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Indian J. Ent., 30 (4) : 272-280

## MASS BREEDING OF *HELIOTHIS ARMIGERA* (HBN.)\*

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Extensive and intensive use of wide variety of insecticides for control of pests have offered an array of problems concerning toxicity hazards, residues, resistance, potentiation etc. Foreseeing such problems, Knipling (1960) suggested the possibility of utilising the autocidal principle for insect control in which mass breeding of host insects is prerequisite.

*Heliothis armigera* (Hbn.) commonly known as gram pod borer is a polyphagous insect causing serious damage to many important crops. It is recorded from almost all the countries of the Old World between 40° north latitude and 40° south latitude (Hardwick 1965). Since it attacks pods or fruits of crops in a number of cases, its control by insecticides offers many toxicity and residue problems. For this reason, a programme for the mass breeding of this insect and its parasites was initiated under a P. L. 480 grant. The results reported here comprise a part of this programme.

No work has been reported to mass rear *H. armigera* in the laboratory. Barber (1936) reared corn earworm (*H. obsoleta*) in the laboratory on milk-stage corn ears but found that the cannibalistic tendency demanded their separation after third instar. Callahan (1962) reared *H. zea* on corn slices in galvanised tin trays having 72 cubicles. Vanderzant *et al.* (1962) aseptically reared four generations of *H. zea* on artificial diet prepared from cold-rolled wheat germ, vitamin free casein with inositol, choline chloride, ascorbic acid, agar and water. However, when reared on purified diet, *H. zea* did not survive beyond the larval stage when their food contained no ascorbic acid (Vanderzant *et al.* 1962—a).

Burton (1964) modified Vanderzant's bollworm diet by adding alphacel, potassium hydroxide, formaldehyde and inhibitor stock solution and reared *H. zea* larvae in vials. The diet prepared in a pressure cooker was directly dispensed into vials. Two freshly hatched larvae were transferred in each vial and then closed by a sterilised cotton plug. The larvae were allowed to pupate in the vial.

### MATERIAL AND METHODS

To start the *Heliothis* culture, a large number of larvae were collected from lucerne, tomato, gram, pigeon pea etc. beginning from June 1963. These larvae were reared individually in 3" x 1" specimen tubes either on leaves or pods of the prevailing host. The larvae were transferred to new vials containing fresh food every day and those about to pupate were transferred into galvanised iron sheet trays measuring 12" x 8" and 6" high containing 4" deep moist soil. The trays were covered with suitable lid and kept away for the larvae to pupate. The trays were examined after 5 or 6 days to collect the pupae. The pupae were then buried about 1" deep in moist soil contained in a glass jar. The mouth of the jar was covered

\*This research has been financed in part by a grant made by the United States Department of Agriculture, Agricultural Research Service, under P. L. 480.

by a piece of muslin cloth bearing 6 to 8 strips of cloth draped into the jar for the moths to rest. The sex of the adult could be readily determined by the colour of the adults (Ramiah 1966).

The adults were confined in a mating cage having wet urethane foam on the bottom. About 10 to 15 cotton-wool plugs dipped in 5 to 10 per cent honey solution were suspended with the help of pins from the sides of the cage or kept in the polythene caps of the rearing tubes and placed on the floor of the cage. Maximum percentage of mating was obtained if 15 males were caged with 5 females and removed on the third day before they start to lay the eggs (Ramiah 1966). These females were held individually in blotting paper-lined plastic containers measuring 4" in diameter and 2" in height. The top of the container was covered by a piece of fine nylon cloth held in place by a rubber band. A cotton-wool plug soaked with 5 to 10 per cent honey solution was kept on the top cloth and covered by plastic cap of the specimen tube to prevent drying. Most of the eggs were laid on the blotting paper which were utilised for further laboratory work.

