IITA Transforming African Agriculture CGIAR	**Crop: Maize, Cowpea** **Function: Insect Rearing**	**SOP #**	IITA-ENTLAB-SOP01
		Revision #	IITA-ENTLAB-SOP01- 01
		Implementation Date	12/08/2022
Page #	1 of 18	**Last Reviewed/Update Date**	19/08/2022
SOP Owner	Snr. Research Supervisor (Rachael Aderanti)	**Approval Date**	19/08/2022

STANDARD OPERATING PROCEDURE (SOP) FOR MASS REARING OF FAW, MAIZE STEM BORER, COWPEA BORER, CICADULINA, APHIDS, THRIPS AND STORAGE PESTS.

Authors & Contributors: Mrs. Aderanti Rachael(R.Aderanti@cgiar.org), Mr. Odey Jacob(J.Odey@cgiar.org), Mr. Faniran Afolabi(A.Faniran@cgiar.org), Mr. Tijani Abideen(A.Tijani@cgiar.org) and Mr. Fabiyi Samuel(A.Fabiyi@cgiar.org).

1. Introduction

Sesamia calamistis Hmps. and Eldana sacharina Walker are two of the most important stem borers of maize in Africa (Bosque-Perez et al... these proceedings). Resistant varieties have been suggested as one of the most promising means to decrease the economic losses caused by these two pests (Bowden 1976; Girling 1980).

Large numbers of insects are required for resistance studies at the International Institute of Tropical Agriculture (IITA); thus, successful mass rearing techniques are essential for the implementation of the breeding program.

An important consideration when insects are mass reared is to ensure that the laboratory colony exhibits the genetic diversity, aggressiveness, and vitality that the pest population exhibits in nature (Mihm 1983). To accomplish this, we replace our stem borer colony every year.

2. Purpose

The purpose of SOP is to describe the procedure to be followed when screening for maize or cowpea resistance to Spodoptera frugiperda (FAW) is preserved experiments in a reliable resource.

3. Scope

This is SOP covers the materials and ingredients used for mass rearing of Spodoptera frugiperda (FAW), Sesamia calamistis, Eldana sacharina (maize stem borers), Maruca vitrata (cowpea borer), Cicadulina etc. are gotten from USA. The mass rearing of the insects is used for screening of breeders' trials, PHD and master's Students in the universities within and outside the country. Collection of data, insect identification and experimental design are being done.

4. Definition of terms

INFESTATION: Is the process of introducing egg mass or 1st instar larva of stem borer into maize plant (experiment on the field or screen house) so they can damage the plants.

SUSCEPTIBLE: These are plants infested that are damage by the insects.

RESISTANT: These are plants infested with the insects that the insect did not damage it.

5. Roles and Responsibilities

Entomologists are responsible for experimental planning, data analysis and publication.

Research supervisor is responsible for data collection, preparation of diet, infestation of diet, general supervision of laboratory, field and screenhouse work.

Research Technicians are responsible for field, lab and screen house infestation, collection of insects, pupa harvesting, eggs harvesting, filling of planting pots and discarding.

6. Procedure/Protocols

6.1 PREPARATION OF SESAMIA AND ELDANA DIET

6.1.1 Materials used for diet preparation include

1. 2 big pots,
2. Weighing balance
3. Micro flow laminar station
4. Gas cooker
5. Spatula
6. Heavy duty blender
7. Cylinder (1000ml)
8. Diet container (Arena)
9. Fork.
10. Mixer

6.1.2 COMPOSITION OF DIET INGREDIENTS FOR SESAMIA AND ELDANA

COMPONENTS **AMOUNT IN 16 LITERS DIET**.

