

## **New Artificial Diet for Continuous Rearing of the Bean Pod Borer, *Maruca vitrata***

Authors: Wang, Pan, Lu, Peng-Fei, Zheng, Xia-Lin, Chen, Li-Zhen, Lei, Chao-Liang, et al.

Source: Journal of Insect Science, 13(121) : 1-11

Published By: Entomological Society of America

URL: <https://doi.org/10.1673/031.013.12101>

---

BioOne Complete ([complete.BioOne.org](https://complete.BioOne.org)) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at [www.bioone.org/terms-of-use](http://www.bioone.org/terms-of-use).

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

---

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.



## New artificial diet for continuous rearing of the bean pod borer, *Maruca vitrata*

Pan Wang<sup>1</sup>, Peng-Fei Lu<sup>2</sup>, Xia-Lin Zheng<sup>1</sup>, Li-Zhen Chen<sup>1</sup>, Chao-Liang Lei<sup>1</sup>, Xiao-Ping Wang<sup>1</sup>

<sup>1</sup>Hubei Insect Resources Utilization and Sustainable Pest Management Key Laboratory, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, P. R. China

<sup>2</sup>The Key Laboratory for Silviculture and Conservation of Ministry of Education, Beijing Forestry University, Beijing 100083, P. R. China

### Abstract

The bean pod borer, *Maruca vitrata* Fabricius (Lepidoptera: Crambidae), is a serious pantropical pest of grain legumes. A suitable artificial diet is desirable for producing uniform insects for commercial purposes or research. Three previously described artificial diets, 1 newly-developed artificial diet, and cowpea (*Vigna unguiculata* (L.) Walp. (Fabales: Fabaceae)), the natural host-plant of *M. vitrata*, were used for rearing *M. vitrata*, and the life parameters were examined. The results indicated that insects completed a full life cycle only when the larvae were fed cowpea or the diet reported by Onyango and Ochieng'-Odero (1993), called the "D-OO diet." However, the rearing efficiency (i.e., larval and pupal survival, longevity of adults, and fecundity) on the D-OO diet was inferior to the rearing efficiency on cowpea. Subsequently, a new artificial diet was formulated based on soybean powder, *Glycine max* (L.) Merr. (Fabales: Fabaceae), and wheat germ, *Triticum aestivum* L. (Poales: Poaceae). The egg production, egg hatching, larval developmental duration, and pupal survival of the *M. vitrata* reared on the new artificial diet were found to be significantly improved relative to the D-OO diet, but were not significantly better than on the host-plant cowpea. The optimum rearing density was 15–25 larvae per box. There were no significant changes in reproductive potential after 8 successive generations of rearing on the new diet. These results indicated that the newly developed diet could serve as a viable alternative to cowpea plant for continuous rearing of *M. vitrata*.

**Keywords:** reproductive potential

**Abbreviations:** **D-J**, diet reported by Jin et al. (2010); **D-JR**, diet reported by Jackai and Raulston (1988); **D-New**, new artificial diet; **D-OO**, diet reported by Onyango and Ochieng'-Odero (1993)

**Correspondence:** <sup>a</sup> [xpwang@mail.hzau.edu.cn](mailto:xpwang@mail.hzau.edu.cn)

**Received:** 25 February 2012 **Accepted:** 20 October 2012 **Published:** 5 November 2013

**Copyright:** This is an open access paper. We use the Creative Commons Attribution 3.0 license that permits unrestricted use, provided that the paper is properly attributed.

**ISSN:** 1536-2442 | Vol. 13, Number 121

#### Cite this paper as:

Wang P, Lu P-F, Zheng X-L, Chen L-Z, Lei C-L, Wang X-P. 2013. New artificial diet for continuous rearing of the bean pod borer, *Maruca vitrata*. *Journal of Insect Science* 13:121. Available online: <http://www.insectscience.org/13.121>

## Introduction

The bean pod borer, *Maruca vitrata* Fabricius (Lepidoptera: Crambidae), is an important pantropical pest of grain legumes, and has a wide distribution throughout Africa, Asia, South America, and the southern states of Australia (Sharma 1998). In China, *M. vitrata* is the primary boring pest of leguminous vegetable crops, particularly the cowpea, *Vigna unguiculata* (L.) Walpers (Fabales: Fabaceae), and has caused significant yield losses yearly (Ke et al. 1985). Chemical insecticides are widely used to control this pest (Afun et al. 1991; Asante et al. 2001). However, the cryptic behavior of the larvae, the cost, and the environmental risks associated with chemical insecticides impose serious limitations on the use of the chemicals to control this species (Singh and Allen 1980; Jackai and Daoust 1986). Effective control of *M. vitrata* often relies on sound integrated pest management strategies. In order to develop and optimize strategies, studies must be focused on the biology, bionomics, behaviors, and ecology of *M. vitrata*, and on improving cowpea resistance against *M. vitrata*. One priority to conduct these studies is the availability of a continuous and adequate supply of high quality experimental insects.

