
Biology and mass rearing of *Mythimna separata* (Walker) (Lepidoptera: Noctuidae) using young leaves of Corn

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Abstract The results showed that, on average, the total development period from egg to adult of 28.95 ± 1.89 days for the male and 28.43 ± 1.55 days for the female. It passed through six larval instars over 16.07 ± 1.31 days for the male and 15.96 ± 0.5 days for the female larva. The durations in days for the different larval instars: 1st and 5th larval instars, 2.0 for both male and female, 2nd 1.43 ± 0.53 and 1.5 ± 0.58 , 3rd 1.86 ± 0.8 and 1.5 ± 0.8 , 4th 2.14 ± 0.35 and 2.5 ± 0.58 and 6th 3.71 ± 0.49 and 4.0 days for the male and female, respectively; pre-pupal period of 1.57 ± 0.5 days for the male and 1.55 ± 0.5 days for the female. The pupal period was 8.88 ± 1.04 days for the male and 8.45 ± 0.55 days for the female. Female moths laid up to 730 eggs, and adult longevity for males and females were 7.54 ± 2.10 and 8.09 ± 1.88 days, respectively. The native variety of corn, IPB var 6, supported the optimum growth and development of the larvae of *M. separata*. Nineteen generations were successfully reared in the laboratory.

Keywords: Biological study, Mass rearing technique, Paddy armyworm

Introduction

The paddy armyworm (PAW), *Mythimna separata* (Walker), is an invasive species of cereals, pasture, and forage crops (cabi.org/isc/about). It was first recorded infesting corn in the Philippines by Uichangco in 1959 (Cadapan and Sanchez, 1972), a staple food in the country. The paddy armyworm is also known as oriental armyworm, sorghum armyworm, Chinese armyworm, cosmopolitan armyworm, ear-eating caterpillar, rice armyworm, rice ear-cutting caterpillar, southern armyworm, paddy armyworm of Graminae, and paddy climbing armyworm. Nevertheless, the preferred common name is paddy armyworm. The preferred scientific name is *Mythimna separata* (Walker) 1865. Other scientific names are *Cirphis separata*, *C. unipuncta* Haworth, *Leucania consimilis* Moore, *L. separata* Walker, and

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Pseudaletia separata Walker. As a cosmopolitan species, *M. separata* is recorded in 21 countries in Asia, including the Philippines, 11 in Oceania, including Australia, and Russia in Europe (cabi.org/isc/datasheet/45093).

In the Philippines, severe outbreaks of armyworm have been reported by Mackie (1910), destroying rice in Batangas and neighboring provinces as caused by *Spodoptera mauritia* (Boisduval), but Cadapan and Sanchez (1972) attributed this to *P. separata* based on the former external morphological description of the pest. Subsequent reports of the armyworm outbreak in 1949 (Otanés and Sison 1949 and Merino, 1949 as cited by Cadapan and Sanchez, 1972) emphasized the seriousness of the armyworm as a pest. Cadapan and Sanchez (1972) reported that hectares of plants in the field, including corn, sugarcane, and pasture grass, were entirely consumed in a single day. They attributed the damage to several species, namely *S. mauritia*, *S. litura*, *C. unipuncta* (a synonym of *M. separata*), *C. loreyi*, *C. yu*, and *C. compta*. Filipino researchers (Merino, 1949 and Otanes and Sison, 1949) considered that destroying natural enemies and prolonged droughts may contribute to armyworm outbreaks. Severe damage has been reported in rice, wheat, sorghum, and millets in other parts of Asia and Australia (Sharma and Davies, 1983; Jiang *et al.*, 2011; Koyama and Matsumura, 2019). It is a major migratory pest of cereal crops in East Asia, South Asia, and Australia, resulting in significant losses (Li *et al.*, 2021). Prolonged summer droughts were causing outbreaks of *C. unipuncta* (also known as *M. separata*) in Canada (Cadapan and Sanchez, 1972). The powerful flight capability, high fecundity of female adults, and high voracity of older larval instars make outbreaks difficult to predict and prevent (Li *et al.*, 2021). Liu *et al.* (2017) identified host plants of adults of *M. separata* used during long-distance migration. Other researchers reported the effect of radiation on the development, longevity, and reproduction of *M. separata* (Ali *et al.*, 2016), responses of *M. separata* to three plant volatiles (Lihuang *et al.*, 2017), effect of egg age as host for *Trichogramma* species (Hou *et al.*, 2018), effect of feeding on defense response of the host (Malook *et al.*, 2019), cross-resistance studies to insecticides (Rasul *et al.*, 2021), application of CRISPR-Cas9 genome editing (Tang *et al.*, 2021), and host for entomopathogenic fungi (Mantzoukas *et al.*, 2022).

