Research Note

SUITABILITY OF THE INSTITUTE OF PLANT BREEDING ASIAN CORN BORER (IPB ACB) ARTIFICIAL DIET FOR LABORATORY MASS REARING OF THE FALL ARMYWORM, *Spodoptera frugiperda* (J.E. Smith) (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

One of the innovations in laboratory insect mass rearing developed in the Entomology Laboratory, Institute of Plant Breeding (IPB), is the IPB Asian corn borer (ACB) Artificial Diet for *Ostrinia furnacalis* (Guenée). This diet has been successfully and continuously used in mass producing the test insects for various bioefficacy studies since 2000, including other major lepidopteran insect pests of corn. In this study, the suitability of this artificial diet was assessed in mass rearing the new invasive insect pest, the fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith), under laboratory conditions. Local feral populations of FAW were collected from different provinces in Luzon, Visayas, and Mindanao and mass-reared under laboratory conditions from 2020 to 2022. Previous rearing methods have been modified to properly handle the different larval stages of the insect. Using the IPB ACB Modified Artificial Diet and the optimized rearing procedures, successive generations of laboratory-reared FAW were produced, up to at least the fifth generation and at most the fifteenth generation, indicating that the said artificial diet is suitable for laboratory mass rearing of different FAW populations for multiple generations. The artificial diet can be further developed or modified to rear other insects aside from the Asian corn borer and other lepidopteran pests.

Keywords: artificial diet, laboratory mass rearing, fall armyworm

INTRODUCTION

In the Philippines, there is currently no study or protocol for mass-rearing fall armyworm Spodoptera frugiperda (J.E. Smith) under laboratory conditions. An efficient way of managing a large number of test insects for long durations is the use of an artificial diet that has a long shelf life and can support the growth and development of the test insects by providing optimum nutrition. For the Asian corn borer (ACB), *Ostrinia furnacalis* (Guénee)

(Lepidoptera: Crambidae), the Entomology Laboratory, Institute of Plant Breeding (IPB), University of the Philippines Los Baños has successfully developed techniques in providing large quantities of test insects with high quality through the IPB ACB Artificial Diet developed by Caasi-Lit *et al.*. in 2015. The formulation and composition of this artificial diet were originally derived from the Insect Rearing Laboratory of the International Maize and Wheat Improvement Center [CIMMYT], Mexico (Gabriel and Camarao 1975). This artificial diet was modified by Camarao in 1976 using opaque-2-corn based on the methods of Rangdang (1971). The diet, along with the insect-rearing techniques, has been modified through the years and is now called the IPB ACB Modified Artificial Diet. This artificial diet has also been used to successfully mass-rear other lepidopteran insect pest of corn such as the corn earworm, *Helicoverpa armigera* (Hübner), common cutworm, *Spodoptera litura* (Fabricius), corn semilooper, *Chrysodeixis eriosoma* (Doubleday), and true armyworm, *Mythimna separata* (Walker) (Caasi-Lit *et al.*. 2016), all of which belong to the insect family Noctuidae.

The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith), another noctuid insect, is an economically important pest infesting a wide range of crops, but mainly corn as its main host plant. In the Philippines, FAW is a relatively new insect pest and has been reported to cause damage in all phases of the growth of corn (Navasero *et al.* 2019). It can consume most of the crops in the field within a short period if left uncontrolled (Capinera 2017). Hence, it is necessary to develop effective, coordinated, and flexible strategies to manage FAW populations (Prasanna *et al.* 2018). For this purpose, FAW control studies depend on the availability of uniform, laboratory-reared test insects, requiring efficient mass-rearing techniques. Current bioefficacy and entomological studies in the country involving mass rearing of FAW still use leaves of susceptible maize varieties as fresh diet (Navasero and Navasero 2020; Montecalvo *et al.* 2022; Duza *et al.* 2024; Marri *et al.* 2023). This requires constant supply of freshly harvested leaves and frequent handling in removal of possible decaying leaf tissues. Moreover, the use of a natural diet such as leaves in mass rearing has been observed to affect the development of the insects due to the limited nutritional component that varies per variety, age, and plant parts (Alam *et al.* 2024). To achieve the objective of effective laboratory mass-rearing of FAW, known for its polyphagous nature and capability to be mass-reared on an artificial diet developed for other species (Mihm 1983), the IPB ACB Artificial Diet was utilized as a rearing medium for FAW. The recent study of Caasi-Lit and Marmeto (2022) utilized this diet in laboratory mass rearing of FAW for at most two generations, which was used for bioefficacy studies in Philippine traditional maize. The aim of this study was to test the suitability of the IPB ACB artificial diet for mass-rearing several populations and successive generations of FAW collected from different locations in the Philippines under laboratory conditions.

