4. MASS REARING OF RICE STEM BORERS*

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Introduction

This study is a part of the international effort to control rice stem borers by the biological method in which large numbers of the radiation induced sterile insects are released into the natural populations. The number of insects required for this control method is so large that the rearing of them on their natural host can be very costly in terms of greenhouse space, production of natural foods, labour and so on. Therefore, the objective of the work presented here was to develop a method of mass rearing of the rice stem borers on artificial media, especially *Chilo suppressalis* (Walker).

Materials and Methods

Larval diets

The following diets were used for rearing of *C. suppressalis* and other rice stem borer species:

1. Diet No. 1 (wheat germ diet) was similar to that used for rearing larvae of southwestern corn borer, *Zeadiatraea grandiosella* (Dyar) (Hormchong, 1967) the sugarcane borer, *Diatraea saccharalis* (Fabricius) (Hormchong, 1968) which was developed from Vanderzant *et al.* (1962) and Berger (1963). Its composition is shown in Table 1.

Table 1. Composition of diet no. 1. (wheat germ diet)

Constitutents	Amount
Wheat germ	36 g
Sucrose	32 g
Casein (vitamin free)	42 g
Salt mixture (Wesson's)	12 g
Alphacel	6 g
Vitamin fortification mixture	12 g
Ascorbic acid	5 g
KOH solution 22.5%	6 ml
Formaldehyde 10%	5 ml
Methyl parahydroxybenzoate 15% in 95% ethyl alcohol	12 ml
Propionic acid	1.5 ml
Tetracycline (250 mg per capsule)	1 capsule
Water	1,000 ml
Agar	26 g

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2. Diet No. 9, (Table 2) was a modification of diet No. 1 which was found to be a desirable medium for *C. suppressalis* among eight different diets (Final Report RC No. 758/RB 1970). Brewer's yeast and cholesterol were added to this diet.

Table 2. Diets tested for rearing of *Chilo suppressalis* and other rice stem borer species.

Ingredients (quantities in grams and in ml.)	Diet number					
	1	9	10	11	12	13
Wheat germ	13.5	13.5	13.5	13.5	—	7
Sucrose	12.0	12.0	12.0	12.0	12.0	12.0
Alphacel	4.5	4.5	4.5	4.5	4.5	4.5
Wesson's salt	4.5	4.5	4.5	4.5	4.5	4.5
Casein (hydrolyzed NBC)	15.5	15.5	15.5	15.5	15.5	15.5
KOH (22.5%)	2.3	2.3	2.3	2.3	2.3	2.3
Formaldehyde (10%)	2.0	2.0	2.0	2.0	2.0	2.0
Methyl parahydroxybenzoate (15%)	4.5	4.5	4.5	4.5	4.5	4.5
Propionic acid	0.5	0.5	0.5	0.5	0.5	0.5
Tetracycline R (capsule)	1/2	1/2	1/2	1/2	1/2	1/2
Vitamin fortification mixture (NBC)	4.5	4.5	4.5	4.5	4.5	4.5
Ascorbic acid	2.0	2.0	2.0	2.0	2.0	2.0
Brewer's yeast	—	2.0	2.0	—	—	—
Cholesterol	—	0.5	—	0.5	0.5	0.5
Wolffia	—	—	—	—	13.5	7.7
Agar	18.0	18.0	18.0	18.0	18.0	18.0
Water	400	400	400	400	400	400

3. Diet No. 12 (*Wolffia* diet) was similar to the wheat germ diet, except that dried *Wolffia* was substituted for wheat germ in equal amounts, *Wolffia arrhiza* (Lemnaceae) is a flowering plant similar to duck weed and is a common aquatic plant in many parts of Thailand (Bhanthumnavin and McGarry, 1971).

4. Other tested diets are shown in Table 2.

Source of insects

C. suppressalis were collected from light traps in the Bangsaen area and from Sampran, Nakorn Pratom Province (approximately 50 kilometers west of Bangkok) since July 1970. A culture of this species was continuously maintained in controlled conditions in the laboratory.

