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## Mass Rearing of *Chilo partellus* on Artificial Diet

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### Materials required

Refrigerator	— Incubator
Weighing balance	— Hot plate
Blender (Big and small)	— Pans
Plastic containers with lid (big and small)	— Plastic cups with lid (Indian Airlines Jam containers)
Cora cloth	— Vacuum cleaner
Plastic bottles	— Oviposition cages
Measuring cylinders and pipettes	— White glycine paper
Cellotape	— Plastic bucket with lid
Diet ingredients as per Appendix I	— Sodium hypochlorite

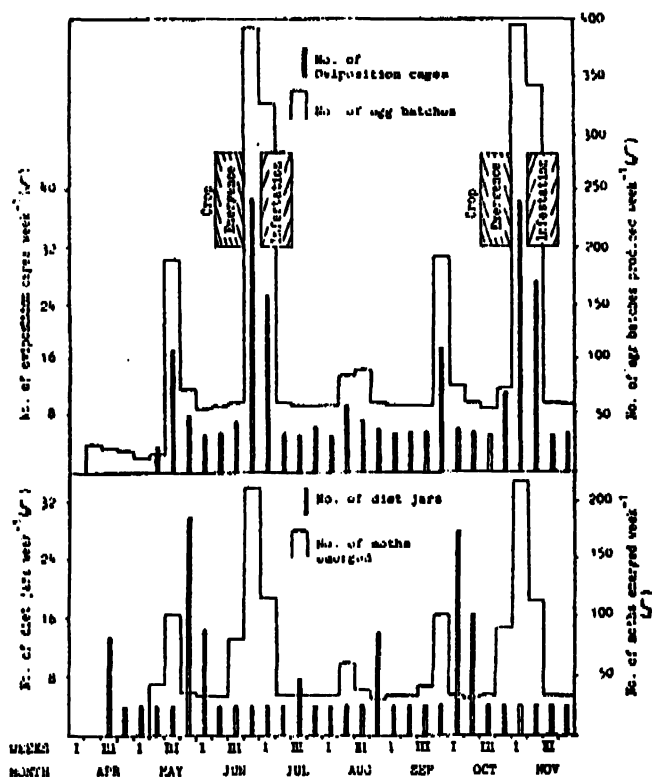


Fig. 8. Schedule of diet preparation, moth emergence, oviposition, and infestation of *C. partellus*, ICRIAT Centre.

time and labour input and mechanization wherever applicable, (ii) maintenance of good hygiene and cleanliness and preventing microbial contamination, (iii) keeping biological records of development times of all species being reared under different environmental conditions, and (iv) checking of insect quality as frequently as possible.

At ICRIAT, culture of *C. partellus* is maintained throughout the year. However production is increased at the time of field infestation. Figure 8 outlines the schedule of diet preparation, moth emergence, oviposition and crop infestation.

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Hatching of the eggs can also be delayed if they are not required immediately. This is achieved by storing black head stage eggs at 10°C under high humidity (>90%). In this case, the plastic bucket complex is kept in an incubator set at 10°C (Fig.7). These conditions can delay hatching upto 10 days without affecting hatchability. First instar larvae can also be stored under these conditions for 24 hrs, if the field conditions do not permit the larval release on a particular day.

### 5. Quality control

The quality of the insects produced in the laboratory can be judged by (i) the performance of laboratory reared insects in a field release situation, and/or (ii) monitoring of particular trait comparable in laboratory reared and field collected insects, and/or (iii) comparing of the laboratory colony with itself over time with respect to insect's growth and reproduction.

For *Chilo* rearing at ICRISAT Center, the quality of the insects reared on artificial diet is monitored through the pupal mass and fecundity of adult females in each generation. Pupal mass and eggs laid by the adult female of the field collected insects are recorded. Similar observations are also recorded from the insects reared on artificial diet in each generation. The male and female pupal mass and fecundity of the adult female of field collected insects and in any of the generations do not differ significantly (Table 3). However in some cases, larvae and pupae look healthier on artificial diet.