MATERIALS AND METHODS

Fall armyworm individuals were collected from January to December 2020 from eight provinces in the Philippines: Pangasinan, Laguna, Isabela, Cagayan, and Cavite for Luzon Island; Cebu and Iloilo for Visayas island group; and Zamboanga for Mindanao Island (**Table 1**). These feral populations represented eight different local FAW populations. It was ensured that the target sites were free from any hazardous pesticide spray prior to field visits. The egg masses of FAW were frequently seen on both the adaxial and abaxial surfaces of the corn leaves, as well as on fully expanded leaves of unopened tassels during the pre-flowering stage. In addition, egg masses were seen on the growing young stalks in the middle portion of the plant when infesting FAW is abundant. Based on previous field collections, the recommended time of egg mass collection is from 35 to 50 days after planting (DAP). Egg masses were collected by cutting the portion of the leaves upon which they were laid on without dislodging. The cut leaves containing the eggs were placed inside a Petri dish lined with moistened filter paper. Early instar larvae were also present on maize plants as early as three weeks old. However, their tendency to feed within the whorl made collecting them more difficult compared to third and later instar larvae which are visibly larger. Signs of pest infestation were holes and frass on the seedling stalk, whorl, and eventually on the young internodes. Larvae were also obtained from the tassel, developing silk, and young ears during the reproductive stage.

Table 1. Initial collections of fall armyworm from Luzon**,** Visayas, and Mindanao in 2020.

Province	Collected Egg mass						Collected Larva					
	Feb	**Mar**	**Jul**	**Sep**	**Oct**	**Nov**	**Feb**	**Mar**	**Jul**	**Sep**	**Oct**	**Nov**
Cagayan							348					
Cavite									32			
Cebu												64
Iloilo											52	
Isabela	11						155					
Laguna	159	19	35	1	1		352	182	116	72		49
Pangasinan							20					
Zamboanga											69	

FAW was collected mainly from target sites in Luzon, accompanied by official domestic transport permits. The collection dates coincided with the known or expected incidence of the pest from February to November 2020.

More than 150 FAW larvae were collected on three occasions in Laguna. Likewise, plenty of larvae were collected initially in Enrile, in the province of Cagayan, and Santo Tomas, in the province of Pangasinan. However, only a few survived upon reaching the laboratory. This could be due to the unfavorable conditions inside the rearing pan during transport which had adverse effects on the survival of the collected insects. Due to the cannibalistic nature of larger FAW larvae, it is possible that the unsuitable transport conditions combined with the limited diet prompted the older and larger instar larvae to consume the younger and smaller individuals. The Cagayan population was not able to continue the succeeding generations due to this high mortality rate. Fifty-two larvae were collected in Iloilo, 69 in Zamboanga, and 79 in Cebu. Larvae from locations other than Laguna were properly disposed of following biosafety measures, such as preservation of discarded larvae in 95% ethanol solution.