Mating chamber

Wire screen cages (5′ × 7′ × 6′) containing a pot of rice plants (approximately 6 week old) were used as mating chambers. Many pairs of moths were placed in the chamber after their emergence. The female moths of *C. suppressalis* deposited their eggs on the upper side of the leaves. Egg masses were collected daily and washed with 3 percent sodium hypochlorite.

Larval rearing containers

The rearing containers used for the larvae were: (1) Flat whisky bottles (325 ml. costing 1 cent each); (2) Erlenmeyer flasks (250 ml.); (3) clear plastic containers (7″ × 4½″ × 2½″); (4) Glass shell vials (4 drams); and (5) Medicine cups (different sizes).

Results and Dicussions

The results of addition of brewer's yeast and cholesterol to diet No. 1 (diet No. 9) in rearing of *C. suppressalis* are shown in Table 3. Diet No. 9 gave a greater number of pupae than diet No. 1, but there was no significant differences in the number of pupae and pupation percentages. A comparison of diet No. 1, diet No. 9, diet No. 10 (diet No. 1 plus brewer's yeast), and diet No. 11 (diet No. 1 plus cholesterol) were also made, the results are presented in Table 4. The differences of the number of pupae and pupation percentages between four tested diets were not significant at 5 percent level ($P>0.05$).

Table 3. Rearing of *Chilo suppressalis* on diet no. 1 and diet no. 9 in flat whisky bottles.

Diet no.	No. first intar larvae started (per bottle)	Average larval period (days)	No. pupae in bottle no.				Total pupae	Average pupae/per bottle	Percent pupation
			1	2	3	4			
1	40	36	23	25	22	24	94	23.5	58.8
9	40	43	22	27	32	23	104	26.0	65.0

Table 4. Rearing of *Chilo suppressalis* on four different diets in flat whisky bottles.

Diet no.	No. first inster larvae started (per bottle)	Average larval period (days)	No. pupae in bottle no.				Total pupae	Average pupae/per bottle	Percent pupation
			1	2	3	4			
1	40	43	16	20	16	22	74	18.5	46.3
9	40	41	25	19	23	21	88	22.0	55.0
10	40	45	23	22	21	34	100	25.0	62.5
11	40	52	21	24	16	8	69	17.2	43.1

Tests involving substitution of dried *Wolffia* for wheat to diet No. 1 (wheat germ diet) with an equal amount (diet No. 12) and one half amount (diet No. 13) of *Wolffia* were conducted. The results presented in Table 5, were based on the number of pupae and pupation percentages obtained. The substitution of 13.5 g of dried *Wolffia* for wheat germ to diet No. 1 resulted in a pupation percentage of 72.0 compared to 41.0 of diet No. 9 and 60.5 of diet No. 13. The differences between diet No. 9 and diet No. 12 were significant statistically ($P>0.01$), while the differences between diet No. 12 and diet No. 13 were not. The average pupal weight obtained from the three diets were slightly different.

Table 5. Comparison of diet no. 9, no. 12 and no. 13 for rearing *Chilo suppressalis* in flat whisky bottles.

Diet no.	No. first instar larvae started (per bottle)	Average larval period (days)	No. pupae in bottle no.				Total pupae	Average no. pupae per bottle	Percent pupation	Average pupal wt. (mg)	
			1	2	3	4				F.	M.
9	50	51.7	13	19	28	22	82	20.5	41.0	72.7	36.3
12	50	57.7	33	33	40	38	144	36.0	72.0	79.1	41.7
13	50	38.0	21	44	26	30	121	30.3	60.5	83.3	43.3

According to the results obtained from the diet No. 12, it seems that *Wolffia* should be a suitable source of proteins and amino acids. Ishii and Hirano (1955) proved that ten amino acids; arginine, histidine, isoleucine, leucine, lycine, methionine, phenylalanine, threonine, tryptophane, and valine are indispensable for the larval growth of *C. suppressalis*. Quantity analysis of *Wolffia* for amino acids has been made. It was found that dried *Wolffia* contained 18 amino acids* including ten amino acids which are mentioned above.