Table 3. Pupal mass and fecundity of *C. partellus* from field collected and laboratory-reared insects

Generation	Pupal mass (mg)		Egg batch- es/female	Eggs/ female
	Male	Female		
Field collected	60.0	122.0	11.0	560
Laboratory-reared G1	59.7	119.2	11.5	525
Laboratory-reared G4	69.3	117.8	10.5	575
Laboratory-reared G6	62.5	128.3	12.0	600
SE	± 2.92	± 5.25	± 0.5	± 28.8
CV (%)	14	13	15	12

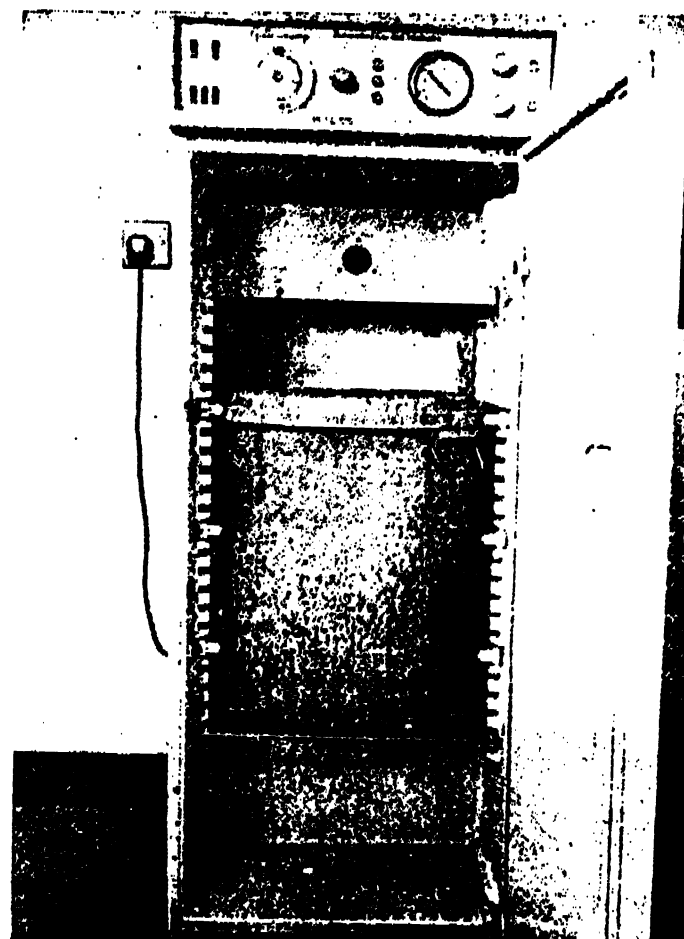


Fig. 7. Storage of *Chilo* eggs at low temperature to delay hatching

### 6. Production

Use of work schedule and forward planning related to the utilization of resources is required to produce sufficient number of insects at desired time. This requires (i) continuous examination of each step in production to reduce

mado in it at regular intervals. White glycine paper (25 x 80 cm) is wrapped around this cage at the time of moth release. Two PVC saucers (25.5 cm diameter) internally fixed with mosquito net are fitted on both ends of the cylinder (Fig. 5).



Fig. 5. Oviposition Cage for *C. partellus*.

Fifty pairs of moths are released in each oviposition cage. A female lays an average of 10-12 egg masses (500-600 eggs) over a period of 4 days, the maximum being laid on the glycine paper through the holes in the cage. The glycine paper is replaced daily with a fresh one without disturbing the moths inside. Maximum eggs are laid when the moths are fed with water only (though honey, sucrose, and glucose were also tested).

#### 4.6. Storage of eggs

The freshly laid egg masses are yellow in colour and the colour changes with embryo development. The eggs turn into 'blackhead' stage on the fifth day after laying. Once at this stage, they hatch within 24 hrs. For normal embryonic development and uniform hatching, the eggs are kept under high humidity (>80%). This is achieved by placing water in a plastic bucket with a lid and keeping the glycine paper hung on a rod (Fig. 6). Room temperature is maintained at  $26 \pm 1^\circ\text{C}$ . These conditions ensure the uniform hatching of the eggs on the 6th day after laying.