The viable egg masses and live larvae collected were sent to the Entomology Laboratory, Institute of Plant Breeding, University of the Philippines Los Baños, Laguna for quarantine and mass production. The portions of maize leaves with the egg masses were cut with scissors and placed in Petri dishes lined with moistened filter paper (**Figure 1a-b**). Wax paper was also used as an alternative oviposition medium. Each Petri dish was secured using rearing pans filled with the solidified artificial diet. Mass rearing was feasible in the laboratory under ambient room temperature. Egg masses placed in Petri dishes lined with moistened filter paper hatched after three days. Laboratory mass-rearing procedures for fall armyworm by Caasi-Lit et al. (2020) were followed.

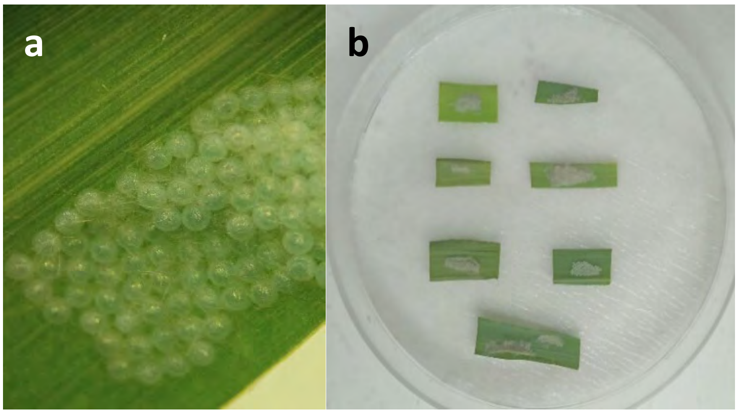

Figure 1. Field-collected fall armyworm egg masses **(a)** on corn leaf and **(b)** lined on a Petri dish with moist filter paper for hatching.

The artificial diet was prepared following the established method of Caasi *et al.* (2015) using the ingredients listed in **Table 2**. The gelatin bar was dissolved by boiling in half the volume of the water (350 mL), which acted as the binder for the diet. The rest of the dry ingredients were mixed with the remaining water in the blender, and the dissolved gelatin was incorporated.

Table 2. Ingredients for the Institute of Plant Breeding modified artificial diet for Asian corn borer (lifted from Caasi-Lit et al.., 2015).

Diet Ingredients	Quantity
Ground corn	96 g
Ground soybean	50 g
Brewer's yeast	40 g
Wheat Germ	2 g
Ascorbic Acid	4 g
Vitamin Complex	5 g
Casein	2 g
Sorbic Acid	1.25 g
Methyl paraben	2 g
Vitamin E	Half capsule=2 drops
Gelatin Bar ("Gulaman")	19 g
Distilled Water	700 ml

The mixture was cooled in diet pans to form. Neonates (**Figure 2a**) that emerged were fed and reared on the IPB ACB Artificial Diet for up to 5-15 successive generations (**Table 3**) with the following rearing conditions: 27°C, 75% relative humidity, 12:12 light: dark photoperiod. The larvae were allowed to develop collectively in the rearing pan until the third instar larval stage. Initial rearing of early instar larvae can be done in rearing pans where the diet was spread into the container and neonates were carefully transferred in these with at most 120 larvae per pan. First and second-instar larvae can be reared in this manner as they are still gregarious. When they reach the third instar, they become cannibalistic. This is the right time to transfer each larva to individual cups placed on a rearing tray for effective rearing. After the third instar stage or the third molting, each larva was placed individually inside an assay cup containing a small piece of artificial diet and sealed with a perforated lid to prevent escape (**Figure 2b-c**). A camel hair brush was used to gently transfer the larvae from the molder into the assay cups. During this growth period, the larvae were not disturbed to limit the stress from frequent handling. Larvae remained feeding on the surface of the diet. The artificial diet was replenished, and frass was removed whenever necessary, during the whole larval period. Later larval instars were appropriately contained individually inside a sealed cup. This is the proper way of handling older larvae to prevent cannibalism.

