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MASS REARING OF *CHILO* SPP. ON ARTIFICIAL DIETS AND ITS USE IN RESISTANCE SCREENING

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Abstract—Several species of *Chilo* are serious pests of cereal crops. The important species attacking cereals are *Chilo agamemnon, C. orichalcociliellus* and *C. partellus* on maize and sorghum; *C. auricilius* and *C. zacconius* on sugar-cane, and rice; *C. suppressalis* on rice; and *C. sacchariphagus indicus* on sugar-cane. Insects are reared in the laboratory on natural and artificial diets for various purposes, namely for insecticide testing, hormone and pheromone manipulation, biological control, host-plant resistance, etc. Rearing of an insect in the laboratory requires rearing facilities, colony establishment, research and development of rearing techniques, resources, and maintenance of insect quality. All these aspects for rearing major *Chilo* spp. have been discussed. However, detailed information is available only for *C. partellus* and *C. suppressalis*. Techniques used for resistance screening and damage evaluation against spotted stem borer, *C. partellus* using naturally occurring population and artificial infestation are described.

Key Words: Mass rearing, artificial diet, resistance screening, *Chilo* spp., oviposition, insectary, artificial infestation

Résumé—Elevage en masse de *Chilo* spp. sur des regimes artificiels et son emploi dans le criblage pour la résistance: Plusieurs espèces de *Chilo* sont des ravageurs dangereux de cultures céréalières. Les espèces importantes qui s'attaquent aux céréales sont *Chilo agamemnon, C. orichalcociliellus* et *C. partellus* sur le maïs et le sorgho; *C. auricilius* et *C. zacconius* sur la canne à sucre et le riz; *C. suppressalis* sur le riz; et *C. sacchariphagus indicus* sur la canne a sucre. L'élevage des insectes en laboratoire sur les régimes naturels et artificiels permet la réalisation de travaux divers, notamment les essais d'insecticides, la manipulation des hormones et de phéromones, la lutte biologique, la résistance de la plante-hôte, etc. Il demande, cependant, la prévision du matériel nécessaire, l'établissement de colonies, la recherche et la mise au point de techniques de l'élevage, les ressources et la conservation de la qualité des insectes. Cet article traite de tous ces aspects de l'élevage de *Chilo* spp. majeurs. Par contre, des informations détaillées ne sont disponibles que pour *C. partellus* et pour *C. suppressalis*.

Sont également decrites, des techniques destinees au criblage pour la resistance et a l'évaluation des dégâts causés par le foreur ponctué du sorgho *C. partellus*; ces techniques font appel à la population naturelle et l'infestation artificielle.

INTRODUCTION

Chilo spp. are a group of Pyralid insects, the larvae of which are pests of cereal crops. The most important *Chilo* spp. attacking cereals are *Chilo agamemnon* Bleszynsky, *C. orichalcociliellus* Strand and *C. partellus* Swinhoe on maize and sorghum; *C. auricilius* Dudgeon and *C. zacconius* Blesz. on sugar-cane and rice; *C. suppressalis* Walker on rice; and *C. sacchariphagus indicus* Kapur on sugar-cane.

Mass rearing is the production of insects in

numbers per generation exceeding 10 thousand to 1 million times the mean productivity of the native female population (Chambers, 1977). Insects are reared for various purposes, e.g. insecticide testing, hormone and pheromone manipulations, biological control, host-plant resistance, male sterilization and genetic engineering, etc. Rearing of an insect in the laboratory requires: (i) establishment of insect colony, (ii) rearing facilities, (iii) research and development of rearing techniques, (iv) resources, (v) quality control and (vi) production. In this paper, we have tried to summarize the available information on all these aspects for the major *Chilo* spp. Detailed information is available for only *C. partellus* and *C. suppressalis*. In the second section, the techniques used for resistance screening against *C. partellus* have been discussed. Similar techniques may be used for screening against other *Chilo* spp. or modified as per the requirements.

MASS REARING

Chilo partellus *Swinhoe*

The spotted stem borer, *C. partellus* is a major pest of sorghum and maize in Asia and Africa. It attacks the crop from seedling stage to harvest and damages all plant parts except the roots. Initial damage symptoms are leaf injury followed by the formation of dead hearts, stem tunnelling, and the production of chaffy panicles. Losses due to this pest have been estimated at 55 to 83% in sorghum (Jotwani et al., 1971) and 24.3 to 36.3% in maize (Chatterji et al., 1969).

C. partellus is mass produced in the laboratory on artificial diets at several institutes for various purposes (Chatterji et al., 1968; Sarup et al., 1985; Taneja and Nwanze, 1988). The main objective of rearing *C. partellus* at ICRISAT in India is to ensure uniform and timely infestation of sorghum while screening for resistance to spotted stem borer. This species is also reared at the Mbita Point Field Station of the International Centre of Insect Physiology and Ecology (ICIPE) in Kenya.