In the beginning, newly hatched larvae were reared until about third instar on a bunch of tender branches of tomato plants. The cut ends of the branches were wrapped in cotton-wool or muslin cloth, soaked in water and kept in one gallon capacity glass jar. This method was not found convenient since it was difficult to transfer these young larvae on fresh food every 2 or 3 days and caused high mortality among the larvae in the early instars. For this reason, rearing of the early instar larvae was done on pot-grown plants. Earthen pots measuring 10" in diameter and 9" in height were filled with soil and 50 to 60 gram seeds were sown in about 7" diameter area of the pot surface. When the plants grew about 4 to 6 inches in height, newly hatched one hundred larvae were transferred on the plants. The plants were then covered by 8 inches diameter circular cage made of iron bars and covered all around and on the top by fine nylon cloth. Such pots were kept in galvanised iron sheet trays containing about 1" deep water so that the soil in the pot remained wet enough to support the growth of the plants and at the same time ants, spiders and other predators could be prevented from going in the cage (Fig. A). The larvae were allowed to develop until third instar on these plants. Since the larvae develop cannibalistic tendency in later instars, they were reared separately in 3" x 1" specimen tubes on any of the natural host plants. However, changing tubes and food everyday was laborious, time consuming and favoured rapid dissemination of nuclear polyhedrosis. For these reasons, it was quite necessary to develop an artificial diet for rearing the larvae. Since the insect breeds on lucerne in nature, it was felt that use of lucerne leaf powder may suit the larvae better than the wheat germ used by Burton for *H. zea*. Drawing from Burton's formula, the following constituents were used to prepare the larval diet.

Powdered lucerne	100 g.
Agar	3 g.
Inhibitor stock solution*	5 c.c.
Acetic acid	5 c.c.
Formaldehyde	4 drops
Multivitamin syrup	0.5 c.c.
Water	300 c.c.

\*Inhibitor Stock Solution—4.0 g. sorbic acid, 3.0 g. methyl parahydroxybenzoate dissolved in 34 ml. ethyl alcohol.

To prepare lucerne leaf power, green lucerne fodder was obtained during the best period of the crop and allowed to dry in open air under shade. When the material was dry, it was shaken vigorously to separate the leaves and tender parts of the plants. This was then ground in a mill and sieved through 20 mesh screen. The lucerne leaf power thus prepared was green and uniform in texture. It was filled in plastic lined gunny bags and stored for use subsequently.

*Preparation of the diet* : Agar was dissolved by boiling it in approximately half of the water. It was then thoroughly mixed in the lucerne powder. Inhibitor stock solution was added in one-fourth quantity of the water and thoroughly mixed in the diet mixture. This mixture was cooked for half an hour at 15 pounds per square inch in a pressure cooker, taken out and allowed to cool. Measured quantities of formaldehyde, acetic acid and vitamin syrup were added in the remaining one-fourth quantity of water and mixed thoroughly in the cooled diet.

The diet thus prepared was filled to about one-half inch height in 3" × 1" transparent plastic specimen tubes and a third instar larva was introduced in each tube. The tubes were closed by plastic screw caps, arranged in trays and held on racks at  $80 \pm 2^\circ\text{F}$  (Fig. B). The larvae fed and developed on this diet quite satisfactorily. The tubes were examined everyday without opening the cap and those containing the larvae that died of polyhedrosis or any other reasons were taken to a distant cleaning place, removed and thoroughly cleaned to avoid the contamination. Slight carelessness and negligence in the rearing procedure resulted in heavy mortality of the larvae due to nuclear polyhedrosis. Proper care of the culture and sanitation has to be maintained to keep the disease under control (Patel *et al.* 1968). After completing the larval development, the larva pupated in a cell made in the remaining of the diet at the bottom of the tube or in a cell made from pallets of excrement. When the larvae transformed into pupae, the tubes were emptied to collect them. After this rearing procedure was established in the laboratory and nuclear polyhedrosis was checked very satisfactorily, different combinations of acids in the diet were tried to determine the best combination for rearing *H. armigera*. Finally, the larvae were reared right from the first instar until pupation on this diet. The duration of larval and pupal stages as well as the egg laying capability of moths reared on artificial diet were studied in the laboratory maintained at  $80 \pm 2^\circ\text{F}$ .

#### RESULTS AND DISCUSSION

Since the larvae had to be reared on pot-grown plants until their third instar, it was necessary to select a host plant which could be grown in pots relatively easily and which is preferred by the larvae. Lucerne and Bengal gram are two important hosts of which one had to be selected. Table 1 gives the comparative development of *Heliothis* larvae from first instar to third instar on pot-grown hosts.