Fig. 6. Storage of *Chilo* eggs laid on glycine paper

days earlier than females. However sex ratio is 1:1. From each jar about 75-80 moths emerge over a period of 10 days (Fig. 3).

#### 4.4. Moth collection

A vacuum cleaner attached to a PVC pipe with multiple various outlets is used to collect the moths (Fig. 4). A bifurcating flexible tube is fixed to the outlet, which terminates at the mouth of a half cut plastic bottle. The lid of the moth collecting plastic bottle is fitted with a pointed tube, while the bottom has few holes of 1-2 mm. A circular

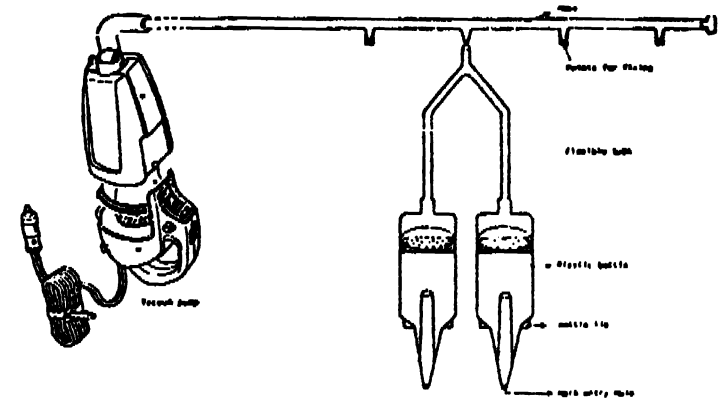


Fig. 4. Sketch of stem borer moth collection device employed at ICRISAT Center

piece of foam is fixed at the base of this bottle and it is attached to the half cut plastic bottle at the time of moth collection. As the vacuum cleaner is switched on, air is sucked through the entry holes of the moth collecting bottles. With this suction, moths are collected in the bottles, which can be replaced with a fresh one after a specified number of moths are collected. With the help of two collection bottles, male and female moths are collected separately (males being smaller in size with dark forewings and smaller pointed abdomen) and transferred to the oviposition cage. Air suction can be regulated to allow smooth collection of moths without damaging them. This device of moth collection helps to collect moths quickly in a short time and also prevents moth escape. During the peak period of moth emergence, about 8000 moths can be collected daily within two hours by three laboratory technicians.

#### 4.5. Oviposition

An oviposition cage consists of an open cylinder (25 cm high and 25 cm diameter) made of galvanised iron wire net of 36 mm openings. The cylindrical shape is made by joining the ends of the net piece. A thin georgette cloth is fixed around this cylinder and uniform holes (6 mm) are

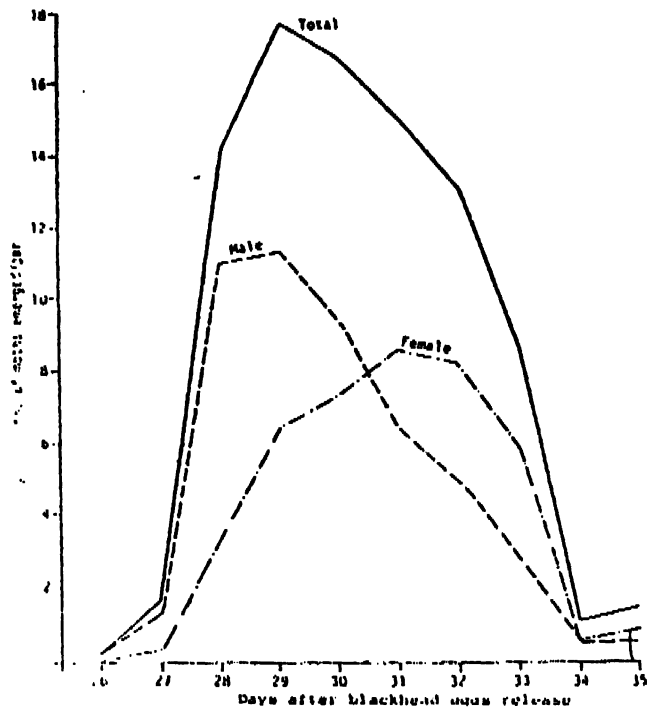


Fig. 3. Pattern of moth emergence from a dirt jar inoculated with 100 eggs.