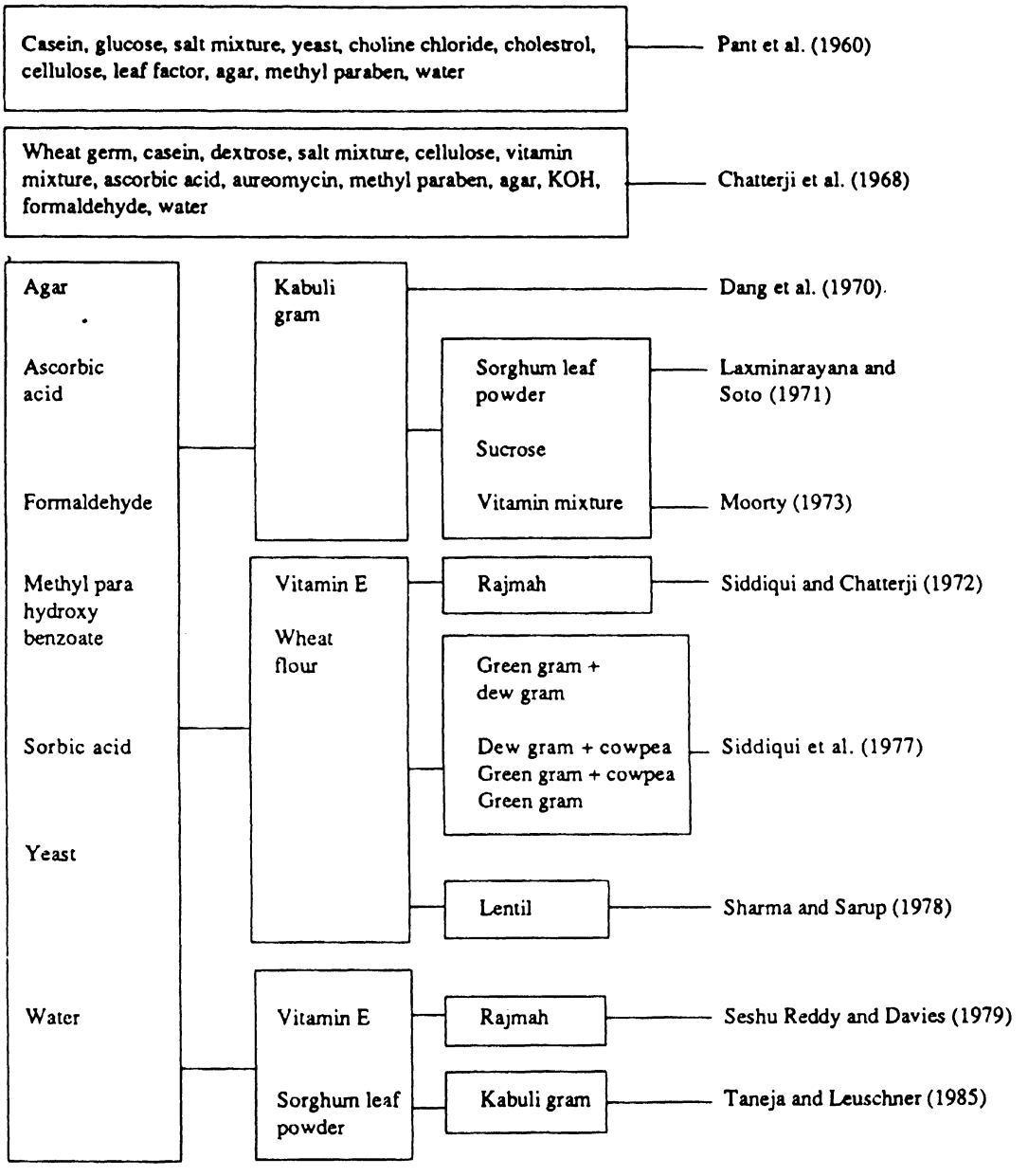
Research facilities. A rearing facility should provide reliable control of environmental conditions (temperature, humidity and light), and maintain a high standard of hygiene. The rearing laboratory at ICRISAT Center is equipped with an independent air conditioning system and environmental conditions are independently regulated in each room. All rooms are well maintained and are leak proof. They are also rodent-proofed and regularly sterilized against microbial contamination.

Research and development of rearing techniques. The first artificial diet used to rear *C. partellus* included casein, glucose, salt mixture, yeast, choline chloride, cholestrol, cellulose, leaf factor, agar, methyl paraben and water (Pant et al., 1960). Chatterji et al. (1968) reared *C. partellus* on a wheat germ-based diet which was earlier used by Keaster and Harrendorf (1965) for rearing *Zeadiatraea grandiosella*. The major breakthrough in mass rearing of *C. partellus* came with the use of Kabuli gram based diet (Dang et al., 1970). This diet had fewer and more readily available ingredients. Most of the diets used in India either delete, add or change the quantity of one or other ingredients of the Kabuli gram-based diet (Tables 1 and 2). The diet employed at ICRISAT Center is presented in Table 2 (Taneja and Leuschner, 1985).

