

MASS REARING AND ARTIFICIAL INFESTATION METHODS FOR *ELDANA SACCHARINA* WALKER (Lepidoptera : Pyralididae)

By. P. R. ATKINSON

South African Sugar Association Experiment Station, Mount Edgecombe

Abstract

A laboratory culture of *Eldana saccharina* Walker is maintained for several purposes. A casein and chick-pea culture mixture is used, contained in 1 kg sweet or preserve jars with screw tops and stainless steel gauze lids. Antimicrobial agents in the mixture are deleterious to development and survival but are nevertheless essential for routine culture methods in which the jars cannot be checked daily for contamination. They can, however, be omitted singly from the mixture if necessary. Pupae are kept on sand moistened with dilute formaldehyde or benomyl solution. Adults breed in large boxes lined with sheet plastic and eggs are laid in paper towelling. The system provides an adequate supply of all stages, and requires a minimum of labour. A method is outlined for artificially infesting cane, both in the glasshouse and in the open, which ensures uniform distribution and infestation rates for experiments such as insecticide trials.

Introduction

All stages of the cane borer *Eldana saccharina* Walk. are required for various purposes such as laboratory studies on predation, rearing imported parasites, adult trapping and pheromone research, cohort planting and artificially infested insecticide trials. A mass rearing method capable of meeting these demands is required involving a minimum of labour. Although cost is important it is secondary to these requirements. The standard method, which is subject to continued improvement, is outlined here, together with some of the techniques which have been tried and discarded.

Natural infestation of cane in the field is usually patchy, and artificially infested cane offers the advantage of uniform distribution and infestation rates, for example for insecticide screening. A method which has been evolved for artificial infestation both in the insectary and in the open, is described.

Culture Methods

Media, for culturing lepidopterous larvae tend to be rather similar, consisting of sources of protein and carbohydrates, plus gels and preservatives^{1,3,4,6,7,11}. Two simple media were tried in the present case (see Table 1).

Mixture (a) had to be discarded because the bean supplies tended to contain a spore-forming soil bacterium resistant to all the antibiotics tried. The more expensive mixture (b) has proved satisfactory and early problems with putrefaction were overcome by including chloromycetin (chloramphenicol), which being an ingredient of livestock feeds, is cheap. Several authors have drawn attention to the effects of antimicrobials on development and survival rates^{8,9,10}. For this reason, and because ingredients are sometimes unavailable, the effect of omitting these preservatives, singly and in pairs was assessed. (See Table 2).

In general, if only one preservative is omitted, the rate of deterioration is compensated for by the increased rate of development, and with care pupae can still be obtained from such mixtures. Ascorbic acid has a surprisingly deleterious effect upon development and survival. Its inclusion in culture mixtures is presumably as a reducing agent, preventing oxidation and rancidity, rather than as a nutrient, and for this insect at

TABLE 1

Culture mixtures used for *Eldana saccharina* Walk. (a) from Dr. S. Kamurov, S.A. Citrus Exchange, Nelspruit, S. Africa (pers. comm.) (b) from J. Appert, Laboratoire Centrale d'Entomologie d'I.R.A.T., Centre Gerdat, Montpellier, France.

		(a)	(b)
Proteins and Carbohydrates	White broad beans (soaked)	330g	
	Chick pea flour		120g
	Glucose		20g
	Casein		12g
Vitamins	Brewers Yeast	50g	12g
Preservatives	Methyl p. hydroxybenzoate	8g	1,6g
	Ascorbic acid	5g	4g
	Sorbic acid		2g
	Chloromycetin (Chloramphenicol)	1,4g	0,7g
Gels	Agar	19g	20g
	Fibrous cellulose		28g
Water		11	11

least, it can with advantage be omitted. The levels of methyl paraben and of cloromycetin given in Table 1 are, for mixture (a) well above, and for mixture (b) slightly above, the "safe" levels recommended by Singh & House¹⁰, and could perhaps be reduced. It seems inadvisable to omit any of the other preservatives, in spite of their deleterious effects, unless the culture can be checked daily, which in practice is rarely the case. Vitamin mixtures¹² and salt mixtures² have not proved necessary. The occurrence of adults with crumpled wings was not cured by adding linseed oil⁵. Instead this condition has been reduced by keeping the pupae on river sand which holds them steady whilst the adults emerge.

