Improvement of the Mass Rearing Techniques for the Asiatic Corn Borer, *Ostrinia furnacalis* (Guenée), in the Philippines

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Introduction

The Asiatic corn borer, *Ostrinia furnacalis* (Guenée), has a wide distribution from northern Asia to Australia.[15] The corn borer is the most destructive pest of corn throughout the Asian tropical regions, for instance Malaysia,[25] Indonesia,[24] Thailand,[23] and the Philippines.[4]

Corn plant damage due to the corn borer causes crop losses up to 20 to 80% in the Philippines.[21] It was estimated that the loss is equivalent to multi-million peso[8] when calculated on the basis of the country's annual corn production of 3 billion pesos, or to about 4 tons/ha of grain yield[14] of a hybrid cultivar currently used in the Philippine Government's Masagana Program.

Insecticidal application to control the corn borer involves drawbacks such as high cost, development of insect resistance, adverse effects on natural enemies, and hazard to human health. To compensate these shortcomings, alternative methods are urgently needed. Adoption of resistant varieties could become the most reliable method to protect corn from corn borer attacks.

Artificial infestation of the test plants for the screening of resistant varieties has been made possible by mass-rearing of the insect in the laboratory.

Rearing methods of the European corn borer (*Ostrinia nubilalis*), have already been established by several researchers. Various techniques for rearing the insect were developed by using artificial diets.[1,2,3,18]

Kamano and Inoue (1955) were the first to develop a rearing method of the Asiatic corn borer by using an artificial diet derived from Beck (1953). A diet for the Asiatic corn borer by adding maize pollen to the standard ingredients was eventually developed.[17] Kojima and Nakayama (1979) developed a diet that made it possible to feed several lepidopterous insects including the Asiatic corn borer. Saito (1980), Saito and Nakayama (1981) modified a diet originally developed for mass rearing of the tobacco cutworm[16] for the Asiatic corn borer. This diet includes wheatgerm and kidney bean as major ingredients.

Since 1981, the corn group at IPB (Institute of Plant Breeding) of the University of the Philippines at Los Baños has conducted a research project on breeding for the corn borer resistance. An artificial diet, on which the Asiatic corn borer can complete its growth and development, is urgently needed for the mass rearing to supply test insects for screening resistant corn varieties.

General methods

1) Collection of egg masses

Egg masses were collected in corn fields at Los Baños, the Philippines, and placed in

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a petri dish with a moist filter paper. They were kept at 25°C in the laboratory until just before hatching, and then inoculated onto the diet without sterilization.

2) Preparation of diet

Acrylic round containers (185 mm in diameter and 80 mm high) with tight-fitting lids were used as rearing vessels. The lid was perforated and fitted with a fine mesh brass screen net (115 mm in diameter). The vessels and lids were disinfected by dipping them for one day in 1% sodium hypochlorite solution, washed in water, and dried, before use.

The basic component of Jamornmarn diet[9] which had been used at IPB, and of Saito diet[19] modified by the authors (hereafter referred to modified Saito diet) is shown in Table 1. To apply the latter to Philippine conditions, methyl paraben and sorbic acid as fungicides and aureomycine as bactericidal agent were added as in the case of the IPB diet. Major components, such as ground corn, ground soybean, wheatgerm, and brewers' yeast, were soaked in sterile distilled water (450 m*l*) and mixed thoroughly using a cooking stirrer. In addition, 250 m*l* of water was boiled while adding agar, and mixed with the portion of major components. Other components such as vitamins, bactericide and fungicides were poured into the stirrer. The diet each weighing 500 g was distributed into each rearing vessel.

3) Inoculation of hatching larvae

Before receiving hatching larvae, the surface of the diet was scraped with a table fork. Numerous scratched grooves on the diet facilitated the establishment and hiding of the larvae. Two hundred larvae were transferred from the petri dish to a rearing vessel individually with a small hair pencil moistened with sterile distilled water. The number of larvae was counted by a manual counter during the transference. A population density of 200 larvae/rearing vessel was adopted in each experiment.

4) Rearing conditions of larvae

Larvae were reared at 25°C under natural photoperiodic condition. In most experiments, larvae were fed for about 20 days. When some larvae were observed at the upper part of the vessels at the end of this period, the vessels were opened, and the upper part of the inner wall was lined with the band of pupal tubes which were made of shredded, slightly transparent plastic straws and adhered to masking tape 25 mm wide. The pupal tubes in which the borers were already pupating were removed from the band using a forceps every day, and cut carefully to pick up the pupae. The pupae were separated by sex, weighed, and placed in acrylic containers with several sheets of moistened tissue paper at the bottom.