(1) *Larval rearing.* The preparation of the diet has been previously reported (Taneja and Leuschner, 1985). Black-head stage eggs are introduced into rearing jars (ready to hatch within 24 hr), which are covered with a sterile cloth and a lid. Jars are initially kept in the dark for 2–3 days, by which time the eggs hatch and the larvae settle on the diet. Larval and pupal stages are completed in the diet and adult emergence begins about 26 days from introduction of egg into the diet and continues for about 10 days. Majority of the adults (>95%) emerge within this period. Emergence of male moths start 2 to 3 days earlier than females, however sex ratio is 1:1. Moth collection is facilitated by using a modified vacuum cleaner attached to a PVC pipe with multiple outlets. This device ensures quick moth collection with little damage to the insects and also prevents moth escape. During peak moth emergence, upto 8000 individuals can be collected daily within 2 hr by three laboratory technicians.

(2) *Oviposition and handling of eggs.* The oviposition cage used at ICRISAT consists of an open cylinder (25 cm high and 25 cm dia) made of galvanized iron wire net of 36 mm openings (Taneja and Leuschner, 1985). Fifty pairs of moths are released into each cage for oviposition. On the average, one female lays 10–12 egg masses (500–600 eggs) over a period of 4 days. Maximum

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



oviposition occurs on second and third day. The best results were obtained when moths are fed with water only. Honey, sucrose and glucose have also been used with less satisfactory results.

Egg collection is done daily and the glycine paper on which eggs are laid is replaced daily. Freshly laid egg masses are yellow in colour, but change with embryo development, and turn into the "blackhead" stage on the fifth day. Once at this stage, larvae hatch within 24 hr. Both high humidity (>90%) and temperature (26 ± 1°C) ensure normal embryonic development and uniform hatching.

Hatching of larvae can also be delayed if they are not required for immediate use. This is achieved by storing blackhead stage eggs at 10°C and high humidity (>90%). These conditions can delay hatching for upto 10 days without affecting hatchability. First instar larve can also be stored under these conditions for 24 hr, if field conditions are not favourable for infestation.

Quality control. The quality of the insects

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

Ingredients	Dang et al. (1970)	Laxminarayana and Soto (1971)	Moorty (1973)	Siddiqui and Chatterji (1972)	Siddiqui et al. (1977)	Sharma and Sarup (1978)	Seshu Reddy and Davies (1979)	Taneja and Leuschner (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)	8	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)	3120	1000	1000	380	380	390	4500	3600
Sorghum leaf powder (g)	–	40	40	–	–	–	200	160
Sucrose (g)	–	60	20	–	–	–	–	–
Vitamin fortification 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 (g)*	420	100	100	–	–	–	–	438.4
Rajmah+	–	–	–	74.8	–	–	548	–
Green gram^/ Dew gram**								
Cowpea++	–	–	–	–	75	–	–	–
Lentil##	–	–	–	–	–	75	–	–

Cicer arietinum L.
+*Phaseolus vulgaris* L.
^*Vigna radiata* (L.)
**Vigna aconitifolia* (Jacq.).
++*Vigna unguiculata* (L.)
##*Lens culinaris* Medic.

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 trends in growth and reproduction of the laboratory colony over time.

At ICRISAT Center, the quality of the insects reared on artificial diet is monitored through 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. Our results show that the male and female pupal mass and fecundity of adult females of field-collected insects and in any generation do not differ significantly (Table 3). On the contrary, we have observed healthier larvae and pupae reared on artificial diet.

At ICRISAT, cultures of *C. partellus* are maintained throughout the year although production is increased twice a year (June–July and October–November) during major field infestations (Fig. 1). A new culture is started each year in April with field-collected larvae and pupae.

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

Generation	Pupal mass (mg)		Number of fertile	
	Male	Female	Egg batches/ female	Eggs/ 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
S.E.	± 2.92	± 5.25	± 0.5	± 28.8
CV(%)	14	13	15	12

G1, G4, and G6 represent generations one, four and six respectively.

Chilo suppressalis *Walker*

The rice stem borer, *C. suppressalis*, is one of the most important pests of rice in Asia, Hawaii, the Middle East, and the Mediterranean region. The initial injury by larvae occurs in the leaf sheath and appears as a whitish discoloration. Later on, the larvae bore into the stem and feed on internal tissues. If damage occurs during the vegetative stage, the central leaf whorl does not unfold, turns brownish and dries out resulting in a dead heart. Infestation occurring after panicle initiation, results in a severance of the developing panicle at the node. The dried-out emerging panicles remain straight and are called whiteheads.