FRACTION A

- Water for boiling 7200ml
- Soybean flour 1024g
- Wheat germ 456g
- Salt mixture 152g
- Sugar 184g

FRACTION B

- Water for boiling
  7200ml
- Agar
  150g

FRACTION C

- Ascorbic acid
  100g
- Aureomycin (14.1%)
  15.6g
- Methl-parahydrobenzoate
  26g
- Sorbic acid
  16g
- Streptomycin
  2g

FRACTION D

- Potassium hydroxide (KOH)
  88ml
- Propionic/phosphorus acids
  92ml
- Choline chloride (15%)
  104ml
- Acetic acid (25%)
  200ml
- Formaldyhyde (10%)
  16ml
- Vitamin suspension
  104ml

6.1.3 PROCEDURE FOR PREPARING SESAMIA AND ELDANA DIET

Mass rearing of maize stem borer, cowpea borer and FAW. Preparation of each diet are different from each other, Sesamia calamistis and Eldana sacharina diet preparation are the same, the only different is that we add 2g of streptomycin to Eldana diet, this is because Eldana is prone to contamination.

STEP 1

Measurement of fraction A: soy flour, wheat germ, sugar, and salt mix, are blended with 7.2 liter of distill water pour and boil in a cooking pot. Agar, which is fraction B measured, and boil with 7.2 liter of distill water. Fraction A and B are boiled for ninety minutes, after it has reach boiling point, off the fire and mix A and B together in a pot.

Step 2. The mixture of Fraction A and B will now be cool in water by stirring the liquid diet so that the hotness will be reduced to 60 degrees centigrade. As in (Fig 1)

After it has cooled, the diet will now be poured into a mixer where the Fraction C and D which stock solution will be added one after the other as the mixer is rolling, it will mix very well for another 10minutes, and then set aside.

STEP 3

The mixture of Fractions A, B, C and D which are mixed in the mixer is ready for dispensing into the container 500ml in each container. The containers would have been washed, soaked into 10% bleach solution overnight, then rinse it and leave it to dry in the micro flow. After the containers get dry, the diet is ready for dispense into the containers or arena.

STEP 4

Dish the diet using 1-liter cup into 29 containers 500ml in each container (i.e., one liter cup of diet should be dished into two containers) (Fig.2) and then leave to solidify. Use the fork to make lines on the surface of diet before infestation of the eggs or larva into the prepared diet.

After infestation of 100 egg mass or larva of sesamia calamistis into the diet in the containers, it will now be transferred into the larva holding room with a relative

temperature and humidity of 25 to 27 degree and 60 to 70% RH, using a 12:12 light-dark period.

Development takes approximately 4 weeks for Eldana sacharina, 5 weeks for Sesamia calamistis 3 weeks for Maruca vitrata and 3 weeks for Spodoptera frugiperda (FAW).

E. sacharina often pupate on the paper towel and pupa are covered by silk cocoon, S calamistis pupate most often on the diet. We collect pupae manually sterilized them by immersion in a 5% bleach solution for 2 min, then rinse and dry them and place them in the cages for emergence to adult stage. After collection E. sacharina pupae are removed from the silk cocoon either with scissors or by immersing them in a mild bleach solution for 5 min. removing the cocoon increases adult emergence. Pupa are kept at 22 to 24 degrees centigrade and 80 to 90% RH. Pupa collection of all the maize and cowpea borers are labor- intensive. Pupa are collected when they are 90% pupated, the remaining pre pupa or big larva are kept in plastic container with several layers of tissue paper until they pupated.

After adults emerge, they are transferred to cages (41 x 50 x 71 cm) for mating and oviposition. The eggs are collected daily. The cages are made up of wood and fine plastic netting; the bottoms are lined with paper towel. The adult oviposition is kept at the same temperature and humidity condition as the pupal cages, the light is turn off in the oviposition rooms when no one is working there.

Approximately 50 to 60 pairs of moths are placed in each oviposition cage. The sex of the adults is determined by the sexual dimorphism of the antennae. Two small plastic (20 ml) plastic cups with 5% sucrose solution and piece cotton wool are placed inside each cage for adult feeding.