5) Conditions for oviposition

The pupae were kept in the plastic containers until emergence.

After counting the number of emerging adult moths, 25 pairs of adult moths were put together in the oviposition cage (27×34×27 cm) at 25°C with a sheet of wax paper on the top side. The moths laid their egg masses on the wax paper. The egg masses were collected daily, placed on the filter paper in a petri dish, and kept at 25°C until just before hatching. The wax paper in the oviposition cage was renewed daily.

6) Evaluation of rearing techniques

Suitability of a rearing technique for growth, development and egg production of the corn borer was evaluated by the following biological attributes:

(1) Larval period

The period from hatching to pupation was taken as larval period. The date of pupation was confirmed by macroscopic check of pupation through the slightly transparent plastic straw.

(2) Mean weight of pupae

When the pupae were taken out from the plastic straw shred at the 2nd day after the pupation, they were weighed individually with an electron balance and the mean weight was

Table 1. Composition of modified Saito and Jamornmarn diets*

Ingredients	A	B	C	D	E	F	G
Ground Corn (g)	–	–	–	–	–	–	96
Ground Soybean (g)	100	100**	100	100**	100	100	50
Wheatgerm (g)	100	100	100	100	100	100	2
Brewer's yeast (g)	40	40	40	40	40	40	40
Ascorbic acid (g)	4	4	4	4	4	4	4
Vitamin E (g)	–	–	–	–	–	–	0.15
Vitamin complex (g)	–	–	–	–	–	–	5
Casein (g)	–	–	–	–	–	–	2
Choline chloride (g)	–	–	–	–	–	–	2
Methyl paraben (g)	2.4	2.4	2.4	2.4	2.4	2.4	2
Sorbic acid (g)	1.25	1.25	–	–	1.25	–	1.25
Aureomycin (g)	0.125	0.125	–	–	–	0.125	0.125
Formaldehyde (m*l*)	3.3	3.3	3.3	3.3	3.3	3.3	2.5
L-Cysteine hydrochloride (g)	0.4	0.4	0.4	0.4	0.4	0.4	–
Agar (g)	12	12	12	12	12	12	16
Water (m*l*)	700	700	700	700	700	700	700

* A~F: Modified Saito diet, G: Jamornmarn diet
** Soybean flour was parched at 120°C for 3 hours.

calculated.

(3) Percentage of pupation

The percentage of pupation was calculated from the number of normal pupae and the total number of larvae. Deformed pupae were omitted from the counts.

(4) Percentage of emerged adults

When pupation was completed, male and female pupae were separated and counted (injured and dead ones were omitted). Normal adults emerging from normal pupae were recorded.

(5) Number of egg masses per female moth

Number of egg masses laid by 25 females per cage was recorded daily and averaged. The color of the egg turned black 4 days after oviposition due to the final stage of embryonic development which facilitates counts. Egg masses composed of 9 eggs or less were omitted from the calculation.

(6) Degree of fungal occurrence

Fungal growth on the artificial diet in rearing vessels was rated as follows:

− : no fungal growth
± : trace of fungal growth
+ : occurrence of several fungal colonies
++ : occurrence of large and numerous colonies
+++ : the whole surface is covered with active fungal growth

The rating was carried out 4 weeks after the inoculation of hatching larvae.

Improvement of artificial diets

1) Comparison of Jamornmarn diet and modified Saito diet

Growth and development of corn borer were compared between Jamornmarn diet, which had been used in IPB, and modified Saito diets. As many Japanese workers on artificial diets for lepidopterous insects reported that parched soybean flour (called "Kinako" in Japan) was a satisfactory dietary component.[6,7,11,22] Kidney bean, a major ingredient of Saito diet, was replaced by soybean in the modified Saito diets.

