

5. REARING STUDIES ON THE RICE STEM BORER, *CHILO SUPPRESSALIS* WALKER

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Introduction

The rice stem borer, *Chilo suppressalis* Walker, is generally considered to be the most serious insect pest of rice plant here in Taiwan. A great amount of money is consumed every year for its control.

In order to investigate feasible control measures of the pest, mass production of this species has become the first step to obtain a large quantity of individuals in laboratory.

During the course of rearing in laboratory the diets were often contaminated by the air-borne microorganisms (e.g. *Penicillium* sp., *Rhizopus* sp., *Cladosporium* sp. and some unidentified bacteria), so this study was aimed finding out a better antimicrobial agent which could prevent the prepared media from deterioration for a longer period covering the whole larval stage.

The present paper deals with a series of these rearing tests of rice stem borer on different diets and effects of various antimicrobial agents.

Materials and Methods

For rearing of rice stem borer, *Chilo suppressalis* Walker, the following seven formula for preparing the diets have been employed.

Diet (1)

Water	50.1 ml
Agar	0.7 g
Cellulose	1.0 g
Glucose	0.5 g
Casein	1.0 g
Sucrose	0.5 g
Dry yeast	1.0 g
Wesson's salt	0.2 g
Cholesterol	0.02g
Choline chloride	0.05g
Ascorbic acid *	0.1 g
Sorbic acid **	0.05g
Dry rice stem	0.5 g

Diet (2)

Similar to diet (I) except that the composition of vitamins and carbohydrates.

Vitamins *

Thiamine	0.015mg
Riboflavin	0.07 mg
Nicotinic acid	0.15 mg
Pyridoxine	0.075mg
Folic acid	0.015mg

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Calcium pantothenate	0.15 mg
Carbohydrate	
Fructose	0.5 g
Maltose	0.5 g

Diet (3)

Similar to diet (I) except that the composition of vitamins, carbohydrates and amino acids.

Vitamins *	
Thiamines	0.255mg
Riboflavin	0.125mg
Nicotinic acid	0.15 mg
Pyridoxine	0.425mg
P-aminobenzoic acid	0.25 mg
Calcium pantothenate	0.025mg
Folic acid	0.25 mg
Inositol	2.5 mg
Biotin	0.025mg
Carbohydrates	
Fructose	0.5 g
Maltose	0.5 g
Amino acids	
Arginine	25.0 mg
Histidine	12.5 mg
Isoleucine	12.5 mg
Leucine	25.0 mg
Lysine	12.5 mg
Methionine	12.5 mg
Phenylalanine	12.5 mg
Threonine	12.5 mg
Tryptophan	25.0 mg
Valine	25.0 mg

Diet (4)

Diet (I) + rice bran 1 g

Diet (5)

Diet (II) + rice bran 1 g

Diet (6)

Diet (III) + rice bran 1 g + rice stem 1 g

Diet (7)

Water	42 ml
Agar	0.7 g
Cellulose	1.0 g
Glucose	2.0 g
Casein	1.5 g
Wesson's salt	0.2 g
Cholesterol	0.02g
Yeast	1.0 g
Rice stem extract	0.5-1.0 g
Sorbic acid **	0.75g

* Ascorbic acid, vitamin and amino acid were added after sterilization.

** Sorbic acid used to prevent contamination were added after sterilization.

Rice stem were cut into 1 inch pieces, weighed and press its extract in blender, Each diet ingredients together with rice stem extract were poured into 200 ml flasks, plugged with adsorbent cotten and sterilized in autoclave under 1 Kg/cm, 121°C for 20 min. Vitamins, amino acids and other labile compounds were added to the basal diet after it cooled down to 50°C.

Egg masses used for rearing were disinfected with 0.1% mercuric chloride for 4 minutes and washed with 70% alcohol before hatching (when the egg reached the black head stage). About 50 eggs were transferred into each flask.

The flasks were placed in a rearing room conditioned at 25°C-85% RH under 12 hour illumination by fluorescent light per day. Feeding behavior and growth conditions were observed. Plastic straw was used for pupation and pupae were weighed individually. 20 pupae were placed in each glass cylinder lined with wax paper. Emergence, percentage of pupation, and egg mass laid by the female were observed and recorded.

Judging from the rearing results, the following diet was selected for antimicrobial agent studies.

Water	1000 ml
Agar	20 g
Cellulose	20 g
Glucose	10 g
Casein	20 g
Sucrose	10 g
Dry yeast	10 g
Wesson's salt	4 g
Cholesterol	0.4g
Ascorbic acid	2 g
Rice stem extract	20 g

Compounds tested to determine their antimicrobial effects were sodium benzoate, potassium sorbate, formaldehyde, sorbic acid, methyl paraben, butyl paraben and antibiotics such as streptomycin sulfate, aureomycin and tetracycline. The first three compounds were dissolved in water and the remainders in 95% alcohol. Test agents were divided into 7 groups:

A. Sorbic acid	0.2 %
Methyl paraben	0.1 %
B. Sorbic acid	0.1 %
Methyl paraben	0.1 %
Streptomycin sulfate	0.02%
Aureomycin	0.01%
C. Methyl paraben	0.1 %
Potassium sorbate	0.1 %
D. Potassium sorbate	0.1 %
Methyl paraben	0.1 %
Streptomycin sulfate	0.02%
Aureomycin	0.01%
E. Sorbic acid	0.2 %
Butyl paraben	0.2 %
F. Formaldehyde	0.05%
Methyl paraben	0.15%
G. Sorbic acid	0.1 %
Sodium benzoate	0.1 %
Methyl paraben	0.2 %

Approximately 15 ml hot, liquid basic diet with different kind of antimicrobial agents were poured into 2.5 × 8.0 cm rearing vials. The vials containing the materials were plugged with adsorbent cotton.