Information on the life history, host plants, relative abundance, and natural enemies of *M. separata* in the Philippines has been initiated by Cadapan and Sanchez (1972); and recorded six larval instars. Catindig *et al.* (1994) reported 31 plant species that supported complete larval development of *M. separata*. In rice and weeds associated, larval survival to pupation was highest on *Leptochloa chinensis* (58%), *Isachne globosa* (54%), *Paspalum paspalodes* (53%), and rice (51%). Larval development was shortest on rice (19.2 days),

followed by *L. chinensis* (21.7 days), and longest on *Imperata cylindrica* (34.8 days) and *Brachiaria distachya* (37.8 days). They reported five larval instars of *M. separata*.

The objective of the study was to determine the different life stages and mass rear the paddy armyworm for efficacy testing of entomopathogens.

Materials and methods

Stock culture of M. separata

The larvae of *M. separata* were collected from a corn field in Barangay Bocoohan, Lucena City, province of Quezon, Philippines. The larvae were reared on young leaves of corn. Eggs were held in plastic plates until hatching, and newly hatched larvae were reared in plastic pans. Pupae collected on the same day were placed in plastic plates/pan for holding and adult emergence. A male and female adult emerging on the same day were paired in a cylindrical Mylar cage for oviposition. The laboratory colony was maintained using this rearing procedure. Neonates for life history determination, fecundity, and longevity were obtained from this colony.

Life history

To determine the durations of the larval and pupal stages, 50 neonate larvae were randomly selected from one batch of egg clusters and individually reared until pupation in Petri dishes numbered consecutively. Individuals were fed with pieces of young corn leaves (IPBvar6), 10-25 DAS (days after sowing). The cultures were checked daily for larval molting, pupation, and adult emergence.

Larval mortality was recorded daily from the first day to the 6th instar until pupation. Larvae were considered dead if they could not move in a coordinated manner when prodded with a blunt probe.

Rearing conditions included a temperature of $27 \pm 1^{\circ}\text{C}$, 70% RH, and photoperiod of 12L:12D. Pupae were sexed and placed in separate containers. Adults were transferred to Mylar cages and provided wax paper for oviposition, and eggs were collected and counted daily. Pre-oviposition period and lifetime fecundity were determined following methods employed in previous studies.

Lifetime fecundity per female was calculated according to the number of eggs laid per paired female maintained in Mylar plastic cylinders. The experiment was replicated ten times.

Data analysis

The obtained data are presented as mean \pm SD. The differences between sexes were determined using a t-test.

Habits and behavior

Oviposition and hatching of eggs

Ten pairs were caged separately in Mylar cages for oviposition and hatchability of eggs. When oviposition was observed, the eggs were counted, and the pairs were transferred to a new oviposition cage (one pair per cage). This was repeated until the females stopped laying eggs and died. Egg hatchability was determined. The mortality of males was also noted.

Larval feeding and molting

Newly hatched larvae from clusters of eggs from the stock culture and those from the individual rearing set-up were observed closely for larval movement and feeding.

Pupation

The larvae from the life history study and those in the stock culture were reared continuously, and pupation behavior was observed.