(1) Materials and methods

Components of the modified Saito diets and Jamornmarn diet are shown in Table 1. The diets A, B, C, D, E, and F are modified Saito diets and G is Jamornmarn diet. For diets B and D ground soybean was parched at 120°C for 3 hr, while for A and C fresh soybean flour

Table 2. Rearing of corn borer on modified Saito and Jamornmarn methods*

Biological attributes	A	B	C	D	E	F	G
Percent pupation	69.3	67.7	44.5	53.0	52.5	6.8	68.3
Sex ratio (male/male+female)	0.514	0.489	0.561	0.515	0.536	0.909	0.490
Days from hatching to pupation ±S.E.	23.0±0.13	23.3±0.14	23.2±0.18	24.3±0.20	22.0±0.14	21.7±0.44	26.3±0.17
Weight of male pupae(mg) ±S.E.	71.7±0.80	76.1±0.82	69.4±1.01	72.9±1.06	76.4±0.75	56.8±1.86	72.7±0.99
Weight of female pupae(mg) ±S.E.	101.2±1.31	104.8±1.54	87.4±1.88	98.2±1.66	102.9±1.60	72.3±7.01	103.6±1.44
Percent emergence	88.3	86.6	89.4	90.8	90.4	85.0	89.4
Days from hatching to emergence ±S.E.	29.9±0.14	30.0±0.12	29.9±0.14	31.1±0.15	29.0±0.12	28.3±0.34	32.3±0.14
No. of egg masses/female	2.2	1.8	2.7	1.4	2.8	—	3.0
No. of eggs/female	44.8	34.5	51.9	31.2	52.4	—	54.0
Egg-mass size ±S.E.	20.6±0.77	19.8±0.81	20.0±0.77	21.3±1.15	18.4±0.94	—	18.1±0.54
Degree of fungal occurrence	—	—	++	++	+	+++	+

* Means of 3 replications

Table 3. Rearing of corn borer on Saito diet with soybean or mung bean, and Jamornmarn diet*

Biological attributes	Saito diet with soybean	Saito diet with mung bean	Jamornmarn diet
Percent pupation	78.4	90.7	66.7
Sex ratio (male/male+female)	0.528	0.541	0.555
Days from hatching to pupation ±S.E.	25.9±0.13	23.5±0.11	33.6±0.23
Weight of male pupae(mg) ±S.E.	72.5±0.87	78.8±1.06	70.0±0.79
Weight of female dupae(mg) ±S.E.	96.3±1.49	110.5±3.16	92.3±1.63
Percent emergence	90.6	90.7	93.7
Days from hatching to emergence ±S.E.	34.4±0.13	31.7±0.12	42.9±0.24
No. of egg masses/female	2.3	4.0	1.2
No. of eggs/female	51.3	107.8	22.7
Egg-mass size ±S.E.	23.0±0.99	26.1±0.81	18.6±0.84
Degree of fungal occurrence	—	—	+

* Means of 3 replications

was used.

(2) Results and discussion

As shown in Table 2, the larval period on any of the modified Saito diets was shorter than on the Jamornmarn diet. Percentages of pupation on diet A, B, and G were almost identical and they were higher than those on C, D, E, and F. The number of eggs and egg masses on the diets B and D were less than on the diets A and C. These facts suggest that the modified Saito diet containing soybean as a major ingredient can be used for rearing the corn borer in the Philippines and is as satisfactory as Jamornmarn's diet. Furthermore, there is no need to parch soybean powder in preparing the diet. The definitely low percentage of pupation shown on diet F was apparently caused by heavy fungal contamination. In the Philippines, it is essential to add fungicides such as sorbic acid to diets.

2) Mung bean, a substitute for soybean

Legacion, one of the present authors, suggested in his preliminary experiment that mung bean may be used as an ingredient of corn borer diet.

(1) Materials and methods

Two modified Saito diets: one with mung bean and the other with soybean were compared with Jamornmarn diet. Other components of them were the same as those of diet A in Table 1.

(2) Results and discussion

As shown in Table 3, the diet containing mung bean gave higher percentage of pupation with increased weight of pupae as compared with other diets, while it reduced the duration from hatching to pupation. Egg production was also improved by increasing the number of eggs and egg masses as well as egg size.

Improvement of the oviposition cage for egg mass production

Germplasm screening of resistant corn in the field requires a large supply of egg masses. In the Entomology Laboratory of IPB, the University of the Philippines, the egg production per female moth was estimated at 3.0 egg masses on the average. Kuwayama (1931), who described numerous biological characters of the Asiatic corn borer in Japan, stated that the number of egg masses per female ranged from 5 to 36 with an average of 16. In the Philippines, Camarao (1976) showed the average of 18 egg masses per female moth taken from the field. Therefore an experiment was conducted to find out a way to increase egg productivity of the adult moths reared on artificial diets.

