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A SEMISYNTHETIC MEDIUM FOR MASS REARING THE TOBACCO LOOPER (PLUSIA ARGENTIFERA GUEN.) (LEPIDOPTERA : NOCTUIDAE)

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SUMMARY

Plusia argentifera **has been reared for several years without any apparent loss of fecundity on a semisynthetic diet containing wheat germ, wheat germ oil, casein, brewer's yeast, ascorbic acid and traces of several nutritional ingredients.**

I. INTRODUCTION

The tobacco looper (*Plusia argentifera* Guen.) is a major foliage pest of tobacco in Australia. To enable progressive biological studies on this insect to be made in the laboratory it was necessary to develop an artificial medium which would permit continuous mass rearing of larvae. It was also necessary that the rearing be done under comparatively aseptic conditions to avoid epizootic occurrences of the diseases *Entomophthora megasperma* and *E. sphaerosperma.*

From overseas work on related species of the Plusiinae it was possible to select a basic artificial medium easily prepared from readily available components. Such a medium for mass rearing of *Plusia acuta* Walk. in the laboratory in South Africa had been developed by Bot (1966). Preliminary trials with *P. argentifera* on this medium, however, were unsatisfactory because the moths failed to develop fully on emergence from the pupal stage. Grau and Terriere (1967), in work on the mass rearing of *Trichoplusia ni* (Hb.) in California, using a comparable basic medium, found that the saponifiable fraction of wheat germ, necessary for normal wing development, was destroyed by high temperature. The final step in the preparation of the medium used by Bot (1966), however, involved heating the medium to 90°C for 30 min. Trials therefore were conducted to develop a medium most suitable to *P. argentifera* under existing laboratory conditions at Mareeba in north Queensland.

II. MATERIALS AND METHODS

The composition of the standard rearing medium is given in Table 1.

TABLE 1

COMPOSITION OF THE STANDARD REARING MEDIUM

Component	Ingredient	Quantity (g)
A	Wheat germ	40
	Vitamin-free casein	8
	Irradiated brewer's yeast	48
B	Methyl parahydroxy benzoate ("Nipagin M")	1·6
	Cholesterol	0·5
C	Bacto agar	5·0
D	Ascorbic acid	4·0
	Inositol (Meso)	0·16
	Choline chloride	0·16
E	"Tween 80"	2·4
	Wheat germ oil	6·0
	Water	300·0 ml

The wheat germ, casein and brewer's yeast (component A) were mixed either by hand or by blender. The "Nipagin M" and cholesterol (component B) after being dissolved in 40 ml of denatured ethanol were added to component A and the mixture allowed to stand in large shallow trays with a little warmth as outlined by Bot (1966), to eliminate the ethanol by evaporation.

The agar (component C) was added to 100 ml of water in a cotton-wool stoppered conical flask and dissolved in an autoclave at 121°C and 15 lb/sq in pressure for 15 min.

Ascorbic acid, inositol and choline chloride (component D) were dissolved in a further 100 ml of cold water. Similarly the "Tween 80" and wheat germ oil (component E) were dispersed in the remaining 100 ml of water, preferably warm.

Components A, B, D and E were combined, thoroughly mixed in a large beaker and warmed in a water-bath to 50°C. The dissolved agar was cooled to 50°C and combined with the mixture of components A, B, D and E. The final mixture was poured into 10 cm x 2·5 cm clear glass flat-bottomed specimen tubes, allowed to set and after several hours stoppered with rolled cotton-wool plugs.

Newly hatched first instar larvae were transferred with a fine camel's-hair brush onto the medium, a cylinder of which approximately 2 cm in height in a tube was more than ample to rear two looper larvae to maturity.

A screening trial was undertaken on several variations of this medium and results compared with those obtained with the medium outlined for *Plusia acuta* by Bot (1966). The various media and their components are given in Table 2.