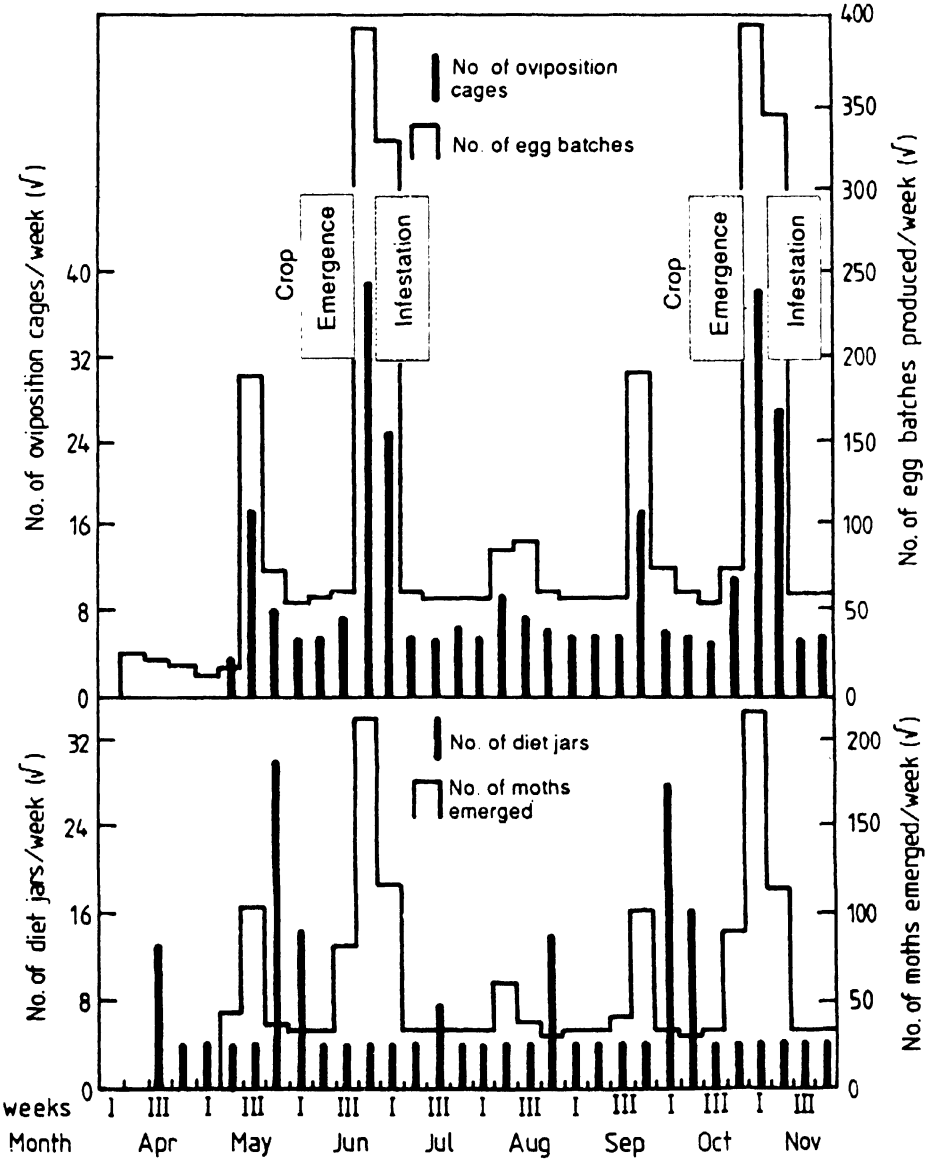


Fig. 1. Schedule of diet preparation, moth emergence, oviposition, and infestation of *Chilo partellus*, ICRISAT Center.

C. suppressalis has been reared on several artificial diets (Ishii, 1952; Kamano, 1971). However, it was found that there was a gradual decrease in vitality due to inbreeding. This was corrected by Sato (1964) who developed a mass rearing method using rice seedlings. Using this method, the vitality of this insect has been maintained for 70 generations over 10 years. Details of rearing *C. suppressalis* on artificial diet has been provided by Kamano and Sato (1985).

Rearing is carried out in an insectary or large incubator maintained at 28 ± 2°C, 50–70% r.h. and a 16:8 light-dark period provided by fluorescent lights (100–500 lux). The founder colony is established from field-collected moths which are released into a polyethelene bag within which is a rice plant. They are kept overnight in a darkened room at 20–30°C. Egg masses are laid on the rice leaves, which are cut and incubated at 28°C.

For subsequent generations in the insectary, an oviposition box (45 x 90 x 130 cm) with wire mesh screen which accommodates potted rice plants, plastic trays for pupae, and absorbent sponges are used. The eggs laid on the leaves are collected regularly and potted rice plants are changed every other day.

The egg masses are sterilized in the black-head stage in 70% ethanol for a few seconds and then in 0.1% aqueous solution of mercuric chloride for 4 min. Then they are fully washed with 70% alcohol. The larvae of *C. suppressalis* is reared in the laboratory both on potted rice plants (natural host) and on artificial diet.