The results show that the development of larvae on gram was much better than that on lucerne as indicated by 68.94 per cent recovery of third instar larvae as against 49.16 per cent recovery on lucerne. Further, lucerne is difficult to grow in pots, takes more time to develop and was quite susceptible to aphids and thrips. On the other hand, gram could be grown in pots very easily and offered no problem in rearing of the larvae.

Rearing of first instar larvae on pot-grown gram plants had to be continued since a very large number of first instar larvae died when they were reared directly

on artificial diet. Over 25,000 larvae reared on pot-grown gram plants from June 1966 through May, 1967, yielded 16356 third instar larvae. This worked out to 64.5 per cent recovery of third instar larvae.

TABLE I

Comparison of development of *Heliothis* larvae upto third instar on pot-grown lucerne and gram plants

Host	Period	No. of sets	I instar larvae placed	III instar larvae recovered	Per cent Recovery (total basis)	Per cent recovery	
						(Set-wise)	
						Min	Max.
Gram	May 65	29	3660	2429	66.4	50.0	93.0
	June 65	33	4019	2865	71.3	27.5	96.6
	Total	62	7679	5294	68.94		
Lucerne	May 65	5	500	244	48.8	32.0	80.0
	June 65	14	1160	572	49.3	28.0	82.6
	Total	19	1660	816	49.16		

**Rearing Third Instar Larvae on Artificial Diet:** The *Heliothis* larvae after third instar were reared individually on artificial diet prepared out of lucerne powder. 42 sets each having 16 to 100 larvae were reared during July and August 1966. The average percentage of healthy pupae obtained from these sets was 77.59. The remaining 22.41 per cent included 12.75 per cent dead pupae, 2.46 per cent deformed pupae, 1.43 per cent cracked pupae, 0.46 per cent dead prepupae and 5.32 per cent dead larvae. The cracked pupae had improper sclerotisation between the margin of the wing-pad and the fourth abdominal segment on the ventral side. This crack like area remained very delicate and pale yellow and slight injury in this part caused bleeding. If this area was extensive, the moths failed to emerge. The average weight per pupa was found to be 0.240 g. These results indicated high mortality among the pupae and the pupae also appeared to be smaller in size. To improve the percentage of pupation and the growth of the larvae, ascorbic acid was added in the diet. Several sets of larvae were reared to compare the effect of the diet with and without 0.25 per cent ascorbic acid. The results of these sets are summarised in table 2.

The results in table 2 indicate appreciable improvement in the suitability of the diet when ascorbic acid was added. The percentage of healthy pupae obtained from the larvae reared on diet with ascorbic acid averaged 85.91 as against 74.69 obtained from larvae reared on diet without ascorbic acid. The mortality in the pupal stages dropped by 9.43 per cent when the larvae were reared on diet with ascorbic acid. The average weight per pupa also increased appreciably when the larvae were reared on diet containing ascorbic acid. The mortalities in the larval and prepupal stages were also low in case of larvae reared on diet with ascorbic acid.

The artificial diet with ascorbic acid was therefore used to rear *Heliothis* larvae from August 1966 onwards and 22 generations were bred in the laboratory until October 1967. During the course of study, it was found that varying proportions of ascorbic acid, hydrochloric acid and acetic acid in the diet showed marked effect on the development of the larvae. Studies were therefore made to determine the best combination of these acids. The following variations were made in the general formula (without vitamin) of the diet as far as acetic acid is concerned.

TABLE 2  
Showing the effect of ascorbic acid in the diet of *Heliothis* larvae

Sr. No.	Observations	Diet	
		Without Ascorbic acid	With Ascorbic acid
1.	No. of sets studied	33	42
2.	Total No. of larvae reared	1719	1724
3.	Total No. of healthy pupae obtained	1284	1481
4.	Per cent of healthy pupae	74.69	85.91
5.	Per cent of cracked pupae	2.21	2.84
6.	Per cent of deformed pupae	3.03	3.07
7.	Per cent mortalities in—(a) larvae	3.90	2.26
	(b) prepupae	1.86	1.04
	(c) pupae	14.31	4.88
8.	Average weight per pupa (in g.)	0.269	0.303