The S. calamistis females lay their eggs on wooden (66cm) long stick wrapped with wax paper, a smooth surface with closely overlapping edges similarly to the leaf sheaths of maize plants appears to be preferred by S. calamistis for oviposition (Jackai and Raulston 1982). E. sacharina lay their eggs on paper towels folded diagonally (23 x 2cm) twenty papers are provided per cage. Eggs are collected daily, and new paper towels provided for oviposition. The eggs are incubated at 24 to 26 degrees centigrade and to 80 to 90% RH. The eggs are sprayed with distilled water every morning.

Egg masses can be stored at 10 degrees centigrade for up to 5 days to delay development.

6.2 PREPARING MARUCA VITRATA DIET

6.2.1 MATERIALS USED FOR DIET PREPARATION

1. Medium size pot
2. Blender
3. Spatula
4. Fork
5. Diet container
6. Weighing balance
7. Micro flow
8. 1000ml cylinder

COMPOSITION OF DIET INGREDIENT

COMPONENTS DIET	**AMOUNT IN 4 LITERS**
FRACTION A	
• Cowpea flour	400g
• Wheat germ	127.2g
• Ascorbic acid	25g
• Wesson salt mix	44.4g
• Aureomycin (14.1% active)	3.9g
• Sugar	60g
• Methyl-parahydrobezoate	3.6g
• Sorbic acid	6.8g
• Water for blending	2000ml
FRACTION B	
• KOH (4m)	22ml
• Choline chloride (15%)	2.6ml

- Acetic acid (25%) 50ml
- Formaldehyde (10%) 26ml
- Vitamin suspension 2 30ml

FRACTION C

- Water for boiling agar 2000ml
- Agar 59.2g

6.2.2 PROCEDURE FOR PREPARING MARUCA VITRATA DIET

STEP 1

Using a 1000ml calibrated measuring cylinder, measure out 2000ml of distilled or clean water and pure into the pot on fire. Measure out the specified quantity of Agar 59.2g and pour into the pot on fire and leave to boil.

STEP 2

Measure out all the components of fraction A according to their stipulated measurements and pour inside the blender add 2000ml of water for blending, blend for 1min.

Measure out also components of fraction B and pour into the blender too and blend for 3mins, and then set aside the blender.

STEP 3

Wait for fraction C to boil, then bring it down and cool it down to 60 degrees centigrade pour the boiled agar into the blender (i.e., mixture of fraction A+B+C blended) and blend the whole ingredients for 5mins and then set aside.

STEP 4

Rinse the diet containers that has been soaked with bleach or sodium hypo chloride and place it under the micro flow laminar workstation, 300ml of diet to be poured in each

container (i.e., the one-liter cup of diet should be dished into 3 containers) and then leave to solidify. Use fork to scarify the surface of the diet before infestation of the eggs or larva, this is for easy penetration of the 1st instar larva into the diet to feed.

Another method of maruca vitrata rearing is the use of germinated cowpea seeds. (Fig 5)

6.3 MASS REARING of Spodoptera frugiperda (FAW)

6.3.1 MATERIALS USED FOR DIET PREPARATION

1. 1 medium size pot
2. Weighing balance
3. Micro flow laminar station
4. Gas cooker
5. Thunderbirds
6. Folk
7. 60ml syringe

6.3.2 PROCEDURE FOR MASS REARING of Spodoptera frugiperda (FAW)

STEP 1

The following ingredients Cowpea flour, wheat germ, brewer's yeast, ascorbic acid, sorbic acid, methyl parahydroxybenzoate, are measured and blend with 2000ml of distilled water for 3minutes, Vitamin suspension and Formaldehyde (40%) measured and blended in the same blender.

STEP 2

Agar is measured and pour in the pot for boiling with 2000ml of distill water. After boiling, set it aside in water to cool and then pour the agar in to the blender and blend for 5minutes.

STEP 3

Rinse 200 thunderbirds and arrange them in the micro flow, the diet is now ready to dispense with the big syringe into the clean dry thunderbirds 10ml of diet. (Fig. 3)

Minimum of two hours later the diet is ready to be infested with 1st instar larva, meanwhile the surface must be scarified with folk for easy penetration of the larva.