Pupae were placed in a oviposition cylinder lined with wax paper (15 cm × 40 cm). Rice seedlings in petri dish were supplied to each cylinder in order to stimulate the mating behavior and oviposition. Egg massess laid on the wax paper or rice leaves were removed and placed in a petri dish and kept on a wet filter paper and incubated at 28 C. (The filter paper was sealed with 0.02% sodium benzoate solution.). 5 newly hatched larvae were transferred into each vial by sterilized brush. The vials should be put upside down, in order to make the feeding early.

Feeding behavior and growth conditions were observed every day. When the larvae mature, they crawled up to the top to pupate. The pupae were put in a moist petri dish. After emergence, five pair of adult moths (male: female=1:1) reared on the same diet were placed in the glass cylinder as described above.

Results and Discussions

Results of this experiment are shown in the following tables.

By substituting the dry rice stem in diet with water extracts of fresh rice stems, fairly satisfactory results were obtained. This suggested, possibly, that this substance

Table 1. Results of the experiment on the rearing of *Chilo suppressalis* Walker on seven diets. (2nd generation)

Diet	No. of eggs incubated	% of hatching	Duration of larval stage (day)	% of pupation	Weight of pupa (mg)		% of adult emergence	Average eggs laid per female
					Range	Mean		
Diet 1	750	79	39	50	43.2~46.7	39.9	97	47
Diet 2	750	81	37	96	43.2~46.7	45	94	53
Diet 3	750	90	29	93	49.5~54.3	52.6	96	42
Diet 4	750	62	33	93	52.6~61.2	58.8	90	71
Diet 5	750	76	35	89	54.1~62.4	60.1	86	75
Diet 6	750	71	33	82	64 ~69.5	68.6	81	71
Diet 7	750	85	30	95	55.2~64.7	62	96	80
Rice stem	750	90	28	96	62.6~65.8	64.1	97	99

Table 2. Results of rearing on different diets. (4th generation)

Diet	No. of eggs incubated	% of hatching	Duration of larval stage (day)	% of pupation	Weight of pupa (mg)		% of adult emergence	Average eggs laid per female
					Range	Mean		
Diet 1	350	70	42	63	33.7~37.4	35.4	92	40
Diet 2	350	71	38	78	40.2~43.6	42.4	93	27
Diet 3	350	65	32	83	43.7~51.3	49.6	96	42
Diet 4	350	76	32	73	57.3~60.4	60	96	66
Diet 5	350	71	35	70	56.8~63	59.6	97	66
Diet 6	350	69	36	75	58.7~61.6	59.6	95	61
Diet 7	350	75	33	85	58.6~62.5	60.5	96	71
Rice stem	350	80	30	87	61.3~62.9	62.5	86	94

Table 3. Results of the rearing on the diet incorporated with seven groups of antimicrobial agents. (1st generation)

*Diet	No. of larvae reared	% of pupation	Weight of pupae (mg) (mean value)	% of adult emergence	Average eggs laid per female	Duration of larval stage (day)
A	200	79	55.6	89	93	37.6
B	200	75	56.2	84	89	40.3
C	200	67	52.7	86	52	41
D	200	83	63.5	92	54	39.8
E	200	73	54.1	93	61	40.6
F	200	67	51.3	83	74	39.2
G	200	78	55.8	87	98	36.4
Rice stem	200	90	66.4	98	120	31.8

* Diet incorporated with different antimicrobial agents.

Table 4. Results of the rearing on the diet incorporated with seven groups of antimicrobial agents. (2nd generation)

*Diet	No. of larvae reared	% of pupation	Weight of pupae (mg) (mean value)	% of adult emergence	Average eggs laid per female	Duration of larval stage (day)
A	200	77.5	54.2	88	89	38.4
B	200	74	57.1	85	85	39.1
C	200	64	51.4	85	47	40
D	200	79.5	61.3	91	47	42
E	200	71	50.3	91	56	40.2
F	200	65	49.2	84	71	41
G	200	75	56.4	85	94	38.8
Rice stem	200	88	67.1	96.6	113	31.2

* Diet with different antimicrobial agents.