1) Materials and methods

As shown in Fig. 1, oviposition cages (27×34×27 cm) used at IPB (IPB type) were covered with plastic sheets on the lateral, top and bottom sides (Improved type I). In addition, a one inch thick plastic foam mat soaked with water was placed at the bottom (Improved type II). Twenty five pairs of moths reared on Saito diet with soybean, Saito diet with mung bean or Jamornmarn diet were put into an oviposition cage of each of three types.

2) Results and discussion

As given in Fig. 2, the mean egg mass size was not influenced to an appreciable extent by the kinds of diet and the types of cage.

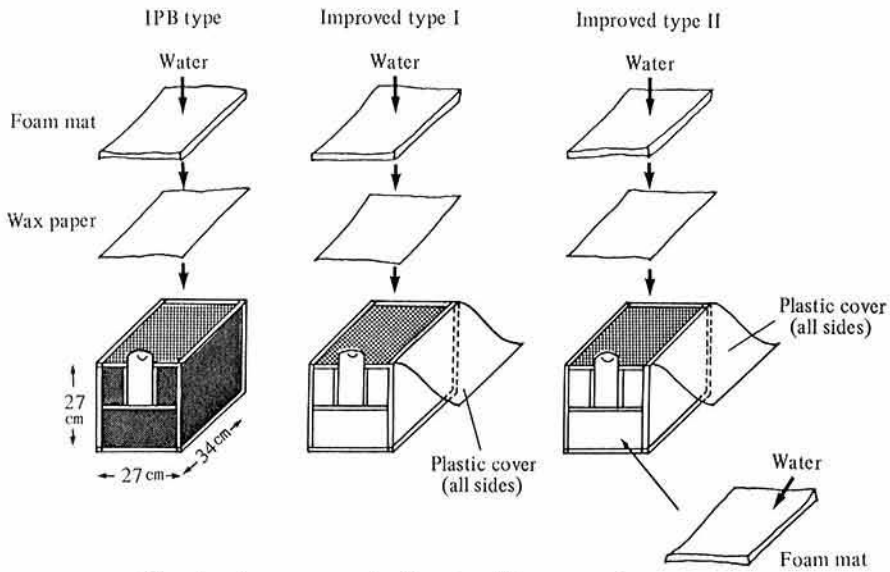

Fig. 1. Improvement of oviposition cage for corn borer

However, the moths reared on the modified Saito diet with mung bean and kept in the oviposition cage of Improved type II produced the largest number of eggs (Fig. 3) and egg masses (Fig. 4). This result indicates an importance of humidity inside the oviposition cage for egg production of corn borer moths. As a matter of fact, a simple device to increase humidity in the cages resulted in nearly 4-fold to 7-fold increase in the number of egg masses produced.

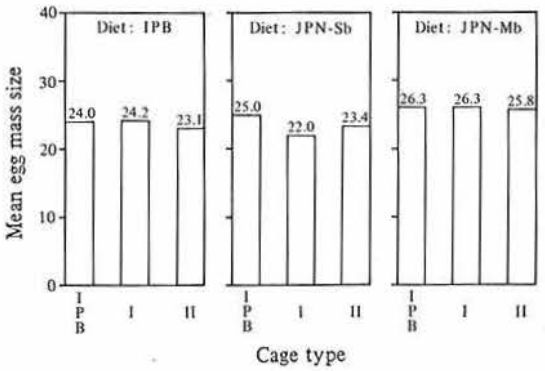

Fig. 2. Size of egg-masses produced from moths, reared on 3 diets and kept in 3 types of oviposition cages
Diet; IPB: Jamornmarn method, JPN-Sb: modified Saito diet with soybean, bean, JPN-Mb: modified Saito diet with mung bean
Cage type; IPB: IPB type, I: Improved type I, II: Improved type II

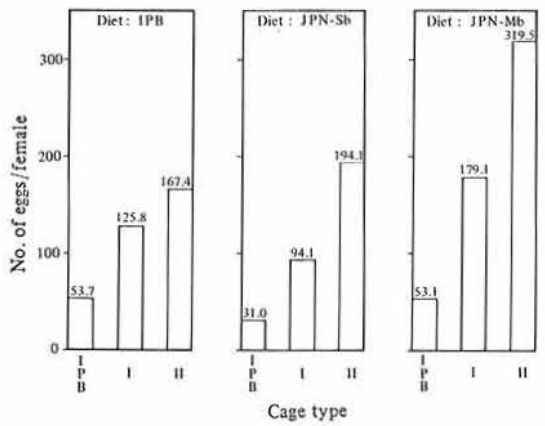

Fig. 3. Mean number of eggs per female moth reared on 3 diets and kept in 3 types of oviposition cages
See Fig. 2 for the legends.