After 14 days in the rearing room, the insect trays were inspected daily for pupation. Pupated individuals were harvested from assay cups and placed inside a Petri dish using sanitized forceps (**Figure 2d**). The pupae were sorted according to the insects' sex under a stereomicroscope using the morphological distinction common to Lepidoptera species (Zhang *et al.* 2013) (**Table 3**). The males were identified by the relatively shorter gap between the

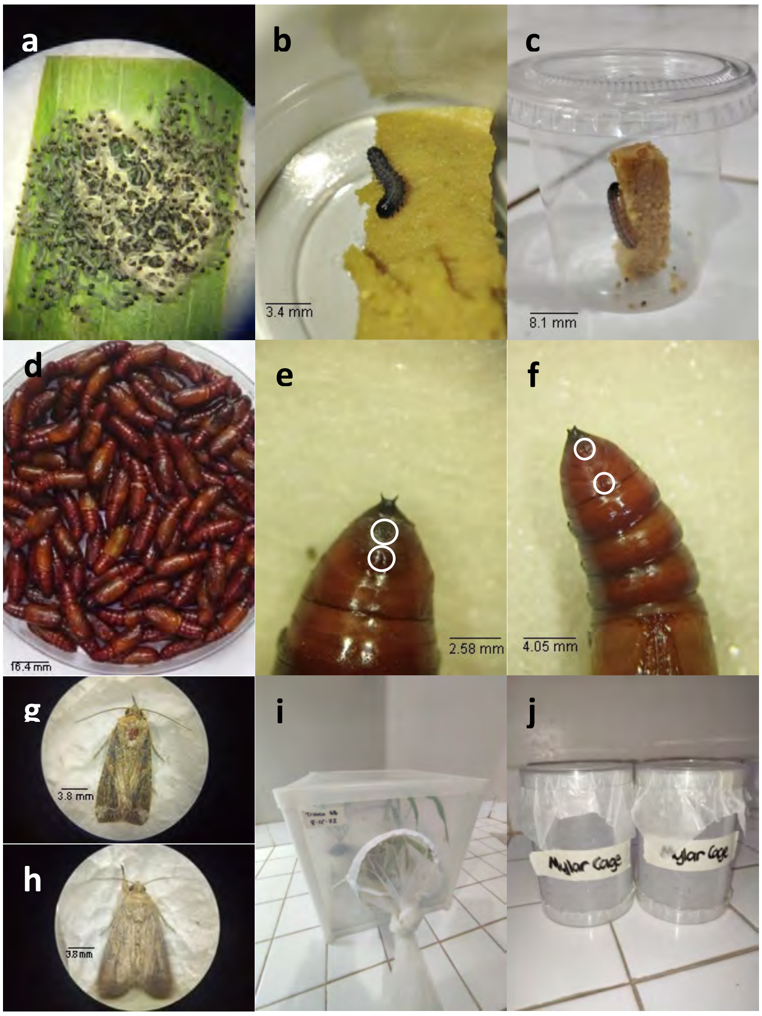

Figure 2. Laboratory mass rearing of the fall armyworm: **(a)** newly hatched larvae; **(b-c)** third instar larva inside an acrylic plastic cup containing a small piece of the Institute of Plant Breeding Asian corn borer artificial diet; **(d)** harvested pupae placed inside a Petri dish; **(e)** male and **(f)** female pupae; **(g)** male and **(h)** female adult moths; **(i)** oviposition cage using young corn plant; **(j)** mylar cages using wax paper as oviposition medium.

genital and anal slits (**Figure 2e**) compared to those of the females as viewed ventrally on the posterior abdominal segments (**Figure 2f**). Pupae were placed separately according to sex in acrylic pans with mesh lids to allow emergence from the cocoon. This separation was necessary to prevent untimely mating and possible oviposition on the plastic containers. Emerged adults were placed inside an oviposition cage (**Figure 2g-h**). Corn seedlings and pieces of wax paper served as media for egg laying. A 30 cm^3 nylon insect rearing cage was used to contain a potted corn seedling (IPB VAR6) (Fig. 2i), and an 8.5-cm x 12-cm (diameter x height) cylindrical mylar cage lined with wax paper on the

Table 3. Laboratory mass rearing of different fall armyworm populations using the Institute of Plant Breeding modified artificial diet based on quality parameters.