Diet Type	Details of combination of acids
A	1.25 % Acetic acid (general formula)
B	1.5 % Acetic + 0.25 % Ascorbic acid
C	2.0 % Acetic + 0.25 % Ascorbic
D	1.0 % HCl + 0.25 % Ascorbic + 0.5 % Acetic
E	2.0 % HCl + 0.5 % Ascorbic
F	0.75 % Ascorbic + 2.5 % Vitamin suspension*
G	1.0 % HCl + 0.25 % Ascorbic
H	1.0 % Acetic + 0.25 % Ascorbic
I	2.0 % HCl + 0.25 % Ascorbic
J	0.5 % Acetic + 0.5 % Ascorbic + 2.5 % Vitamin
K	1.5 % HCl + 0.25 % Ascorbic
L	1.25 % HCl + 0.5 % Ascorbic
M	1.0 % HCl + 0.5 % Ascorbic
N	1.0 % HCl + 0.25 % Acetic + 0.25 % Ascorbic

\*Dissolve 12 mg. Biotin in 100 C. C. water and add Nicotinic acid (Niacin) 600 mg; Calcium Pantothenate 600 mg; Riboflavin 300 mg; Thiamine Hydrochloride 150 mg; Folic acid 150 mg; Pyridoxine Hydrochloride 150 mg. and Vitamin B-12 2 mg.

Several sets of larvae were reared on these diets and the results obtained are summarised in table 3 below :

TABLE 3  
Effect of different diets on the development of *H. armigera* larvae (Third instar until pupation) as judged from percentage of pupation and weight of pupa.

Diet Type	No. of sets studied	No. of Larvae		No. of Pupae			% Healthy pupae	Wt. per pupa in g.
		Reared	Dead	Healthy	Dead	Deformed and cracked		
A	75	3468	200	2641	469	158	76.153	0.2494
B	9	519	31	272	142	74	52.408	0.2592
C	4	243	18	138	71	16	56.790	0.2318
D	11	627	31	468	48	80	74.641	0.2834
E	17	954	50	737	102	65	77.253	0.2684
F	18	961	73	758	102	28	78.876	0.2630
G	118	6153	221	5063	498	371	82.285	0.2942
H	51	2629	93	2187	167	182	83.187	0.3009
I	36	2045	42	1709	185	109	83.569	0.2318
J	100	5262	203	4404	364	291	83.694	0.2734
K	7	411	13	348	34	16	84.671	0.2618
L	5	302	—	257	28	17	85.099	0.2694
M	13	733	12	637	33	51	86.903	0.3158
N	30	1803	40	1596	47	120	88.519	0.3176

C. D. at 5 % = 0.0515 g.

C.V. % = 18.98 % for weight per pupa.

The analysis of the results of rearing the larvae on different diet combinations has revealed significant differences in the weight of pupae reared on these diets. The heaviest pupae were obtained when the larvae were reared on a diet containing 1 per cent HCl, 0.25 per cent acetic acid and 0.25 per cent ascorbic acid. The percentage of healthy pupae obtained from the larvae reared on this diet was 88.52 which was also the highest among all the pupae developing on other combinations. Thus, this being the best diet among all the combinations tried, *H. armigera* larvae were reared on this diet for the purpose of mass breeding. This procedure to rear the larvae after hatching till pupation in two phases yield 64.5 per cent third instar larvae on pot-grown plants and 86.52 per cent of these third instar larvae developed into healthy pupae on artificial diet. Thus, on the whole, 57.1 per cent of the first instar larvae completed their development into healthy pupae when they are reared in these two phases.

**Rearing First Instar Larvae on Artificial Diet :** The method of rearing the *Heliothis* larvae in two phases was not convenient and since polyhedrosis could be checked very satisfactorily, it was possible to rear the larvae on the artificial diet

larva from the first instar until pupation. For this purpose, a first instar larva was placed on a leaflet of gram plant with the help of moistened camel hair brush and the leaflet was then placed in the tube containing artificial diet. The larva readily settled on the leaflet and later began to feed on the diet. The results of 9 sets of experiments in this way are summarised in table 4.

TABLE 4

Duration of larval stages of *B. thausi* larvae from first instar until pupation when reared on artificial diet containing 1 per cent HCl, 0.25 per cent ascorbic acid and 0.25 per cent acetic acid.