Natural host. (1) *Seed preparation.* Dry rice seed is immersed in salt water (specific gravity 1.14) and floating seeds are discarded. The remaining seeds are rinsed under running water and dried at room temperature for 3 days. The seed is stored at 5–10°C to preserve germination ability.

(2) *Seedling preparation.* Seeds are immersed in a bucket of water overnight at 20–30°C and washed thoroughly. Wet seeds (60 g) are put into a rearing jar and 15 ml water is added and the jar is sealed with the metal cap. The jar is transferred in the insectary or incubator at 28 ± 2°C under illumination (100–500 lux) for 5–7 days at the end of which, the seedlings are ready for use.

(3) *Inoculation with egg masses.* The seedlings are inoculated with about seven egg masses (blackhead stage) per jar. An amount of 15 ml of water is added and the jar is resealed with a metal cap which is provided with a cotton stopper. It is then kept for 10 days after which larvae are collected and transferred into fresh seedlings at the rate of 100 larvae per jar.

This procedure is repeated after 7 days and 50 larvae are transferred into new seedling jars and resealed. Most of the mature larvae bore into the cotton stopper in the metal cap and pupate 7–10 days after the second transfer. The cotton stoppers are collected and transferred to oviposition boxes for egg laying as moths emerge, or to the refrigerator for storage.

Table 4. Ingredients of artificial diet for rearing *C. suppressalis* in the laboratory

Ingredient	Quantity/10 flasks
Agar	10 g
Cellulose	10 g
Sucrose	15 g
Starch	20 g
Casein	15 g
Wheat germ	10 g
Salt mixture*	4 g
Cholestrol	0.2 g
Choline chloride	0.5 g
Sodium ascorbate	3 g
L - Cysteine HCL	0.3 g
Vitamin Solution*	10 ml
Water	490 ml

*Salt mixture: K_2HPO_4 49.75 g, KH_2PO_4 18.0 g, $MgSO_4$ 16 g, $Ca(H_2PO_4)_2.H_2O$ 8.0 g, Nacl 5 g, $Fe_2(SO_4)_3.6H_2O$ 2.0 g, $MnSO_4.H_2O$ 0.5 g, $CuSO_4.5H_2O$ 0.25g, $Zn(C_2O_2H_3)_2.2H_2O$ 0.05 g.

*Vitamin solution: Thiamine hydrochloride 200 mg, Riboflavin 100 mg, Nicotinic acid 200 mg, Pyridoxine hydrochloride 100 mg, Calcium pentothenate 200 mg, Folic acid 20 mg, Biotin 20 mg, p-Amino benzoic acid 200 mg, Inositol 2000 mg, Water 1000 ml.

(Source: Kamano and Sato, 1985).

Artificial diet. (1) *Diet composition.* The diet used to rear the larvae is based on wheat germ (Table 4), which provides all the essential nutrients to the developing larvae. Diet preparation is fully described by Kamano and Sato (1985).

(2) *Larval rearing.* Sterilized egg masses are kept inside a flask containing artificial diet. Flasks are plugged with cotton wool and kept in the insectary or incubator at 28 ± 2°C. The cotton wool stopper is removed when pupation starts. The mature larvae are transferred to the pupation

cages containing cellophane tubes. Pupation takes place inside the cellophane tubes.

(3) *Adults*. The sex ratio is approximately 1:1. The first male and female emerge about 25 days after egg hatching. Males emerge before females. Wings of males are darker than the female moths and can also be distinguished by morphological differences in the abdominal tip (ovipositor and clasper). Emergence is complete in 50 days with a peak occurring in 35–40 days. Adult survival from hatching larvae is about 60%.

Holding insects at low temperature. Moths cannot be held under 20°C since mating and flight activity cease. However, egg masses (all ages) attached to the leaves of rice plant can be safely kept for 1–2 weeks at 5°C in a Petri dish containing moistened filter paper. Larvae (all stages) feeding on seedlings in jars can also be safely kept at 5°C for 1–2 weeks until new seedlings are available. Pupae can be kept at 5°C safely for 1–2 weeks. The ability to hold eggs, larvae and pupae make it possible to regulate the emergence of moths.

Life cycle data. Life cycle and survival data of various stages under optimum rearing conditions of 28°C, 50–70% r. h. and LD 16:8 photoperiod are as follows: (1) Incubation period 5–6 days, (2) larval period 25–35 days and (3) pupal period 6–7 days. Total development time from egg to egg is 37–49 days. Egg hatchability is 80–90% and survival rate from neonate larvae to adult is 50–60 days. Pre-oviposition period lasts for 1 day and the peak oviposition period is 2–3 days after emergence. Moth longevity is 5 days with mean fecundity of 235 eggs per female. Mean pupal weight for male and female is 45 and 55 mg, respectively.

Chilo agamemnon *Bleszynski*

The oriental corn borer, *C. agamemnon* is a major borer of maize in subtropical and tropical regions of Mid-East, and Africa (Mihm, 1985). The early instars of this species feed in the leaf whorls of maize seedlings. The later instars attack the stem and other plant parts.