Table 2. Amount of diet ingredients used for rearing *C. partellus* in the laboratory

Ingredients	Dang et al. (1976)	Edmunds, Meyer and Soto (1971)	Ward (1965)	Siddiqui and Chatterji (1972)	Siddiqui et al. (1977)	Sharma and Sarup (1978)	Seshu Reddy and Davies (1979)	Taneja and Leusch- ner (1985)
Agar (g)	51	25	20	5.1	5.1	6.0	51	40.8
Ascorbic acid (g)	13	4.3	4	1.3	1.3	1.5	13	10.4
Formaldehyde (ml)	8	2.7	2.6	1.0	1.0	1.0	10	3.2
Methyl P-hydroxy benzoate (g)	6	2.7	2.6	0.8	0.8	0.9	8	6.4
Sorbic acid (g)	4	1.5	1.0	0.4	0.4	0.5	5	4.0
Yeast (g)	40	13.3	13.2	4.0	4.0	5.0	40	32.0
Water (ml)	3122	1000	1000	380	380	390	4500	3600
Sorghum leaf powder (g)	—	40	40	—	—	—	200	160
Sucrose (g)	—	60	20	—	—	—	—	—
Vitamin fertilification mixture (g)	—	5	1	—	—	—	—	—
Vitamin E (g)	—	—	—	0.1	0.1	0.1	5.2	4.6
Wheat flour (g)	—	—	—	20	20	20	—	—
Pulses:								
Kabuli gram <sup>1</sup> (g)	420	100	100	—	—	—	—	438.4
Rajmah <sup>2</sup>	—	—	—	74.8	—	—	—	—
Green gram <sup>3</sup>	—	—	—	—	—	—	—	—
Dew gram <sup>4</sup> , Cowpea <sup>5</sup>	—	—	—	—	75	75	—	—
Lentil <sup>6</sup>	—	—	—	—	—	—	—	—

1. *Cicer arietinum* L.; 2. *Phaseolus vulgaris* L.; 3. *Vigna radiata* (L.); 4. *Vigna acontifolia* (Jacq.)  
5. *Vigna unguiculata* (L.); 6. *Lens culinaris* Medic.

#### 4.3. Larval rearing

The diet in each jar is inoculated with surface sterilized, blackhead stage (ready to hatch within 24 hrs) eggs. The white glycine paper containing about 100 eggs is placed in the diet through a needle (Fig. 2). Egg inoculation is carried out under the laminar flowhood to avoid contamination. The jars are then covered with a sterile cloth and a lid. The central portion (7.5 cm dia.) of the lid is fixed with a 80 gauze brass mesh. These jars are kept in dark for 2-3 days, by which time the eggs hatch and the larvae settle on the diet. The glycine paper, needle and the cloth are removed and the jars are shifted to the larval rearing room.

In the larval rearing room, temperature is maintained at  $28 \pm 1^\circ$  C, relative humidity at 60-70% and 12 hrs light. Larval and pupal stages are completed in the diet. The adult emergence starts 26 days after egg inoculation and continues for about 10 days. Majority of the adults (>95%) emerge within this period. Emergence of male moths starts 2 to 3



Fig 2. Diet inoculation with 'black head' stage *Chilo* eggs

emerging male and female moths are collected and released (in 1:1 ratio) for oviposition. The egg masses are surface sterilized by dipping them in 10% formaldehyde for 2 min and washing them thoroughly with distilled water and are used to start the insect culture on artificial diet.

#### 4. Research and development of rearing techniques

##### 4.1. Insect diet

The successful formulation of an artificial diet should be based on a sound, basic understanding of insect nutrition, the chemical composition of the insect's natural food, and the knowledge of its habitat and feeding behaviour. The selection of an insect diet depends on the purpose of rearing, number of insects to be reared, availability, and cost of the ingredients. The principal requirements in formulating a diet are: (i) it must be physically and/or chemically attractive to stimulate the insect to feed on an unfamiliar food (ii) it must possess all the essential nutrients in balanced proportion for normal growth, development, and reproduction, and (iii) it must be free from microbial contamination.