Quality parameters	Fall Armyworm Populations (Mean ± Standard Error)						
	Laguna	Cavite	Pangasinan	Iloilo	Zamboanga	Cebu	Isabela
No. of successive generations	15	13	11	11	11	6	5
Mean no. of individuals per generation	675 ± 52	489 ± 18	554 ± 46	650 ± 139	529 ± 44	369 ± 35	512 ± 62
Mean no. of egg mass per generation	221 ± 20	104 ± 16	131 ± 15	114 ± 17	89 ± 7	115 ± 9	65 ± 12
Mean no. of males per generation	374 ± 36	282 ± 18	296 ± 28	332 ± 68	289 ± 27	193 ± 18	277 ± 36
Mean no. females per generation	301	207	258 ± 19	318 ± 71	240 ± 18	176 ± 17	235 ± 28
Development period (days)	18-37	32.1-34	30.3-32.3	28.3-36	31.2-38.3	30-38.7	32.8-36.2
Minimum	18 ± 0.71	32.1 ± 0.34	30.3 ± 0.14	28.3 ± 1.58	31.2 ± 0.56	30 ± 0.93	32.8 ± 1.46
Maximum	37 ± 1.30	34 ± 0.35	32.3 ± 0.40	36 ± 1.71	38.3 ± 1.27	38.7 ± 0.98	36.2 ± 1.62

inside was prepared **(Figure 2j**). These two types of oviposition set-up for moths were consistently followed in the laboratory. Adult pairs were fed with sugar solution provided through pieces of cotton balls for both set-ups. The FAW adults have a white hindwing with a dark-brown margin. However, males have more distinct patterns with triangular white spots at the tip of each forewing.

RESULTS AND DISCUSSION

The local FAW populations were able to undergo several successive generations using IPB modified artificial diet (**Table 3**). The acceptable quantity of test insects in this study was at least 300 live larvae produced per generation. If this parameter was not met, the rearing was terminated. The number of successive generations was highest in Laguna with 15, and lowest in Isabela with five generations. Laguna also had the highest average number of individuals and egg mass per generation with 675±16 and 221±20, respectively. In contrast, Cebu and Zamboanga recorded the lowest with 369±35 and 89±7, respectively. The number of male offspring being greater than those of females was consistent across all populations. It is not clear whether this affects fecundity in females. This is an area of laboratory research that needs to be further investigated.

The shortest range of developmental period (egg to adult) was observed in Laguna population with 18±0.71 days, and longest in Cebu population with 38.7±0.98 days. One of the factors that prolonged the developmental period was the placement of the egg mass inside the medical refrigerator which delayed the incubation for about a week instead of the usual three days. Indeed, FAW eggs hatched in 2 - 3 days in a recent study under insectary conditions of 27 ± 2°C, 75 ± 5% relative humidity, and 12:12 light: dark photoperiod (Marri *et al.* 2023). This notwithstanding, placing the egg masses in a refrigerator allowed the research team to anticipate newly hatched larvae in accordance with the protocols made during the pandemic since daily visits to the laboratory were prohibited. Further studies need to be conducted on methods of storing FAW egg mass and its effects on viability.

This study represents the first comprehensive study of the IPB ACB Artificial Diet as an effective rearing medium for FAW. The FAW collected from Luzon (Laguna, Cavite, Pangasinan, and Isabela), Visayas (Iloilo and Cebu), and Mindanao (Zamboanga) had survived for at least five generations and demonstrated the quality parameters for insect rearing. Therefore, the IPB ACB Artificial Diet is suitable for mass-rearing FAW under optimum laboratory conditions. Locally, this is the first attempt at rearing the fall armyworm using an artificial diet. In the future, the research team would like to perform a series of studies to determine the comparative performance of FAW on IPB ACB Artificial Diet and other rearing media. Laboratory experiments may include a comparison of the insect life history parameters using an artificial diet and maize leaves as treatments.

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