Mass rearing

The improvised rearing units were commercially available plastic pans (30 cm long, 8.7 cm high, and 21.5 cm wide) with some modifications. Each pan was provided with an aeration window by cutting a portion, 22 cm x 14 cm area at the cover. The opening was covered with a bigger fine-mesh muslin held in place using glue. The bottom of the pan was lined with a paper towel fitted to the bottom, but plain paper may also be used. The paper towel served as the receptacle for the larvae excreta and also absorbed the excess moisture for easy disposal. The rearing pans were emptied, cleaned, and disinfected with 70% ethyl or isopropyl alcohol before re-use. The top of the rearing pan was covered with a kitchen towel before placing its cover to prevent the escape of small larvae.

Mass rearing procedure

Mating and oviposition

Day-old adult female and the male moth were confined per Mylar cage, with a cotton ball moistened with 10% sugar solution as food. When adult

females started laying eggs, usually 2 to 3 days after pairing, the pair with eggs were carefully transferred to a new Mylar cage with the same provision as before, after which the egg masses were clipped off from the wax paper and pooled in a plastic pan for incubation. All eggs laid on the same day were pooled to obtain homogenous test material. Eggs laid until the 3rd or 4th laying days were obtained for mass rearing, after which the adults were discarded properly.

Larval feeding and pupation

Five to ten seedlings of young corn (cut from the base) of 10-15 DAS were placed per pan as food for neonates of about 500- 1,000 individuals. After 24H, fresh corn stalks (5 stalks) were placed on top of the previous ones, allowing the larvae to transfer to the fresh food freely. After another 24H, a new set of fresh corn stalks were placed on top of the previous ones, and this was repeated after 24H, after which, the oldest left-overs were carefully removed. When the larvae were already on the 3rd instar stage, the culture was split into two; and after 3 to 4 more days, the culture (in a pan) was split again into half. Another splitting was done when necessary. When pre-pupae developed, these were segregated from the still-feeding larvae and pooled in another rearing pan for pupation. The process was repeated until all the feeding and surviving larvae stopped feeding. Pupae observed on the same day were collected, pooled in another container, and sexed to segregate the males from females.

Results

Life history and behavior

Egg

The eggs were laid between the leaf sheaths and the stem near the joint of the leaf sheath and the leaf blade, in folded/creases of wax paper cover; or near the rim of the Mylar oviposition cage. The eggs were spherical, measuring 0.46 mm in diameter with a finely ridged surface when freshly laid (Figure 1a, Table 1). They are pale yellow but become dark brown to black before hatching. The incubation ranged from 4 to 5 days but mostly 4 days (Table 2).

Larva

Newly hatched neonates were small, pale in color, with brown heads (Figure 1b). They aggregated and hid in protected sites of the leaves and chewed-up small portions underneath the leaf surface, leaving transparent

cellulosic areas. It measured on the average of 1.31 mm long x 0.19 mm in width (Table 1). The second instar (Figure 1c) had a pale brown head capsule after molting, turning brown after a few hours. It turned yellowish green after feeding, stayed in groups underneath the leaf surface, and fed in patches. An individual larva measured 3.06 mm long x 0.36 mm in width. The third instar had a pale head body when newly molted (Figure 1d) and fed in patches underneath the leaf surface. It measured 6.58 mm long x 0.84 mm in width. The fourth instar was transparent yellowish brown (Figure 1e), turning dark with distinct dorsal and lateral lines extending from the prothorax to the anal region. It was generally pale in color and measured 17.06 mm long x 2.19 mm in width. The larva voraciously fed from the leaf margin inward, leaving the midrib or consuming it all. The fifth instar was bigger than the 4th instar (Figure 1f), measuring 23.96 mm long x 2.91 mm width. It was a voracious feeder than the 4th instar. The sixth instar (Figure 1g) was the biggest and most voracious, measuring 41.40 mm long x 5.18 mm in width. The last two days of the 6th instar called pre-pupa are non-feeding (Figure 1h). Total larval periods for the male and female were 16.07 ± 1.31 days and 15.96 ± 0.50 days, respectively (Table 2).