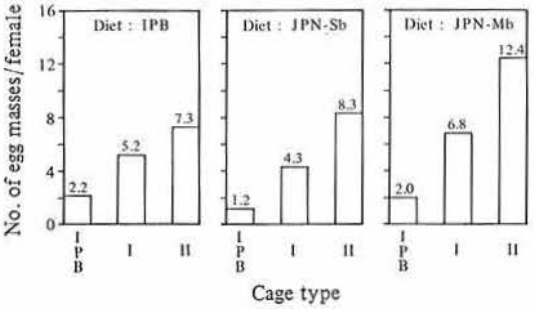

Fig. 4. Mean number of egg masses per female moth reared on 3 diets and kept in 3 types of oviposition cages
See Fig. 2 for the legends.

Further improvements of artificial diets

Although sorbic acid was added to the Saito diet to the suppression of fungal occurrence, its effect still remained unsatisfactory: fungal contamination of diet in the rearing vessels was often observed at the pupal stage of the insect.

1) Supplementary addition of propionic acid

(1) Materials and methods

Propionic acid was added to the modified Saito diet containing mung bean. The rate of application was 0 (control), 0.1, 0.25, 0.5, 1, and 2% on dry weight basis.

(2) Results and discussion

Fungal occurrence was suppressed by the addition of propionic acid at the rate of 2, 1, 0.5 and 0.25%. However, the number of days from hatching to pupation and emergence was increased at high contents of propionic acid. The difference of about 2 days was observed between 2% and 0% propionic acid. It shows that propionic acid at high contents inhibits larval and pupal growth and egg production of the corn borer (Table 4).

2) Reduction of diet quantity

Table 4. Effects of propionic acid on growth and development of corn borer reared on modified Saito diet with mung bean and suppression of fungal contamination*

Biological attributes	Percentage of propionic acid (dry weight basis)					
	2.0%	1.0%	0.5%	0.25%	0.1%	0%
Percent pupation	69.8	84.5	87.3	88.0	90.3	87.7
Sex ratio (male/male+female)	0.537	0.534	0.492	0.537	0.537	0.527
Days from hatching to pupation ±S.E.	25.2±0.12	25.0±0.12	24.3±0.12	23.9±0.11	23.8±0.11	23.3±0.11
Weight of male pupae(mg) ±S.E.	67.5±0.95	71.5±0.80	69.5±0.83	69.3±1.14	67.8±1.07	68.1±1.05
Weight of female pupae(mg) ±S.E.	92.4±1.72	94.5±1.86	93.4±1.60	87.7±1.82	95.9±1.95	92.1±1.69
Percent emergence	94.2	93.6	93.5	96.1	97.1	93.3
Days from hatching to emergence ±S.E.	33.9±0.12	33.7±0.12	33.0±0.12	32.6±0.11	32.5±0.12	32.0±0.12
No. of egg masses/female	6.0	8.8	9.4	11.9	10.7	10.7
No. of eggs/female	211.5	294.9	314.3	335.5	317.5	314.2
Egg-mass size ±S.E.	35.5±1.02	33.4±0.76	33.4±0.75	28.1±0.60	29.6±0.66	29.4±0.74
Degree of fungal contamination	—	—	—	—	—	##

* Means of 3 replications

As expensive imported materials have to be used to prepare artificial diets for the corn borer in the Philippines, an experiment to reduce the quantity of the diet without affecting growth and egg production of the corn borer was carried out.

(1) Materials and methods

The amount of modified Saito diet (with mung bean) supplied per rearing vessel was 500, 450, 400, 350, 300 or 250 g, and 200 hatching larvae were fed per vessel.

(2) Results and discussion

The result shown in Table 5 indicates that the insects fed on 350 g of the diet achieved normal growth and adequate egg production. However, less amount of diet, 300 and 250 g, resulted in decreased egg production. It shows that we can save the quantity of diet by 30%.

Conclusions

To conduct germplasm screening of resistant corn in the field, good timing between plant growth and the preparation of egg masses of corn borer in the laboratory is needed. As the growth of test plants depends on weather conditions in the field, corresponding adjustment is needed in insect rearing.