Table 5. Results of the rearing on the diet incorporated with seven groups of antimicrobial agents. (3rd generation)

*Diet	No. of larvae reared	% of pupation	Weight of pupa (mg) (mean weight)	% of adult emergence	Average eggs laid per female	Duration of larval stages (days)
A	200	76.8	54.7	89.6	87	37.4
B	200	70	54.3	84.3	83	41.3
C	200	62	52.4	87.4	43	44.1
D	200	75.6	62.1	89	48	38.9
E	200	71.3	49.6	85	59	40
F	200	64	51.4	83	63	41
G	200	76.2	58.2	87.8	83	39.6
Rice stem	200	86.3	61.4	93	109	34

* Diet incorporated with different antimicrobial agents.

Table 6. Results of the rearing on the diet incorporated with seven groups of antimicrobial agents. (4th generation)

*Diet	No. of larvae reared	% of pupation	Weight of pupa (mg) (mean value)	% of adult emergence	Average eggs laid per female	Duration of larval stages (days)
A	200	74.5	53.0	93.96	85	39.3
B	200	64	52.3	83	80	38.4
C	200	58	50.8	86	38	43. ?
D	200	74	60.0	80	42	44.3
E	200	72	48.2	80	61	41.4
F	200	64	52.1	84	56	42
G	200	73	57.2	91	75	40.1
Rice stem	200	85.5	64.9	92	108	35

* Diet with different antimicrobial agents.

play a very important role for the normal growth and development of rice stem borer. The nutritive values of fructose, maltose and sucrose for the rice stem borer were noted by Hirano and Ishii (1957). Based on the above, results a combination of glucose and sucrose (1:1) appeared to be a favorable carbohydrate source in the diet.

Comparing the egg masses laid, larvae stage covering a generation and percentage of hatching, pupation, emergence, the Diet 7 seemed to be the promising one. Egg masses laid by females were not significantly less than that from females reared by rice stem, but the fecundity of females seemingly decreased from generation to generation. Occasionally, a number of adults showed twisted, unexpanded wings or could not emerge from the pupae. Inbreeding and lacking of cyclic temperature could possibly be the reasons which need further study.

Seven agents were tested to prevent the medium from contamination by air-borne microorganisms, and their effectiveness and influence to the development of rice stem borer were observed and recorded. Tables 3, 4, 5, 6 indicated that when the diet containing the antimicrobial agents either group A (Sorbic acid 0.2% + Methyl paraben 0.1%) or group G (Sorbic acid 1% + Methyl paraben 0.2%) would give higher percentage of pupation, emergence and egg-laying of the insect. In table 5 we also found that group A and G could be exposed to unaseptic condition for a longer period of 33-34 days without a contamination of the microbe.

Table 7. Period of inhibition of microbial growth on diet incorporated with different antimicrobial agents.

Kinds of antimicrobial agents and concentration	No. of days inhibited
Sorbic acid 0.2% + Methyl paraben 0.1%	34
Sorbic acid 0.1% + Methyl paraben 0.1% + Streptomycin sulfate 0.02% + Aureomycin 0.01%	32
Methyl paraben 0.1% + potassium sorbate 0.1%	31
Potassium sorbate 0.1% + Methyl paraben 0.1% + Streptomycin sulfate 0.02% + Aureomycin 0.01%	30
Sorbic acid 0.2% + Butyl paraben 0.2%	32
Formaldehyde 0.05% + Methyl paraben 0.15%	29
Sorbic acid 0.1% + Sodium benzoate 0.1% + Methyl paraben 0.2%	33

Effects of antimicrobial agents on growth, development and survival were related to the concentration of antimicrobial agents; the higher concentration resulted in less contamination and decrease in growth rate, development and percentage of pupation.

Although abnormalities frequently occurred in the rearing colony of rice stem borers in the past two years, no evidence of any significant side-effect on the growth and development of rice stem borer caused by the test agents was observed.

Abstract

On the basis for establishing satisfactory artificial diets for the large-scale rearing successive generations of the rice stem borers, *Chilo suppressalis* Walker in the laboratory, seven different diets were tested. The rice stem borers were successfully reared for four generations with high survival and normal growth on various diets. The antimicrobial agents we employed, namely, sodium benzoate, potassium sorbate, formaldehyde, sorbic acid, methyl paraben, butyl paraben, aureomycin, tetracycline, and streptomycin sulfate were incorporated respectively with basal diet. The results showed that antimicrobial agent group A (Sorbic acid 0.2% + Methyl paraben 0.1%) and group C (Sorbic acid 0.1% + Sodium benzoate 0.1% + Methyl paraben 0.2%) appeared to be the better antimicrobial formulation for mass rearing rice stem borers under unaseptic condition. Furthermore, there was no evidence of side-effect of these antimicrobial agents. The tests were preliminary ones. Further study will be needed.

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Discussion

- M. T. Ouye :** You mentioned that you found clear body larvae. Have you examined the larvae for virus infection?
- D. F. Yen** We examined the larvae only for bacterial infection and no bacterial infection was found.
- M. Fukaya :** I found very low virus infection in my cultures. In rice stem borer rearing we need not to worry about the viral infection.
- T. Hormchong :** Have you had any contamination in the yeast used in the diet medium?
- D. F. Yen** No, I don't think so.