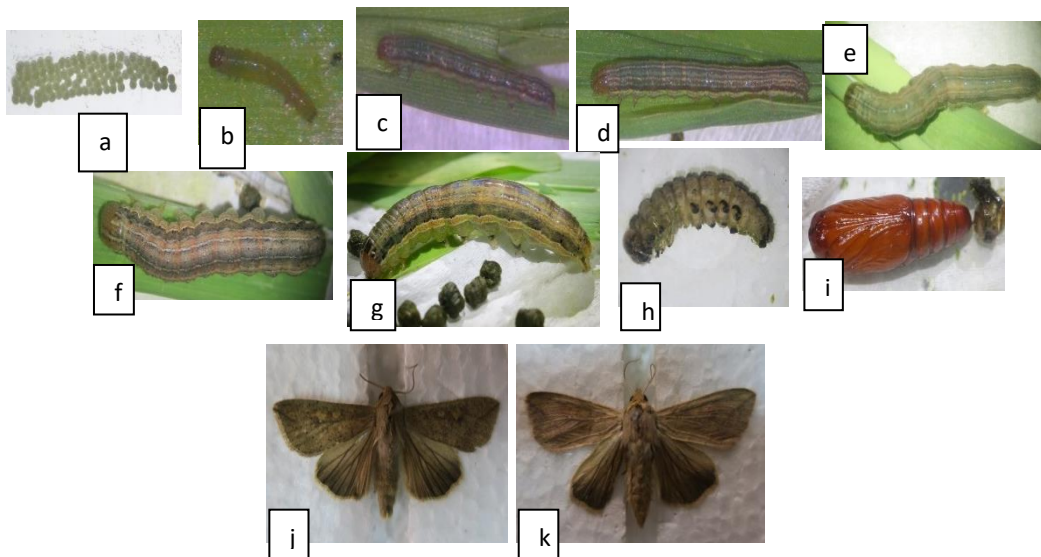


Figure 1. Life stages of *Mythimna separata* are as follow: a.) eggs, b.-g.) 1st to 6th instar, h.) pre-pupa, i.) pupa, j.) male adult, and k.) female adult

Pupa

The newly molted pupa was pale yellow turning brown to black (Figure 1i) before adult emergence. It pupated in the soil, field, and between leaves and tissue paper under laboratory conditions. The male pupa measured 16.78 mm long x 5.07 mm in width. The female pupa measured 16.48 mm long x 5.15 mm in width.

Adult

The adult male was darker in color, forewings are brownish gray with some markings (Figure 1j). The body measures 16.90 mm long, thorax 4.04 mm, and wing expanse 38.22 mm. It lived for 7.54 days. The female (Figure 1k) was lighter in color; her forewings yellowish gray with some markings. The body was 17.29 mm long; thorax 4.05 mm in width; wing expanse 41.95 mm. Eggs ranged from 100-730 per female with a mean of 375.63 eggs within the 1.63 ± 0.62 days oviposition period (Tables 3 and 4). The female lived for about 8.09 days.

Laboratory rearing of *M. separata*

Laboratory rearing set-up and the different life stages are kept separately in rearing pans (Figure 2). Larval survival was 94 percent, percent pupation of surviving larvae was 100, and adult emergence was 96 percent.

Table 1. Measurements (mm) of the eggs and larval instars of *Mythimna separata* (Walker) reared on corn under laboratory conditions

Stage	Length			Body			Head Capsule			Wing Expanse		
	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD
Egg (diameter)	0.36	±	0.01									
1st instar	1.31	±	0.17	0.19	±	0.03	0.24	±	0.02			
2nd instar	3.06	±	0.16	0.36	±	0.03	0.32	±	0.08			
3rd instar	6.58	±	0.29	0.84	±	0.10	0.63	±	0.03			
4th instar	17.06	±	1.03	2.19	±	0.32	1.56	±	0.09			
5th instar	23.96	±	1.44	2.91	±	0.33	2.25	±	0.11			
6th instar	41.40	±	2.05	5.18	±	0.23	3.34	±	0.10			
Pupa												
Male	16.78	±	1.04	5.07	±	0.44						
Female	16.48	±	0.99	5.15	±	0.25						
Adult												
Male	16.90	±	1.39	4.04	±	0.31				38.22	±	1.42
Female	17.29	±	0.61	4.05	±	0.42				41.95	±	3.55