Pupal production of *C. suppressalis* in flat whisky bottles, presented in Table 6, showed that more pupae were obtained when larger number of first instar larvae per container were used (60–80). However, the best percentage pupal yield was obtained when 20 and 40 larvae per bottle were infested. The size of the rearing chambers and the amount of the first instar larvae infested effected pupal production.

Table 6. Production of *Chilo supperessalis* pupae in flat whisky bottle.*

Test no.	No. first inster larvae/bottle	Bottle no. & no. pupae				Total pupae	Average no. pupae per bottle	Percent pupation
		1	2	3	4			
1	20	13	14	16	12	55	13.8	68.8
2	40	24	9	14	26	73	18.3	69.1
3	60	39	36	23	31	129	32.3	53.8
4	80	44	29	29	28	130	32.5	40.6

* Flat whisky bottle, 325 ml.

An experiment was conducted to determine the length of larval periods. The first instar larvae of *C. suppressalis* were reared on rice seedlings in sterilized round whisky bottles which contained sandy soil about 2 inch deep at the bottom. These larvae were kept in the bottles until the completion of the third instar. The fourth instar larvae were then transferred to round glass jars (9 inch diameter) containing 4–6 week old rice plants. These larvae were kept in this condition until they pupated. The data persented in Table 7, showed that the average larval period from the first instar to the third instar on the rice seedling was 12.8 days, and from the fourth instar to pupation on the rice plants was 16.4 days.

* Analysis made by the Division of Nutrition, Department of Health, Ministry of Health, Bangkok, Thailand.

Table 7. Rearing of *Chilo suppressalis* on rice seedlings in whisky bottles and glass jars.*

Test no.	No. larvae started/ bottle	Larval period		Total larvae	No. pupae	Average pupal weight (mg)	Percent pupation
		1st-3rd instar on young rice seedlings in bottle (days)	4th instar-pupation on 6 weeks old rice plants in glass jar (days)				
1	30	14	15	29	18	35.3	60.0
2	30	110	16	26	20	39.4	66.6
3	30	14	18	32	22	41.1	73.3
4	30	11	17	28	23	36.7	76.6
4	30	15	16	31	17	37.2	56.6

* Round whisky bottles and 9 cm. diam. glass jar.

Conclusions

A greater number of pupae was obtained by addition of brewer's yeast and cholesterol to diet No. 1, although differences between means was not significant. The substitution of dried *Wolffia* for wheat germ to diet No. 1 with an equal amount indicated highly significant differences in number of pupae and percentage of pupation.

Pupal production of *C. suppressalis* in flat whisky bottles showed that more pupae were obtained when larger numbers of first instar larvae per container were used, but the percentage yield was lower.

The average larval period from the first instar through the third instar on the rice seedling was 12.8 days and from the fourth instar to pupation was 16.4 days.

References

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Discussion

T. Saito: Have you tried mass rearing of *T. incertulas?*

T. Hormchong: Various diets which were used for *C. suppressalis* were tried for the mass rearing of *T. incertulas* but no success has been achieved. We could only rear *T. incertulas* larvae up to the 4th instar after which all larvae died. In case of *T. inotata* some larvae pupated and emerged

as adults but female did not lay fertile eggs.

M. Sakai: Why is there difference in the length of larval period of *Chilo* in your data and data collected in Japan?

Hormchong: This difference may be due to the difference in temperature and humidity of the laboratory. Moreover, the life cycle of *C. suppressalis* is different in Thailand.

J. S. Hyun: What is the sex ratio of *C. suppressalis?*

T. Hormchong: No work has been done to determine the sex ratio but it has been observed that there were more males than the females.