Various methods for rearing larvae were tried (Fig. 1). Isolated blocks of medium (a) and flat trays (b), dried out too quickly and larvae tended to wander from the former. Single tubes (c) proved too laborious. The "Swiss roll" method of alternating layers of medium and plastic sheet (d), was ineffective because larvae tended to eat through the intervening plastic layers, defeating their purpose, which was to reduce wandering and hence cannibalism, within the medium. The preserve or sweet jar method (e) has proved simple and effective with 20-40 larvae per jar, and although cannibalism undoubtedly occurs it is unimportant at this density. Cardboard or plastic containers are easily penetrated by the larvae so that only glass or metal containers can be used. The jars are kept on their sides at ambient room temperature and humidity, although the room is warmed during winter.

Pupae are cut free of their cocoons because the latter are often covered with rotting culture mixture. The sexes are kept separately on clean river sand moistened with 0,03% formaldehyde or 0,005% benomyl solution to maintain a high humidity and to discourage fungi.

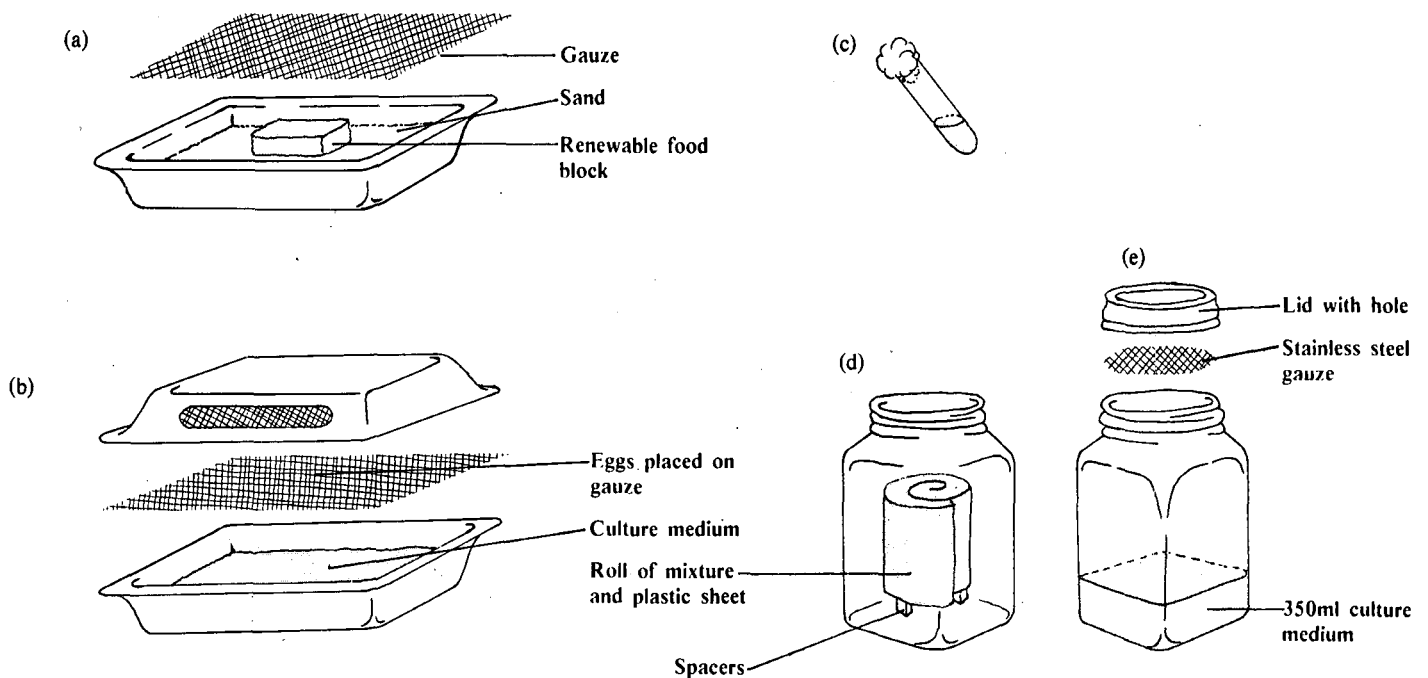
Adults are transferred to breeding boxes measuring 60 x 30 x 10 cm, lined with plastic sheet to reduce laying in crevices or around the lid. Most of the eggs are laid in the folds or plies of paper towelling provided for that purpose. Egg batches are cut out of the paper towelling daily or peeled off the plastic sheet lining, and kept in sealed plastic bags until hatching. Various other