No. of larvae reared	No. of larvae		No. of pupae obtained			% healthy pupae	Wt. per pupa in g.
	Survived	Dead	Healthy	Dead	Cracked & deformed		
9	608	161	439	5	3	72.2	0.309

The results in the above table show very satisfactory results yielding 72.2 per cent healthy pupae which are much better than that obtained when the larvae are reared in two separate phases. During these trials, it was observed that about 5.3 per cent of the larvae remained very small for several days. These larvae readily resumed growth when they were fed with host leaves.

Studies were also made to find out the effect of this diet on the larval and pupal durations as well as on the emergence of moths. The larvae which failed to grow on artificial diet were not included in this study. The results are summarised in table 5.

TABLE 5

Duration of larval and pupal stages and emergence of moths when reared on artificial diet containing 1 per cent HCl, 0.25 per cent ascorbic acid and 0.25 per cent acetic acid.

No. of larvae observed	Larval duration in days			No. of pupae observed	Pupal period in days			% emergence
	Min	Max	Ave		Min	Max	Ave	
374	16	27	20.71	163	10	19	12.24	89.57

The results in the above table indicate that the duration of larval stage has varied from 15 to 27 days with an average of 20.71 days. The average pupal period was 12.24 days. 89.57 per cent of the pupae developed into healthy moths. Thus, the results of rearing the larvae on artificial diet right from first instar until pupation are quite encouraging.

**Handling of Pupae:** The pupae collected from rearing tubes were transferred to a 1' x 1' x 1' wooden cage containing 1.5" deep moist soil on the bottom and were covered over by about 0.5" deep moist soil. Three sides of the cage were fitted with plastic sheets and the fourth with a piece of muslin cloth having a sleeve

The results in the above table show very satisfactory results yielding 72.2 per cent healthy pupae which are much better than that obtained when the larvae

breeding of *Heliothis*. A mass of plastic-wool was kept in the cage for the emerging moths to rest and stretch their wings.

**Egg Laying Capability of Moths:** To determine the egg-laying capacity of moths obtained from larvae reared on artificial diet, mated females were held in plastic container having blotting paper on the bottom as well as along the wall. The moths laid eggs on the blotting paper as well as on the plastic which cover. Cotton-wool swab dipped in 5 per cent honey solution was provided on the cover to feed the moths. The results are given in the following table.

**TABLE 6**  
Egg laying capability of moths obtained from larvae reared on artificial diet containing 1 per cent HCl, 0.25 per cent acetic acid and 0.25 per cent ascorbic acid.

Serial No.	Emerged on	Pre-oviposition period in days	Oviposition
1	Dec. 5, 1967	5	2
2	Dec. 5, 1967	5	2
3	Dec. 5, 1967	5	2
4	Dec. 5, 1967	5	2
5	Dec. 15, 1967	5	2
6	Dec. 15, 1967	5	2
7	Dec. 15, 1967	5	2
8	Dec. 9, 1967	5	2
9	Dec. 9, 1967	5	2
10	Dec. 9, 1967	5	2
11	Dec. 11, 1967	5	2
12	Dec. 11, 1967	5	2
<b>Total</b>		<b>51</b>	<b>32</b>
<b>Average</b>		<b>4.25±0.91</b>	<b>2.66±0.21</b>

The results in table 6 show that the egg laying capability has varied from 510 to 1676 with an average of 1442.3 ± 360.6 eggs per female. The maximum egg laying capacity of the moths obtained from the larvae reared on their natural food plant was found to be only 578 eggs per female during the early part of work. The low fecundity may be due to some faults in rearing techniques.