Twenty-four tubes were made of each treatment medium listed and divided over four replications each of six tubes. Two newly hatched unfed first instar larvae were placed in each tube. The six tubes of the same medium replicate were placed together in a marked brown-paper bag. The media were prepared immediately prior to hatching of the larvae.

Survival and development were assessed on pupation. The pupae were weighed and placed in emergence cages. Further records were made of the number and appearance of emerged adults.

TABLE 2

MEDIA USED IN TREATMENTS

Treatment	Medium	Components
1	Medium after Bot (1966)	A,B,C,D
2	Modified Bot's medium	A,B,C,D, with ethanol leachate from 160g of green tobacco leaf
3	Standard medium as defined in Table 1	A,B,C,D,E
4	Modified standard medium with linseed oil	A,B,C,D, with the medicinal linseed oil replacing wheat germ oil in component E
5	Reduced and modified standard medium with dried tobacco leaf	A,B,C,D, with 20g ground dehydrated green tobacco leaf replacing wheat germ in component A
6	Modified standard medium with dried tobacco leaf	A,B,C,D,E, with dried tobacco leaf replacing wheat germ in component A
7	Tobacco leaf, field-grown	
8	Reduced and modified standard medium with dried tobacco leaf	B,C,D,E, with equivalent weight of dried tobacco leaf replacing the whole of component A

III. RESULTS

Details of pupae and emergence of adults are given in Table 3.

TABLE 3

DETAILS OF PUPAE AND EMERGENCE OF ADULTS

Treatment No.	No. of 1st Instar	No. of Pupae	Mean Pupal Weight (g)	No. of Adults Emerging Fully
1	48	27	0·2014	..
2	48	15	0·1823	10
3	48	34	0·2072	32
4	48	24	0·2036	20
5	48	..	..	..
6	48	..	..	..
7	48	35	0·2035	34
8	48	..	..	..

The duration of the larval stage on media in which development was completed (treatments 1, 2, 3, 4 and 7) compared favourably with that of larvae reared on normal field tobacco leaf. In these treatments the duration of the larval stage was 13-15 days (S.D. 0·7 days).

All larvae on media in which dried tobacco leaf had been substituted for any ingredient of the standard medium failed to survive beyond the first instar (treatments 5, 6 and 8).

Moths emerging in treatment 1 had grossly distorted wings (Figure 1, left and centre). Subsequent dispersal, mating and oviposition were not possible because all moths were incapable of flight. This sharply contrasted with moths emerging in treatment 3 (Figure 1, right).

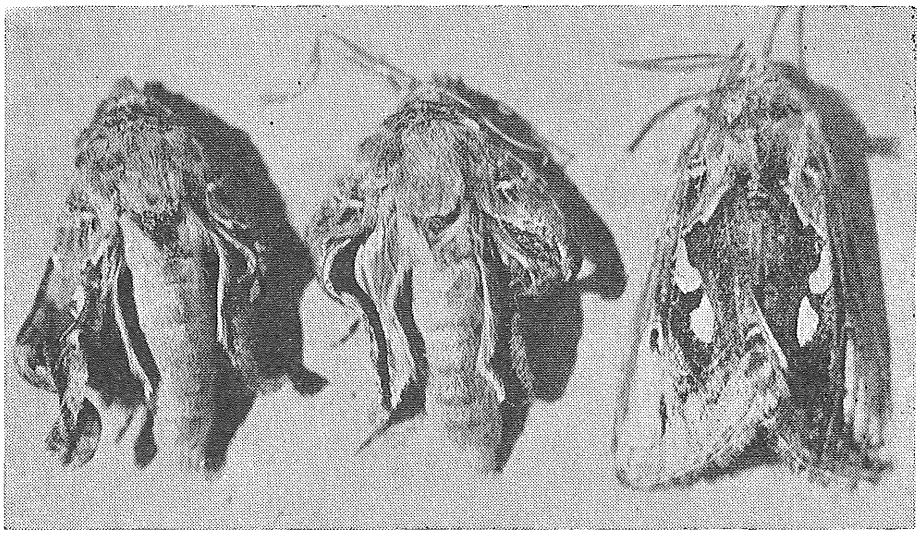

Figure 1.—Adults of *P. argentifera* from semisynthetic media. The two moths at left had fed as larvae on medium without wheat germ oil supplement. The moth at right had fed as a larva on medium containing wheat germ oil.