The first artificial diet used to rear *C. partellus* included casein, glucose, salt mixture, yeast, choline chloride, cholesterol, conc. cellulose, leaf factor, agar, methyl paraben and water (Pant *et al.*, 1960). Chatterji *et al.*, (1963) reared *C. partellus* on a wheat germ based diet which was earlier used by Kenster and Harrendorf (1965) for rearing *Zea diatraea grandiosella*. The major breakthrough in mass rearing of *C. partellus* came with the use of Kabuli gram based diet (Dang *et al.*, 1970) which required the least number and readily available diet ingredients. Ever since, a number of diets have been used in India by deleting, adding, or changing the quantity of one or other ingredients (Table 1 and 2).

The diet used at ICRISAT Center is given in Table 2 (Taneja and Leuschner, 1985). All the ingredients (except sorghum leaf powder) are locally available. For sorghum leaf powder, green leaves of 35-40 days' old plants of susceptible cultivars (e. g. CSH 1 or CSV 11) are collected, washed and dried. The dried leaves are ground and autoclaved before use in the diet.

Table 1. Diets used for rearing *C. partellus* in the laboratory

Casein, glucose, salt mixture, yeast, choline chloride, cholesterol, cellulose, leaf factor, agar, methyl paraben, water			Pant <i>et al.</i> (1960)
Wheat germ, casein, dextrose, salt mixture, cellulose, Vit. mixture, ascorbic acid, aureomycin, methyl paraben, agar, KOH, Formaldehyde, water			Chatterji <i>et al.</i> (1963)
- Agar	- Kabuli gram		Dang <i>et al.</i> (1970) ✓
- Ascorbic acid		- Sorghum leaf powder	Laxminarayana and Jato (1971)
- Formaldehyde		- Sucrose	
		- Vit. mixture	Moorty (1973)
- Methyl para hydroxy benzoate	- Vit. E	- Rajmah	Siddiqui and Chatterji (1972)
- Sorbic acid	- Wheat flour	- Green gram + dew gram	
- Yeast		- Dew gram + Cowpea	Siddiqui <i>et al.</i> (1977) ✓
- Water		- Green gram + Cowpea	
		- Green gram	
		- Lentil	Sharma and Sarup (1978)
	- Vit. C	- Rajmah	Sahu Reddy and Davies (1979)
	- Sorghum leaf powder	- Kabuli gram	Taneja and Leuschner (1985)

##### 4.2. Diet preparation

All the ingredients (Table 2) except the sorghum leaf powder, agar, and formaldehyde are blended for 1 min. in an electric blender. Sorghum leaf powder is soaked in 2 l of warm water (70°C) and blended for 3 min. Agar is boiled in 1.6 l of water, cooled to 40°C and poured in the blender. Formaldehyde is finally added and all the constituents are blended for 3 min. The diet is then poured into the 1 l capacity plastic containers. About 300 g diet is poured in each container, which is sufficient for 100 larvae to develop successfully.

or may be modified to suit the specific behaviour and requirements of various growth stages.

## REARING MANAGEMENT OF *C. partellus*

### Objectives

The main objective of rearing *C. partellus* on artificial diet at ICRISAT Center is to ensure field infestation of sorghum plants to screen for stem borer resistance. Screening for stem borer resistance is carried out during the rainy (June - October) and post-rainy (October-March) seasons. In each season, artificial infestation is done in about 3 ha area comprising 400,000 plants. Each plant is infested with 7 to 8 first instar larvae at 15 to 20 days' old seedlings. It implies that all the infestations must be completed within a period of 10 to 15 days and sufficient larvae should be produced during this period.

### 2. Design of the rearing laboratory

The design of the rearing laboratory should provide reliable environmental control (temperature, humidity, and light), and maintain good hygienic conditions (air flow and ventilation). The rearing laboratory at ICRISAT Center (Fig.1) has an independent air conditioning system and the environmental conditions can be regulated in each room. Each room has a thermostat, hydrostat, and timers which regulate temperature, humidity, and light respectively.