TABLE 2

The effects of omitting ingredients singly and in pairs, from mixture (b), on the rates of development and survival of Eldana

	INGREDIENTS OMITTED							
	1 Normal	2 Cellulose	3 Casein	4 Casein+ methyl paraben	5 Sorbic acid	6 Methyl paraben	7 Ascorbic acid	8 Ascorbic acid+ Methyl paraben
TRIAL (1)								
No. of Larvae added	200			200	200	200	240	
Total survivors	92			98	193	95	194	
% Survivors Stage 4	0,6			1,6	8,0	23,5	1,4	
5	13,7			4,9	20,5	20,0	24,4	
6	16,2			42,5	68,0	4,0	25,4	
Pupa Total	46,0			49,0	96,5	47,5	80,8	
Mixture condition	Good			Poor	Fair	V. Poor	Poor	
TRIAL (2)								
No. of Larvae added	120	120	120		120	120		120
Total survivors	52	54	37		99	69		111
% Survivors Stage 3		10,8						
4	29,2	30,8	12,5		3,3	22,5		
5	14,2	3,3	18,3		39,2	32,5		5,0
6					19,2	2,5		0,8
Pupa Total	43,4	45,0	30,8		20,8	57,5		86,7
Mixture condition	Good	Shrinkage	Good		V. Poor	Poor		Poor

Figure 1. Methods tried for culturing Eldana saccharina Walker larvae.



adult breeding containers have been tried including sweet jars lined with paper or plastic bags, or simply inflated bags, each containing one pair of moths, but this was more laborious than the present method.

Aseptic conditions are very difficult to maintain in a large

routine culture and no attempt has been made to do so. Eggs are not surface-sterilized because they are not brought into contact with the medium, the preservatives in which tend to kill them anyway⁸. Utensils are cleaned with detergent and 'Dettol', but are not autoclaved.

Feral larvae and pupae from both sugarcane and indigenous host plants are frequently introduced into the culture to maintain its vigour. Shorey and Hale¹¹, for example, noted that behavioural changes occurred in their isolated cultures and concluded that this was only one manifestation of other changes which undoubtedly took place as the insects became increasingly adapted to the culture conditions. In the present case, provided that the eggs or young larvae are protected from rain and predation, and the young larvae prevented from dispersing, by the methods outlined below, insects from the culture readily transfer to cane or to indigenous host plants.

Infestation methods

Sugarcane tends to be an unsuitable host for *Eldana saccharina*, and one reason for this is the difficulty which young larvae apparently experience in penetrating the stalk. The results of one experiment to demonstrate this are given in Table 3.

TABLE 3

(a) Mean numbers of larvae becoming established on tillers of two cane varieties, which were uncut or cut to facilitate larval penetration.

(b) Analysis of variance for the same. Raw data were transformed to log (x+1)

(a)

	N55/805 (soft)		NCo 376 (Hard)	
	CUT	UNCUT	CUT	UNCUT
No. of sticks	17	18	17	19
Mean larvae / stick	3,35	2,00	2,82	0,58

(b)

Source	d.f.	Sums Squares	Mean Squares	F	P
Varieties	1	0,4054	0,4054	9,77	0,01
Cut/Uncut	1	1,1686	1,1686	28,16	<0,01
Interaction	1	2,9302	2,9302	70,61	<0,01
Residual	67	2,7780	0,0415		
Total	70	7,2822			