Table 2. Durations (days) of the different developmental stages of male and female *Mythimna separata* (Walker) reared on corn under laboratory conditions

Development Stage	Male			Female		
	Mean	±	SD	Mean	±	SD
Egg	4 - 5			4 - 5		
Larva						
1st instar	2.00	±	0.00	2.00	±	0.00
2nd instar	1.43	±	0.53	1.50	±	0.58
3rd instar	1.86	±	0.90	1.50	±	0.58
4th instar	2.14	±	0.38	2.50	±	0.58
5th instar	2.00	±	0.00	2.00	±	0.00
6th instar	3.71	±	0.49	4.00	±	0.00
Pre-Pupa	1.57	±	0.50	1.55	±	0.50
Total Larval period	16.07	±	1.31	15.96	±	0.50
Pupa	8.88	±	1.04	8.45	±	0.55
Total Development Period (egg-adult)	28.95	±	1.89	28.43	±	1.55

Table 3. Post developmental periods (days) of female *Mythimna separata* (Walker) reared on corn under laboratory conditions

Post-developmental stage	Range	Mean	±	SD
Pre-oviposition	2 - 5	4.00	±	0.83
Oviposition	1 - 3	1.63	±	0.62
Post-oviposition	0 - 7	3.00	±	1.97

Table 4. Fecundity and survival (days) of *Mythimna separata* (Walker) reared individually on corn during the larval stage under laboratory conditions

Parameter	Range	Mean	±	SD
Fecundity				
Egg mass/ female	2 - 10	5.92	±	2.35
Eggs/ female	25 - 730	382.54	±	205.76
Eggs hatched/ female	102 - 730	405.50	±	205.01
Survival				
Male	5 - 12	7.54	±	2.10
Female	5 - 11	8.09	±	1.88