IV. DISCUSSION

The largest yield of both pupae and normal moths of *P. argentifera* in the laboratory was obtained by rearing the larvae on normal field-grown tobacco leaf (treatment 7). In practice, during certain periods of the year this technique has been most unsatisfactory for the purpose of mass rearing due to unavoidable contamination with oospores of species of *Entomophthora* which under laboratory conditions caused epizootics resulting in decimation or complete destruction of the larval cultures. Furthermore, this technique is laborious and time-consuming, with the added difficulty of continuously growing tobacco in the field without using crop protection chemicals.

Although tobacco is a natural and preferred host of *P. argentifera* (Cunningham, unpublished data) all treatments incorporating ground dried green tobacco leaf in the medium (treatments 5, 6 and 8) were fatal to the larvae while still in the first stage. This suggests that some toxic constituent becomes dominant in dried tobacco leaf. The simple addition of some of the insect's natural food in this way did not simulate the natural state and did not facilitate laboratory rearing.

The use of an ethanol leachate of green tobacco leaf (treatment 2), however, did enable some larvae to complete development satisfactorily and to pupate but with an obvious reduction in mean pupal weight. The number of pupae obtained was low and some inability to emerge as normal moths further reduced adult yield.

The direct use of the medium outlined by Bot (1966) for rearing *P. acuta* in South Africa (treatment 1) proved reasonably successful in enabling larvae of *P. argentifera* to complete development and to pupate but with a reduced mean pupal weight. None of the emerged moths were normal. The typical wing

deformity (Figure 1) made flight and flight-dependent activities such as feeding and mating impossible. The inability to reproduce made this technique unsatisfactory as a laboratory rearing method.

The standard medium (treatment 3) yielded numbers of both pupae and normal moths (Figure 1) comparable with those with natural tobacco as food. Mean pupal weight was greater than with any other medium.

A modification of the standard medium with linseed oil replacing wheat germ oil (treatment 4) yielded a reasonable number of pupae with a mean weight comparable with that obtained with normal tobacco leaf as food. A number of moths, however, failed to emerge fully.

The deformity of moths of *P. argentifera* emerging from medium-reared pupae was due to a deficiency of satisfactory ingredients. This was proved by the use of the standard medium (treatment 3) and the modified standard medium (treatment 4), which contained all the ingredients of Bot's formulation but were supplemented with wheat germ oil and linseed oil respectively. Bot's formulation, although containing wheat germ, involved heating to 90°C for 30 min and the evidence supports the view of Grau and Terriere (1967) that the saponifiable fraction of wheat germ necessary for wing development was destroyed by heat. The successful standard medium (treatment 3) as well as the modified standard medium (treatment 4) were heated to only 50°C.

From this evidence separate attempts to use Bot's formulation with heating only to 50°C initially gave promise of success. In further batches results were grossly inconsistent, apparently due to an age-quality factor associated with local supplies of wheat germ. Rather than bioassay the freshness of each supply of wheat germ the difficulty was overcome by the simple addition of wheat germ oil. The wheat germ ingredient, however, has been retained in the standard medium, since this together with yeast comprises the bulk of the medium and thus is the bulk food supply for the larvae.

All the ingredients in the standard medium are readily available locally and are comparatively cheap. Whatever the quality of the wheat germ, the medium is otherwise uniform and cultures providing well-developed *P. argentifera* moths have been maintained for several years without any apparent loss of fecundity.

V. ACKNOWLEDGEMENTS

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