After the infestation of the FAW, the larva will be moved into the larva holding room and they are maintained at 25 degrees centigrade, 70 to 80% RH. Three weeks after, 90% of the FAW will turn to pupa stage and in another 8 to 11 days they will emerge to adult stage, the adults will be kept in the cage both male and female with 5% sugar solution that they will feed on. Tissues are taped in the cages where their eggs are laid 3 to 5 days after they are kept in the cage. The eggs are usually harvested every day from the tissue in the cage and kept in the incubator at 10 degrees centigrade just to slower hatchability.

6.4 PROCEDURES AND TECHNIQUES FOR REARING CICADULINA LEAFHOPPER

Various Cicadulina species have different environmental requirements. Determining temperature, humidity, light condition and host plants that are optimal for selected leafhopper species as a Prerequisite to starting a large-scale rearing operation. Only reproductive potential and a high percentage of active transmitters of maize streak virus in their populations should be selected for mass rearing for resistance screening to this virus. Some species of cicadulina leafhopper are known as efficient vector of maize steak virus, which presently considered highly important in reducing maize yield in sub-Saharan Africa. Maize streak virus outbreak resulting in economic yield losses have been reported or observed in at least 20 African countries (Fajemisin et al. 1976, 1984).

Various species of cicadulina virus differ significantly in the maize streak- transmission efficiency. C.Triangula and C. mbila (Naude) are much more efficient vectors than C storeyi, C. arachidi or C. ghaurii sp. N. (Dabrowski 1987b)

Rearing highly efficient species reduces the number of leafhoppers required high and uniform maize streak virus symptoms on maize seedlings.

7. References

Bowden. J. 1976. Stem borer ecology and strategy for control. Annals of Applied Biology 84:107 – 134

Girling. D.J. 1980. Eldana sacharina as a crop pest in Ghana. Tropical Pest Management. 26: 152 – 156 IITA. 1983. International Institute of Tropical Agriculture, Animal Report for 1982. Ibadan Nigeria. IITA 1984. International Institute of Tropical Agriculture, Annual report for 1982. Ibadan Nigeria. IITA.1984.

International Institute of Tropical Agriculture, Annual report for 1983. Ibadan, Nigeria. Jackai, L.E.N and J.R. Raulston. 1982. Rearing two maize stem borers and legume pod borers on artificial diet. IITA Research Briefs 3(1): 1-6.

Mihm, J.A. 1982 Techniques for efficient mass rearing and infestation in screening for host plant resistance on corn earworm Heliothis zea. CIMMYT. Mexico.

Mihm. J.A. 1983. Efficient mass rearing and infestation tecniques to screen for host plant resistance to maize stem borers. Diatraea sp. CIMMYT, Mexico.

Raulston, J.R. and P.D. Lingren. 1972. Methods for large scale rearing of the tobacco budworm. USDA-ARS. Production Research Report No. 145.

Burton. R.L. 1967 Mass rearing the FAW in the laboratory. United States Department of Agriculture, Agriculture research service Report ARS-33-117.

Alam, M.S.1983 Mass production of Leafhoppers. Annual Report for 1982. International Institute of Tropical Agriculture.

Dabrowski, Z.T. 1983. Identifying and collecting Cicadulina for maize streak resistance screening IITA Research Briefs 4(4): 2-3.

Dabroswki, Z.T. Cicadulina rearing and screening for streak virus resistance. Annual report for 1983. International Institute of Tropical Agriculture, Ibadan, Nigeria. Pp. 41 – 42.

8. Appendix

Figure 1: Cooling of hot diet in water to reduce the temperature.

Figure 2: Dishing of 500ml Sesamia calamistis Diet into the clean containers (Arena)

Figure 3: Dispensing Diet into Thunderbirds for rearing (FAW)

Figure 4: Harvesting of Sesamia calamistis eggs with forceps.

Figure 5: Maruca vitrata rearing using cowpea germinated seeds.