Floors, walls and ceiling are washable and leak-proof, thus avoiding invasion by ants, lizards and other unwanted creatures which may interfere with rearing. The water outlets are also fitted with screen mesh to avoid entry of such creatures. To prevent microbial contamination, all the materials used in the laboratory are surface sterilized in sodium hypochlorite solution (commercial grade 6-8% conc.) and transferred from one room to another through service windows (Fig.1). Used diet containers and oviposition cages are also shifted to the washing area through service windows. The sterilization room, larval rearing rooms and oviposition room are provided with separate exhaust fans to minimize air circulation across rooms.

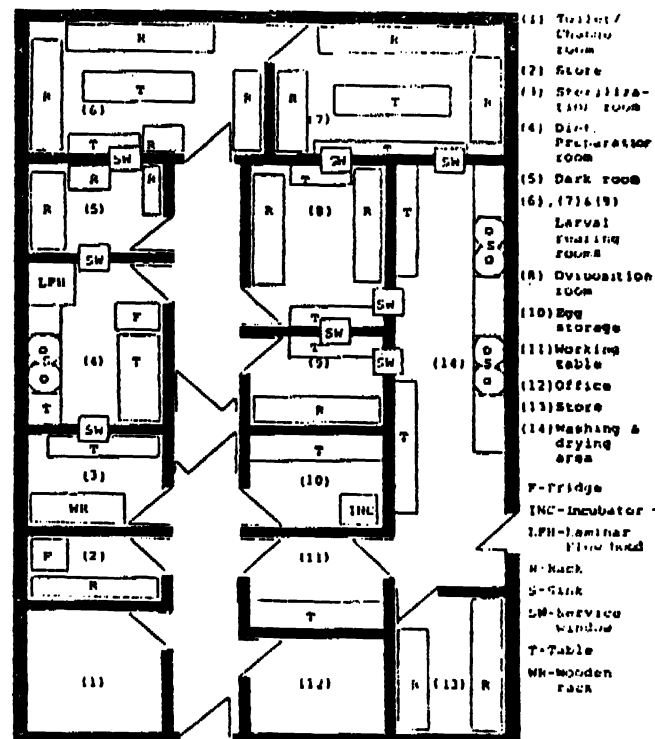


Fig 1. Lay out plan of Insect Rearing Laboratory at ICRISAT Center

### 3. Establishment of insect colony

The first step in starting an insect culture is to have disease free and genetically diverse parental stock. To start a *Chilo* culture, field population of grown up larvae and pupae are collected and kept in plastic containers along with sorghum stems. The plastic containers are of 1 litre (l) capacity (11 cm diameter and 13.5 cm height) and the central portion of lid (7.5 cm diameter) is fixed with 80 gauze brass mesh. This provides air circulation and prevents larval escape. The



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## Mass Production of Spotted Stem Borer, *Chilo partellus* Swinhoe on Artificial Diet

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Mass rearing is the production of insects in numbers per generation exceeding ten thousand to one million times the mean productivity of the native female population (Chambers, 1977). Insects are reared for the benefit of mankind (silkworm, honey bee, lac insects etc.), and/or for the research in the field of physiology, ecology, genetics and insect control techniques. Rearing of insects is required for insecticide testing, hormone and pheromone manipulation, biological control, host-plant resistance, male sterilization, and genetic engineering

Insect rearing management (IRM) is the efficient utilization of resources for the production of insects of standard quality to meet the research goals (Singh and Ashby, 1985). There are seven elements of IRM: objectives, establishment of insect colony, design of the rearing laboratory, research and development of techniques, resources, quality control, and production. All these elements for economic mass production of spotted stem borer, *Chilo partellus* Swinhoe, are discussed in this chapter. Techniques for rearing *C. partellus* may also be used to rear other lepidopterous

# **BIOCONTROL TECHNOLOGY FOR SUGARCANE PEST MANAGEMENT**

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