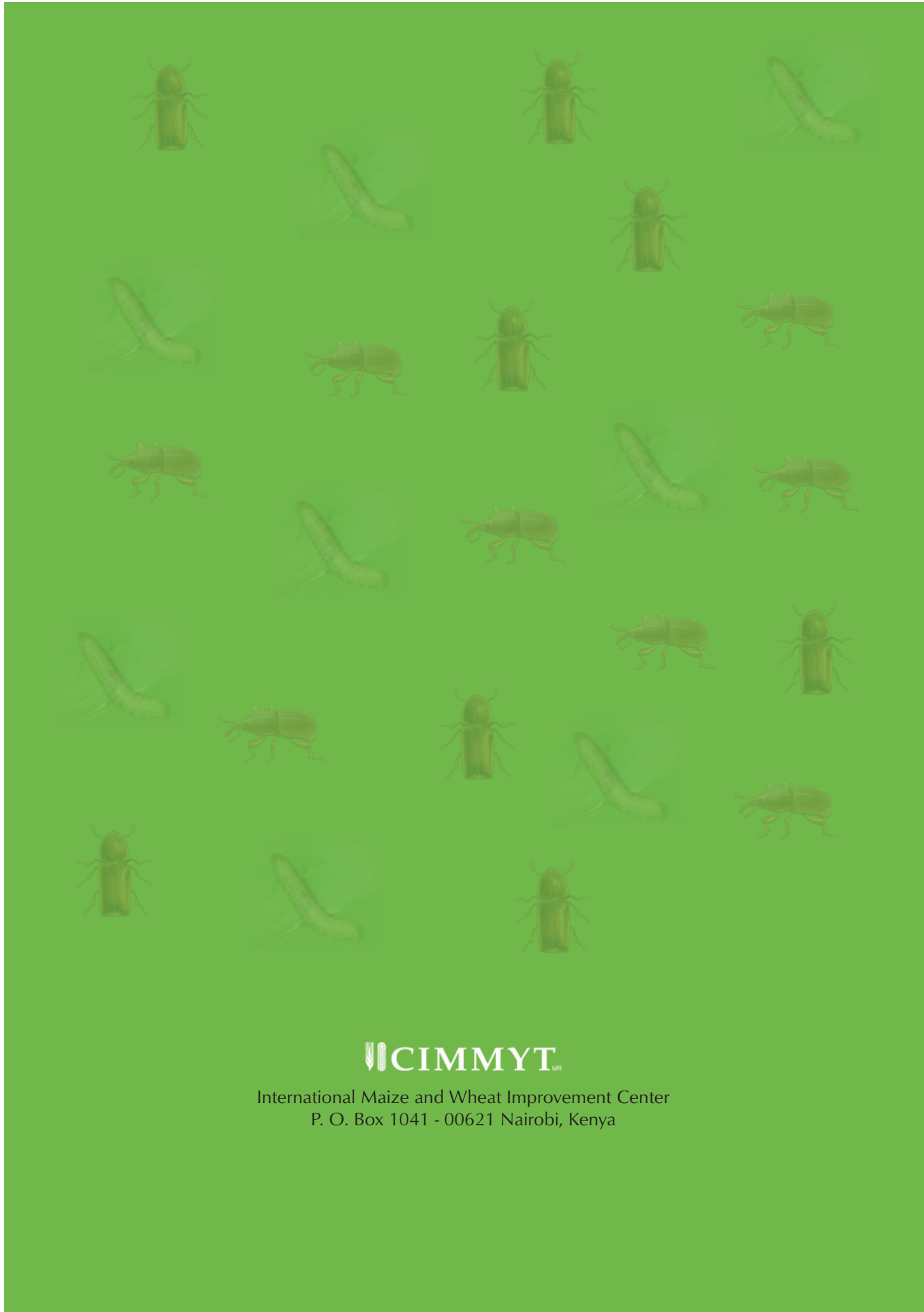

CIMMYT

International Maize and Wheat Improvement Center
P. O. Box 1041 - 00621 Nairobi, Kenya