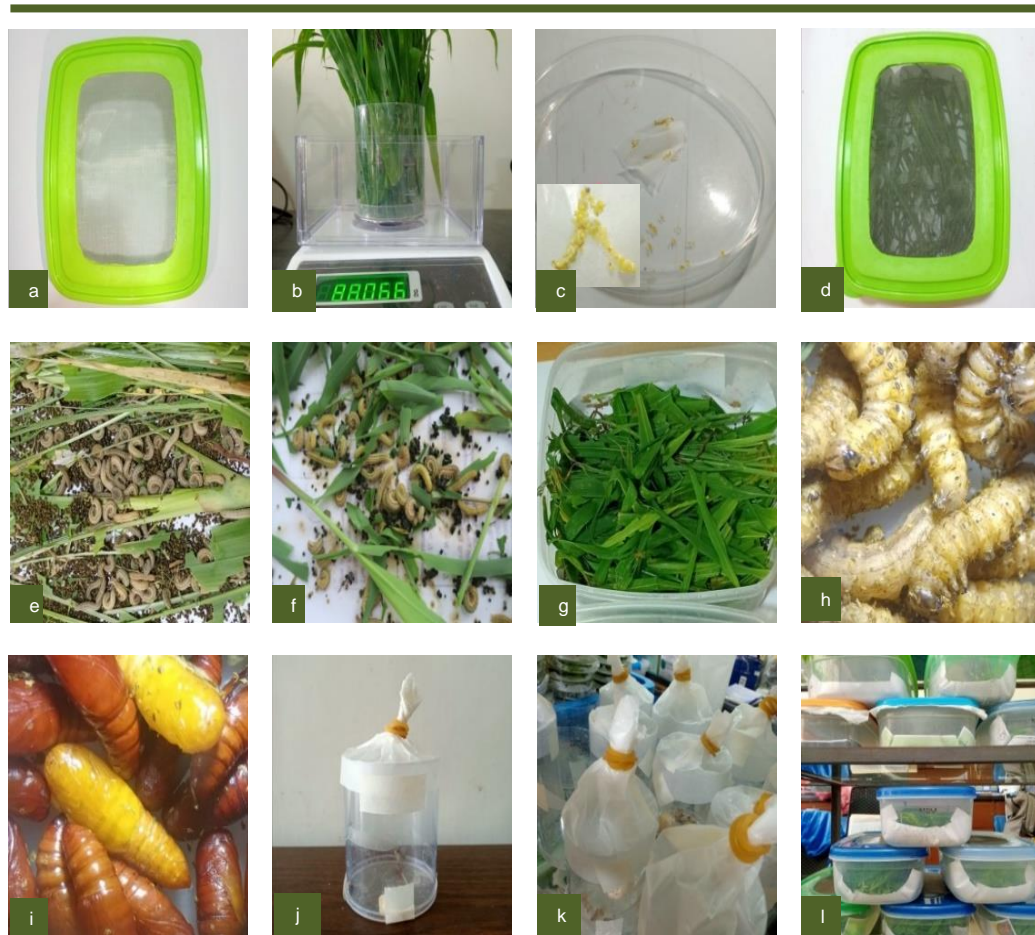


Figure 2. Set-up for mass rearing *Mythimna separata* (Walker) showing the different materials used: a) plastic pan, b) young corn leaves weighed, c) newly harvested eggs, d) incubating newly laid eggs, e) larvae before replenishing food, f) larvae transferred to new rearing pan containing fresh food, g) larvae after replenishing food, h) pooled pre-pupae from mass rearing pan, i) pupae from pooled pre-pupae, j) oviposition cage with a pair of male and female adults, k) set of oviposition cages, and l) shelf for stacking rearing pans

Discussion

In the present study, the freshly laid eggs of *M. separata* were pale yellow and changed coloration to dark brown to black prior to hatching (Cadapan and Sanchez (1972), Catindig *et al.* (1994)) reported that the newly laid eggs of *M. separata* are smooth, shiny, and milky-white or yellowish white, turning

brownish or yellowish black to dark brown before hatching. These observations were similar to the results observed in the current study.

The incubation period of *M. separata* eggs varied from 4 to 5 days in the present study. The exact duration was also reported by Tara and Hussain (2019). Cadapan and Sanchez (1972) and Catindig *et al.* (1994) reported an incubation period of 2 to 3 days and 3 days, respectively, a day earlier than what was observed in the study.

The larvae of *M. separata* passed through six different instars when reared on fresh young leaves of corn (IPB var 6) until pupation. This conformed to Cadapan and Sanchez (1972), who reported six instars of *M. separata* on an undetermined variety of corn, and Tara and Hussain (2019), but not to Catindig *et al.* (1994), who reported five larval instars.

The total larval period for the male was 16.07 ± 1.31 days, and for the female, 15.96 days were within the range reported by Cadapan and Sanchez (1972), 14-25 days for the female, and 15-25 days for the male. Catindig *et al.* (1994) reported five larval stages, on average, of 27.2 days on rice. Tara and Hussain (2019) reported an average of 26.24 ± 1.99 days larval period of *M. separata* when fed with barley.

Sharma *et al.* (2002) reported a longer developmental period for *M. separata* reared on fresh leaves of pearl millet with seven larval instars; larval development periods for instars I-VII were 3.9, 3.3, 3.1, 2.7, 2.2, 1.8 days, respectively. The pre-pupal and pupal periods lasted 1 to 2 and 8 to 12 days, respectively. The entire developmental period lasted for 29 to 39 days. The observed pre-pupal stage of 1.57 ± 0.05 days for the male and 1.55 ± 0.5 days for the female in the study were shorter than those reported by Tara and Hussain (2019) of 2.48 ± 0.35 days in barley and 2 days in rice by Catindig *et al.* (1994).

Mythimna separata as a polyphagous pest, has been reported to feed on 33 plant species and some unspecified grasses belonging to eight families (Sharma and Davis, 1983). Catindig *et al.* (1994) reported that there are 31 plant species that support the development of *M. separata* but most species of Poaceae (= Graminae). It causes severe damage to sorghum and pearl millet in South Central India and population increase rapidly due to high fecundity of females (nearly 996 eggs per female) (Sharma *et al.*, 2002).