Rearing of this pest on artificial diet was first reported by Isa (1972), who used diet given in Table 5. Larvae are reared individually in vials plugged with cotton and aluminium caps at 27°C and kept in the dark. Pupae are collected and placed on moist cotton in clean vials for emergence. Adults are released in glass jars covered with muslin and lined with wax after incubation in Petri dishes.

The mean larval period on artificial diet is 27 days (21.9 days on corn), larval mortality 20% (37.5% on corn), pupation 80% (62.5% on corn), male pupal weight 45.2 mg on diet (40.0 mg on corn).

Chilo auricilius (*Dudgeon*)

C. auricilius is one of the most destructive pests of sugar-cane in northern sugar-cane belt of India. Its attack affects the cane yield and sugar recovery. The larvae of this pest feed on sheathing leaves, the soft part of the rind and also sometimes mine the mid-rib. After feeding on the leaf tissue for a week, they penetrate into the cane and tunnel it. The late water shoots growing in the field are also attacked. The damage seems to be conspicuously marked in top, middle and bottom portions of cane, which is possibly indicative of attack by different broods.

Varma and Avasthy (1973) reared this species on artificial diet based on french bean (Table 5). Rearing is carried out at 22–27°C and 60–75% r.h. Ten neonate larvae are placed in each rearing tube and after 2–3 weeks are transferred to fresh tubes (5 larvae/tube). Pupation takes place near the cotton plug or in pupal cells within the diet 33–58 days after inoculation. Pupae are removed and incubated in 7.5 cm Petri dishes. Adult emergence takes place 6–10 days later and the moths are released in 15 x 10 cm dia glass jars, where they mate and lay eggs on freshly cut 15 cm long sugar-cane leaf sections.

Mean larval period on this diet is 47.0 days, pupation 82.0%, mean pupal period 8.3 days, adult emergence 65.8%, adult longevity 5–6 days for males and 4–5 days for females, eggs laid per female 224.8, sex ratio of male to female 1:0.8. Longevity and fecundity of adults reared on artificial diet are not significantly different from wild population.

Chilo orichalcociliellus *Strand*

The coastal stalk borer, *C. orichalcociliellus* feeds on maize and sorghum and only occurs in the East African coastal area. It is a pest of sorghum in Madagascar. Delobel (1975) reared this species on artificial diet; the ingredients are given in Table 6.

Rearing in the laboratory is carried out at 26–31°C with 12 hr photoperiod or at 18–21°C with 11 hr photoperiod. Neonate larvae are reared individually in 15–45 mm dia transparent plastic

Table 5. Ingredients of artificial diet for rearing *Chilo agamemnon* Bleszynski, *C. auricilius* (Dudgeon), and *C. sacchariphagus indicus* (Kapur) in the laboratory

Ingredient	Quantity		
	C. agamemnon	*C. auricilius*	*C. sacchariphagus indicus*
Agar	3.0 g	8.0 g	9.0 g
Ascorbic acid	0.45 g	1.0 g	9.0 g
Casein	1.5 g	50.0 g	22.5 g
Formaldehyde	–	1.0 ml	2.0 ml
Methyl p-hydroxy benzoate	1.5 ml (20% in alcohol)	1.0 g	2.0 g
Sorbic acid	1.5 ml (10% in alcohol)	0.5 g	1.0 g
Sugar-cane shoot powder	–	50.0 g	75.0 g
Water	150 ml	425 ml	400 ml
Yeast	6.0 g	16.0 g	9.0 g
French beans	–	100.0 g	–
Kabuli gram	–	–	60.0 g
Multivitaplex caps	–	–	5 in number
Vitamin fortification	–	8.0 g	–
Wassens' salt mixture	–	5.0 g	–
Sucrose	1.5 g	–	19.0 g
Hostacycline	–	–	1.5 g
Cellulose	1.5 g	–	–
Glucose	1.5 g	–	–
Powdered rice bran	9.0 g	–	–

Sugar-cane shoot powder is prepared by chopping sugar-cane tops into small pieces, drying them for 3–4 days in the hot sun or in hot air over 10–20°C and then grinding into powder.
(Source: Varma and Avasthy, 1973; Isa, 1972; Easwaramoorthy and Shanmugasundaram, 1988).

Table 6. Diet ingredients for rearing *Chilo orichalcociliellus* Strand and *C. zacconius* Blesz. in the laboratory

Ingredients	Quantity	
	C. orichalcociliellus	*C. zacconius*
Agar	7.0 g	14.0 g
Ascorbic acid	1.4 g	10.0 g
Corn flour	30.0 g	112.0 g
Methyl p-hydroxy benzoate	1.0 g	1.0 g
Water	340.0 ml	600.0 ml
Wheat germ	30.0 g	28.0 g
Yeast	5.0 g	30.0 g
Bactrim	–	0.25 g
Benzoic acid	–	1.2 g
Formaldehyde (10%)	2.0 ml	–
Chickpea flour	30.0 g	–
Maize stem powder	2.0 g	–
Levulose	10.0g	–

(Source: Delobel, 1975; Bordat and Pichot, 1978).

boxes. The diet is renewed weekly. Pupae are sexed and transferred to 208 x 55 mm dia plastic emergence boxes. For oviposition, similar boxes are lined with creased cellophane paper and adults released each pair to a box.