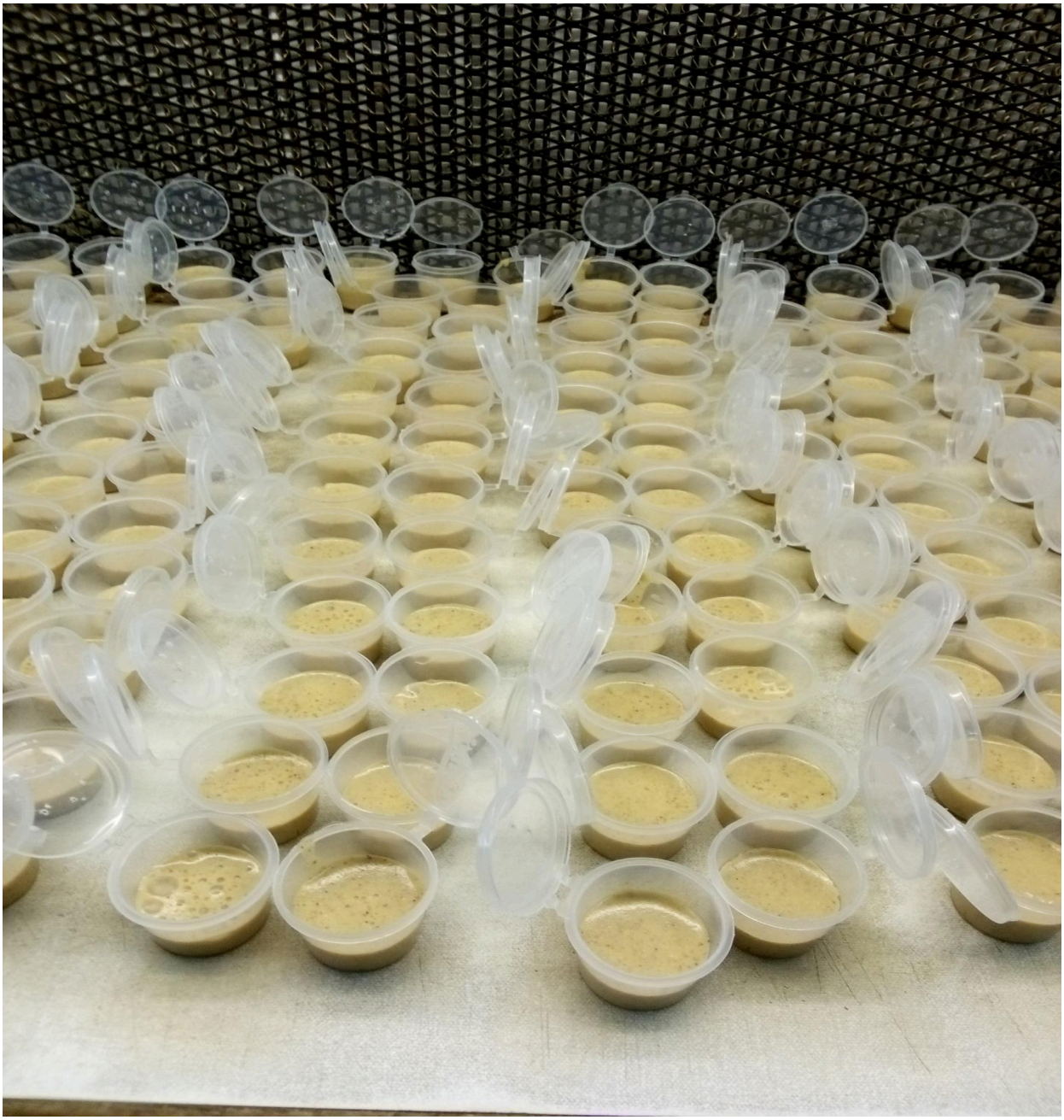

Figure 6: 10 ml of fall armyworm Diet.

Figure 7: Susceptible plants damaged by Spodoptera frugiperda (FAW).