The male and female sex identification of *M. separata* was based on the genital opening (slit) which was widely separated from the anal opening in the female pupa, but close to each other in the male, seen at the ventral side of the abdomen. Lin *et al.* (2020), likewise observed the same morphological differences in genital openings of *M. separata* male and female pupae. They added the presence of a single bristle-frenulum in the male adult but a three-

bristled frenulum in the female as an additional morphological character to differentiate sex at the adult stage.

Mythimna separata have been successfully mass-reared on leaves of young corn (IPB var 6) under laboratory conditions. Pieces of wax paper with eggs were placed in plastic pans lined with tissue paper for incubation. Fresh, young leaves of corn provided a day before hatching allowed newly hatched larvae to start feeding and develop normally. For large production of PAW aimed at producing sufficient number of homogenous test material over a period of time and to enable an optimum and sustained mass rearing keeping each day's harvest of eggs separately allowed eggs to hatch within 1 to 2 days of each other in each pan.

Neonates were maintained in the pan in a mass of about 1,000 individuals, since group feeding promotes growth and development. Larvae of similar developmental stages were kept together so that extraction of the various life stages for bioefficacy testing of entomopathogens become easier.

To ensure the proper growth and development of larvae, cleanliness was always observed. This was done by lining the bottom of the rearing pan with a kitchen napkin to absorb excess moisture and as a receptacle of feces or frass, which was discarded every time food was replaced with fresh ones. The rearing pans were cleaned and disinfected with 70% ethyl or isopropyl alcohol before reusing. Likewise, to maintain the freshness of corn leaves, these were cut from the base early in the morning, washed under running water, and air-dried. Research showed that excessive moisture promotes the development of microorganisms affecting the quality of leaves and encouraging the growth of lethal pathogens.

The larvae were tiny during the early instar stages, and care was employed in feeding. Fresh corn leaves were added daily until the third instar stage, allowing the larvae to transfer freely onto the fresh food. On the fourth day, when the initial food dried up, the culture was split into half and was fed daily, increasing the number of corn leaves. The larvae in the fourth to the sixth instar fed voraciously, hence, the culture was split again. These three instars were the longest and they should be fed well to produce bigger and healthy pupae and adults for longer reproductive periods and higher fecundity.

The pre-pupae were collected from the rearing pans and placed in another pan where they were pooled and allowed to pupate. The pupae were collected from these pans, and those harvested on the same day were segregated from the previous collection to ensure the uniform emergence of adults. Pupae were sexed and marked by sex and date.

Usually, females emerged ahead of the males, fed with 10% sugar solution dispensed in cotton balls. When males emerged the following day,

these were paired in a 1:1 ratio, 10 pairs in 22.5 cm x 14 cm cylindrical Mylar plastic cages. Two or three days after pairing, females laid eggs on the creases of wax paper cover and sometimes on the edges of Mylar cages. The oviposition period lasted for four days, but only eggs laid during the first three days were collected for incubation and rearing of hatched larvae. The adults were discarded properly on the fourth day.

The colony was started in October 2020 and is now at F18 (July 2022), with no apparent decline in vigor and reproduction. Attempts to mass rear the larvae of *M. separata* on leaves of *Brassica rapa*, *Ipomea batatas*, *Allium fistulosum*, *Trianthema portulacastrum* and sliced fruits of *Solanum melongena*, all known plants of the pest were unsuccessful due to low survival rates and prolonged developmental periods. Catindig *et al.* (1994) reported that of the 31 plants species that supported complete larval development of *M. separata*, larval survival to pupation was highest on *L. chinensis* (58%), *I. globosa* (54%), *P. paspalodes* (53%), rice (51%), and lowest in soybeans and mungbean (1%). Artificial diets reported for *M. separata* (Hatori and Atsusawa, 1980; Jia *et al.*, 2019) were not tested.

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