Chilo sacchariphagus indicus (*Kapur*)

The internode borer, *C. sacchariphagus indicus* is an economically important species infesting sugar-cane in India. It infests the crop from internode formation stages to harvest. The damage due to internode borer infestation results both in "field" and "factory" loss. The life cycle is completed in 42–60 days and the pest has six overlapping generations a year.

Laboratory rearing of *C. sacchariphagus indicus* on artificial diet is carried out for various purposes including its use as a host for rearing parasites. The diet used for rearing it at the Sugarcane Breeding Institute, Coimbatore, India is given in Table 5 (Mehta and David, 1978; Easwaramoorthy and Shanmugasundaram, 1988).

Freshly emerged moths (10 pairs) are released for oviposition on potted sugar-cane plants (about 60 days old) covered with iron wire mesh oviposition cage. After 4 days, the eggs are collected and sterilized with 10% formalin for 2 min, washed with distilled water and allowed to dry on filter paper. Eggs are kept at room temperature to the blackhead stage, when 50 eggs are inoculated per diet bottle. The diet bottles are kept upside down in the wooden rack.

Larvae are transferred to fresh diet bottles after 10–12 days, with larval number being reduced from 30/bottle to 15. The larvae are disinfected with 10% alcohol and washed with distilled water. After drying over a filter paper, healthy pupae are transferred onto Petri dishes lined with filter paper and placed in an emergence cage. On emergence, moths are collected and released for egg laying in an oviposition cage.

Chilo zacconius *Blesz.*

The African striped borer, *C. zacconius* is an important pest of rice, and sugar-cane in West Africa. Damage by the insect in rice at the vegetative stage causes "dead hearts" and damaged tillers do not produce panicles. At the flowering stage, insect damage produces "white heads" or panicles with empty or partially filled grains. In sugar-cane, this species destroys the meristematic tissue of the shoot, resulting in dead heart formation. In older canes, the larva bores into, and tunnels the stalk without killing it; this reduces the sugar content of the sugar-cane. The only externally visible symptoms are borer holes on the internode.

Flat, scale-like and overlapping eggs are laid in 2–5 rows on the surface of leaves. Incubation period lasts for 5–6 days. Five larval instars have been reported with a duration of 22–28 days and the pupal period lasts 5–8 days. There could be 5–7 generations in one year.

C. zacconius is reared in the laboratory in France without the need for plants as food or substrate. Bordat and Pichot (1978) used a diet (Table 6), modified from an earlier one (Bordat et al., 1977) after a bacterial disease outbreak in 1977. The antibiotic previously used has been replaced by sulfamide bactrim (a mixture of sulfamethoxazole and trimethoprim).

Oviposition takes place on corrugated paper in a cylindrical polystryene container that also holds synthetic sponge soaked in water. Incubation, larval development, pupation and adult emergence take place in separate round boxes of transparent plastic. The surface of the larval box which contains the rearing medium, is round in order to prevent condensation and drowning of younger larvae. The pupation box contains corrugated paper that is transferred every 48 hr to the emergence box and replaced by fresh paper. Mortality is highest in the early two instars. Five to seven larval instars have been reported. Male adults always emerge first since they undergo fewer larval instars than do females.

SCREENING FOR HOST-PLANT RESISTANCE TO *C. PARTELLUS*

One of the most important prerequisites for a host-plant resistance programme is to have an effective and reliable screening technique through which a large number of genotypes can be screened at a time. An effective resistance screening technique should ensure uniform and desired level of insect infestation. Various techniques have been used to screen sorghum for spotted stem borer resistance using natural occurring population as well as artificial infestation.

Screening under natural infestation

Hot-spots. Hot-spots are locations where pest populations are known to occur naturally and regularly at levels that often result in severe damage to the crop. Such locations can be used for resistance screening under natural pest infestations. Hot-spot locations for *C. partellus* are Hisar in North India, Afgoi and Baidoa in Somalia, Panmure and Mezarbani in Zimbabwe and Golden Valley in Zambia.

Planting date. To screen under natural pest infestations especially at the hot-spot locations, the planting date of the crop should be adjusted in such a way that the susceptible stage of the crop coincides with the peak activity period of the pest. This can be determined by conducting population dynamics studies either using attractant traps or by monitoring pest infestation at regular intervals. Such studies conducted at Hisar have shown that *C. partellus* is most active in August–September. Therefore, a sorghum crop planted between 1st and 3rd week of July suffers maximum damage.