Legacion observed earlier that the pupae stored at 10°C for 20 days are able to emerge into normal moths. This information threw light on the timing problem. However, effect of the pupal storage on egg production has not been studied. Anyway, in relation to the timing problem, practical storage methods should be established.

The present authors carried out a series of experiment on corn borer rearing on artificial diets.

The Saito diet was improved to make it adaptable to Philippine conditions. The modified Saito diet containing mung bean showed the highest yield of pupae, moths, and egg masses.

The fungal contamination of the artificial diet was overcome by adding sorbic acid and propionic acid. However, high contents (>0.25% on dry weight basis) of propionic acid gave adverse effects on larval growth, yield of pupae and moth, and egg production.

A simple device to increase the humidity in oviposition cages exerted a striking effect in increasing egg production of the corn borer

Table 5. Rearing of corn borer with various amounts of diet supply*

Biological attributes	500 g	450 g	400 g	350 g	300 g	250 g
Percent pupation	75.4	87.0	79.7	84.2	83.2	74.2
Sex ratio (male/male+female)	0.469	0.517	0.519	0.509	0.487	0.535
Days from hatching to pupation ±S.E.	21.7±0.13	20.6±0.18	20.5±0.11	20.1±0.10	20.8±0.12	20.8±0.13
Weight of male pupae(mg) ±S.E.	73.3±1.07	74.7±0.87	76.0±0.92	74.8±0.78	75.3±0.88	69.8±0.96
Weight of female pupae(mg) ±S.E.	112.5±1.81	111.4±1.85	117.9±1.56	112.6±1.56	107.7±1.80	95.6±2.03
Percent emergence	96.1	90.3	97.2	94.1	93.6	93.1
Days from hatching to emergence ±S.E.	30.1±0.13	28.9±0.11	30.9±0.17	28.6±0.10	29.4±0.12	29.5±0.12
No. of egg masses/ female	8.2	9.4	10.7	10.1	8.5	6.5
No. of eggs/female	267.8	290.4	340.5	327.8	282.2	199.8
Egg-mass size ±S.E.	32.5±0.81	31.0±0.75	31.9±0.71	32.5±0.73	33.1±0.87	30.9±0.98

* Amount of modified Saito diet/rearing vessel with 200 larvae.
Means of 3 replications

Table 6. Comparison of diet cost between the improved rearing method and the former method

Insect rearing method	No. of plants to be tested*	No. of egg masses needed*	Yield (no.) of egg mass/ female	No. of pairs of moths needed	No. of pairs obtained/ rearing vessel	Total amount of diet required (kg)	Unit cost of diet (Peso/kg)	Cost of diet (Peso)
Improved	31,500	126,000	12	10,500	70	52.5	95.78	5,028.45
Former	31,500	126,000	3	42,000	62	339.0	168.53	57,131.67

* Assuming that 31,500 corn plants have to be screened to select out 500 germplasm for resistance in a year.
To do this job 126,000 egg masses of corn borer are need. Prices of diet materials were those in September 1984.

moths.

These improvements on diet composition and oviposition cage in the mass rearing of the corn borer were evaluated in terms of diet cost.

As given in Table 6, the use of modified Saito diet, containing mungbean, at the rate of 350 g/rearing vessel and of improved oviposition cages proved that the cost of the diet required to supply enough number of egg masses to a screening work dealing with 31,500 plants was reduced to one-tenth that of the rearing technique so far employed in IPB. In this calculation, costs for facilities and equipment, running cost, wages, etc. were not included.

Summary

In breeding corn varieties resistant to the corn borer, artificial infestation of test plants requires mass rearing of the insect in the laboratory. The mass rearing technique so far practiced in the Philippines uses expensive diets but results in low output.

We tried to introduce and modify the rear-

ing method established by Saito (1980) for the corn borer, *Ostrinia furnacalis*. The Saito diet was first modified by using soybean as a substitute for kidneybean, and again modified by using mung bean in place of soybean. Addition of sorbic acid, aureomycine and propionic acid was made. The modified diet containing mung bean allowed better growth of the corn borer and appreciable suppression of fungal occurrence. With this diet, a 30% reduction of the supply quantity was made possible without causing adverse effects on pupal growth and egg production. Covering each oviposition cage with a plastic sheet and supplying a foam mat soaked with water to the bottom of the cage enabled to produce an average of 12 egg masses per female, in contrast to the average of 3 egg masses per female, hitherto noted.

Due to these improvements of the rearing method, the cost of the diet used for producing egg masses was reduced to only one-tenth that of the former rearing method.

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