**SUMMARY**

A technique to mass breed *Heliothis armigera* in the laboratory has been developed. To obtain the eggs, 15 males and 5 females were held in 1' x 1' x 1' wooden mating cage. On the third day, the females were taken out and confined in a blotting paper lined plastic container measuring 4" in diameter and 2" in height for oviposition. Newly hatched larvae were reared upto third instar on *Sorghum* gram plants. The plants were covered by a fine nylon cloth cage supported on a circular frame prepared out of iron bar. The larvae developed on these plants

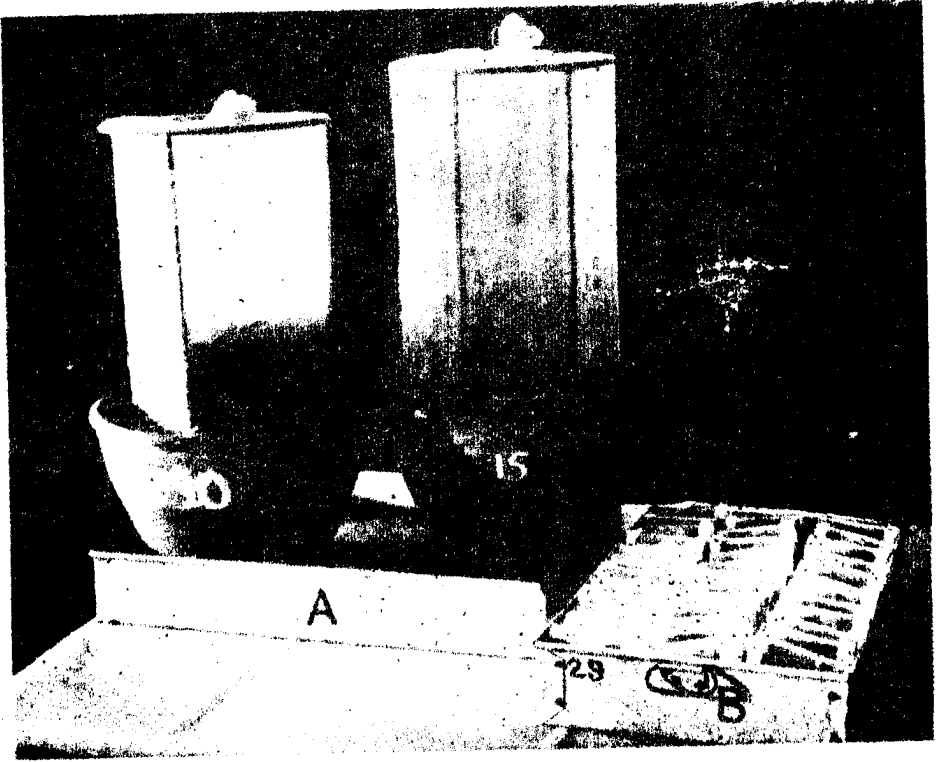


Fig. A- Pot-grown plants covered with nylon-lined cage to rear *Heliothis* larvae until third instar. B- Tray containing plastic specimen tubes to rear *Heliothis* larvae from third instar until pupation.

until third instar and then were reared individually on artificial diet in 3" x 1" plastic specimen tubes. The basic formula used to prepare the diet included lucerne powder, agar, inhibitor stock solution, formaldehyde, multivitamin syrup and water. Later the addition of ascorbic acid, hydrochloric acid and acetic acid in the diet improved both the development of the larvae as well as percentage of pupation. A diet containing 1 per cent HCl, 0.25 per cent ascorbic acid and 0.25 per cent acetic acid yielded 88.52 per cent well developed healthy pupae. When the larvae were reared in two phases viz. early instars on pot-grown gram plants and later instars on artificial diet, only 57.1 per cent larvae could complete the development. However, when polyhedrosis was satisfactorily controlled and the diet was improved, larvae could be reared directly on the artificial diet, so as to yield 72.2 per cent pupae. When reared on this diet at  $80 \pm 2^\circ$  F., the average larval and pupal periods were 20.71 and 12.24 days respectively. The average egg laying capacity of the moths was  $112.3 \pm 360.6$  eggs per female.

#### ACKNOWLEDGEMENTS

The authors are grateful to Dr. M. D. Patel, Director, Institute of Agriculture, Anand; Dr. A. M. Patel, Principal, and Dr. H. K. Patel, Head of the Department of Entomology, A. A. College of Agriculture, Anand for providing the necessary facilities. They are also indebted to Drs. P. B. Dowden, G. W. Angalet, C. F. Richardson and H. L. Burton of the Entomology Research Division, U.S.D.A. for their interest and technical assistance in the work.

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(Accepted: 11-11-1968)