Screening under artificial infestation

Artificial infestation ensures uniform and sufficient level of pest infestation at desired time. Screening under artificial infestation is essential to confirm resistance observed under natural pest infestation as well as to study mechanisms of resistance. For artificial infestation of spotted stem borer, insects are reared in the laboratory using artificial diet as described in the first section of this paper.

Field infestation. For field infestation the "bazooka applicator" developed by Mihm and colleagues at the Centro International de Mejoramiento de Maiz y Trigo (CIMMYT) in 1976 (CIMMYT, 1977) for infesting maize with corn earworm has been modified to suit our requirements. This method requires a carrier for the larvae. First instar larvae are mixed with poppy seed (*Papaver* sp.), locally known as "khas khas". Five hundred blackhead stage egg masses are kept along with 85 g of poppy seeds overnight in a plastic jar with a tight-fitted lid. In the morning the hatched larvae are gently mixed with the carrier and transferred into the plastic bottle of the bazooka. The bazooka is then taken to the field for infestation and with a single stroke, 5–7 larvae fall in each plant whorl.

Plant growth stage. Developmental stage of crop at the time of infestation is an essential consideration for obtaining meaningful damage symptoms. The most critical damage which results in maximum grain yield reduction, is the destruction of growing point, the symptom of which is formation of dead hearts. Maximum dead hearts are obtained when relatively young plants (15–20 days old) are infested. Dead heart formation decreases progressively as infestation is delayed. For stem and peduncle tunnelling, plants may be infested later using bazooka applicator as long as whorls are available.

Time of infestation. Field infestation is generally carried out between 0800 and 1100 hr to avoid larval mortality due to higher temperatures. However, on cloudy days, infestations can be carried out at any time during the day.

Larval density. Generally 5–7 larvae per plant are sufficient to cause appreciable leaf feeding and dead hearts (> 90% damage in susceptible genotype). This corresponds to the natural density levels of infestation in the fields. However the number of larvae per plant can be regulated depending on the requirement. Similarly, a second infestation may be required depending on the weather conditions after the first infestation such as rainfall. The bazooka applicator is agitated regularly (usually after every 10 strokes) to ensure uniformity in larval distribution. There is often an accumulation of water in the plant whorl and in order to avoid larval drowning, the whorl should be gently tapped before infestation.

Control of shootfly. Shootfly infestation interferes with the screening for resistance to stem borer. A selective insecticide may be used that would suppress shootfly without leaving any residual effects on stem borer establishment. Soil application of carbofuran at sowing for shootfly control has a detrimental effect on stem borer establishment. Fenvalerate and endosulfan seem to be of some promise and can be sprayed to suppress shootfly infestation 1 week before artificial infestation with stem borer. At ICRISAT Center, we have found that cypermethrin (a synthetic pyrethroid) applied through electrodyne sprayer 1 week prior to borer infestation effectively controls shootfly without any detrimental effect on borer establishment. Similarly, planting the test material early in the season when shootfly infestation is quite negligible does not require insecticidal application.

Damage evaluation for resistance screening

Stem borer attack in sorghum causes leaf damage, dead heart formation, stem/peduncle tunnelling and production of chaffy panicles. All these symptoms are not necessarily related to yield loss. Leaf injury, which is the first larval feeding symptom, has been found to be related to yield loss only under severe infestation. At ICRISAT stem tunnelling has also not been correlated with reduction in grain yield, although quantity and quality of fodder may be adversely affected. Peduncle damage could be critical in situations of high wind velocities, which would break the peduncle. The most critical damage has been found to be the destruction of the growing point which results in the formation of dead hearts. This parameter is therefore the most important criterion for differentiating degrees of resistance. The second important criterion is the production of chaffy panicles.

Leaf feeding. Leaf feeding count was taken 1 week after artificial infestation, 3 and 6 weeks after crop emergence under natural infestation (Table 7). The parameters to be recorded include, total number of plants, number of plants showing

Table 7. Damage evaluation rating for resistance screening against spotted stem borer in sorghum

Score	Number of leaves with leaf feeding	Leaf area damage (mm^2)	Dead hearts/ chaffy/broken panicles (%)
1	1–2	<150	<10
2	1–2	150–300	10–20
3	2–3	300–450	21–30
4	2–3	450–600	31–40
5	3–4	600–750	41–50
6	3–4	750–900	51–60
7	4–5	900–1050	61–70
8	4–5	1050–1200	71–80
9	5–6	>1200	>80

leaf feeding symptoms, and leaf feeding score. Leaf feeding score should be on 1–9 scale based on the plants showing leaf feeding symptoms.

Dead hearts. Dead heart count was taken 3 weeks after artificial infestation, and 4 and 6 weeks after crop emergence under natural infestation, recording total number of plants, plants showing borer dead hearts, and visual score (1–9) for dead hearts.

Harvest count. At crop harvest, observations are taken on the total number of healthy panicles, number of partial and complete chaffy panicles, number of broken panicles, visual score (1–9) for chaffy/broken panicles and grain weight.

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