The numbers of larvae were counted which became established on marcotted sticks of mature cane of varieties NCo 376 (hard) and N55/805 (soft), which had either been slit with a knife to facilitate penetration, or left unslit. The raw data were transformed to log (x+1) and analysed. The difference between varieties, the difference between cut and uncut sticks, and the interaction were all significant, which suggests that the ability to penetrate cane is one factor limiting high infestation rates. Accordingly, in artificial infestation, the canes are slit in several places. Secondly, culture medium is smeared on the surface as a food source until the larvae are large enough to penetrate. Thirdly, the stems are sleeved with brown paper (glasshouse or insectary experiments) or with light canvas (outdoor experiments), which serves two purposes. It protects the eggs or young larvae, whichever are used, from predation, and it limits dispersal of the young larvae, during which considerable mortality occurs. Using this method (Fig. 2), racks of marcotted canes or tubs of mature stools can be evenly infested with larvae and can be treated at a known age in preliminary insecticide screening experiments (see Table 4). Similarly, by omitting one or more of these procedures from the method, the limiting effects of penetration, predation of the young stages and dispersal, on a planted cohort of eggs or larvae can be assessed.



Figure 2. Artificial infestation of marcotted cane tillers.

TABLE 4

Artificial infestation rates on mature stools in tubs, using various sleeving materials, indoors and outdoors.

Site	Sleeving material	Larvae Applied	Larvae Survived	% Survival
Glasshouse	Brown wrapping paper	80	19	23,7
		140	50	35,7
		120	26	21,7
		80	15	18,8
		100	29	29,0
		80	28	23,3
		120	23	19,2
Outside	Woven plastic sacking I.C.I. "Terram" Light Canvas Plastic impregnated brown paper	80	3	3,8
		80	11	13,8
		80	16	20,0
		80	10	12,5
		80	10	12,5

REFERENCES

- Adkisson, P.L., Vanderzant, E.S., Bull, D.L. and Allison, W.E. (1960). A wheat germ medium for rearing the pink bollworm. *J econ Ent* 53: 759-762.
- Beckman, H.F., Bruckart, S.M. and Reiser, R. (1953). Laboratory culture of the pink bollworm on chemically defined media. *J econ Ent* 46: 627-630.
- Betbeder — Matibet, M., Coquart, J. and Bordat, D. (1977). *Eldana saccharina* Walker: technique d'élevage sur milieu artificiel et observations sur sa biologie en laboratoire. *L'Agronomie Tropicale XXXII*, (2) : 174-179.
- Clark, E.W., Richmond, C.A. and McGough, J.M. (1961). Artificial media and rearing techniques for the pink bollworm. *J econ Ent* 54 : 4-9.
- David, W.A.L. and Gardiner, B.O.C. (1965). Rearing *Pieris brassicae* (L) on semi synthetic diets with and without cabbage. *Bull ent Res* 56 : 581-593.
- Girling, D.J. (1972). Report on investigations on graminaceous stem borers in East Africa (November 1969 - March 1972). Commonwealth Inst. Biological Control report, East Africa Station, Kawanda, Uganda.
- Hensley, S.D. and Hammond, A.M. (1968). Laboratory techniques for rearing the sugarcane borer on an artificial diet. *J econ Ent* 61 : 1742-1743.
- Kishaba, A.M., Henneberry, T.J. Pangaldan, R. and Tsao, P.H. (1968). Effects of mold inhibitors in larval diet on the biology of the cabbage looper. *J econ Ent* 61 : 1189-1194.
- Ouye, M.T. (1962). Effects of antimicrobial agents on micro-organisms and pink bollworm development. *J econ Ent* 55 : 854-857.
- Singh, P. and House, H.L. (1970). Anti microbials: "safe" levels in a synthetic diet of an insect, *Agria affinis*. *J Insect Physiol* 16 : 1769-1982.
- Shorey, H.H. and Hale, R.L. (1965). Mass rearings of the larvae of nine noctuid species on a simple artificial medium. *J econ Ent* 58 : 522-524.
- Vanderzant, E.S. (1957). Growth and reproduction of the pink bollworm on an amino acid medium. *J econ Ent* 50 : 219-221.