Many studies have focused on developing an economical diet formula and cost-effective rearing techniques for *M. vitrata* (Jackai and Raulston 1982, 1988; Onyango and Ochieng'-Odero 1993; Chi et al. 2004; Liu and Hwang 2006; Jin et al. 2010). However, there are many questions and issues related to the development of artificial diets for the continuous rearing of this species. First, some of the components are difficult to find or obtain, such as cowpea flower powder (Onyango and Ochieng'-Odero 1993), Insecta F-II (Chi et al. 2004), Taiwan sesbania leaves, and Taiwan

sesbania seed powder (Liu and Hwang 2006). Second, the rearing density of the larvae in most previous studies was 1 larvae per container (Jackai and Raulston 1988; Chi et al. 2004) or less than 5 larvae per container (Onyango and Ochieng'-Odero 1993; Jin et al. 2010), except for Liu and Hwang (2006). Third, it is difficult to obtain an adequate number of mating pairs to maintain the experimental population by using an artificial diet (Jackai and Raulston 1988). Therefore, artificial diets for this species need to be improved for continuous rearing in the laboratory to produce a large amount of uniform insects.

The aim of this study was to develop an artificial diet suitable for the continuous rearing of *M. vitrata* without a loss of vigor or reproductive potential.

## Materials and Methods

### Experimental insects

*M. vitrata* larvae were collected from the Cihui farm (30° 59' N, 114° 06' E) in Wuhan City, Hubei Province, P. R. China. The population was maintained on the host-plant cowpea at  $26 \pm 1^\circ$  C, with a photoperiod of 14:10 L:D and  $60 \pm 10\%$  RH, except for adult mating and oviposition, when they were kept at  $85\text{--}90\%$  RH. The eggs were collected daily, and the newly hatched larvae were used in the following experiments.

Twenty newly hatched larvae were transferred to transparent plastic boxes (10 cm diameter) and reared with either artificial diets or on cowpea. The artificial diet was replaced every 5–6 days, and the cowpea was replaced every 2 days. A corrugated paper was put into the box as a pupation site when the larvae entered the prepupal stage, which was determined by a change in the body color from pink to light green. The pupae were sexed and kept indi-

vidually in plastic boxes. For mating, 15 pairs of newly emerged moths were transferred into a rearing cage (length × width × height = 30 × 40 × 50 cm) with a 10% honey solution. After 3 days, each pair was kept separately in an ovipositing container with a 10% honey solution. The container was composed of a transparent cylindrical plastic cup (8cm diameter, 9 cm height) with a Petri dish base on the bottom. Oviposition occurred on the inner surface of the cup. The cups were replaced daily. The cups with eggs were kept at  $26 \pm 1^\circ \text{C}$ , with a photoperiod of 14:10 L:D and  $60 \pm 10\%$  RH until hatching.

### Diet ingredients source

The ingredients of all the diets are shown in Table 1. Soybean, *Glycine max* (L.) Merrill (Fabales: Fabaceae), and wheat germ, *Triticum aestivum* L. (Poales: Poaceae), were purchased from a local market. Wesson salt mix and vitamin mixture were prepared as the detailed list shown in the footnote of Table 1. Brewers' yeast was purchased from Angel Yeast Corporation Ltd. ([www.angelyeast.com](http://www.angelyeast.com)). Other ingredients (industrial grade products) were ordered from China National Medicine Corporation, Ltd. ([www.cncm.com.cn](http://www.cncm.com.cn)).

### Experiment 1: Re-evaluation of the reported diets

Three diets described in previous studies (Table 1) were selected to determine their suitability for continuous rearing. The availability of their components and their reported rearing efficiency were also assessed. These artificial diets were prepared following the procedures described in the previous studies (Jackai and Raulston 1988 (D-JR); Onyango and Ochieng'-Odero 1993 (D-OO); Jin et al. 2010 (D-J)). Life parameters were measured, including the survival rates, the developmental durations of the larvae and pupae,

longevity, egg production, egg hatching, and egg developmental duration. Twenty larvae were reared in 1 container, as described by Liu and Hwang (2006). Each diet was replicated 10 times. Eight pairs of adult for each diet were used to measure the adult longevity and egg production.

### Experiment 2: Evaluation of the new artificial diet

The results of Experiment 1 showed that enough adults for continuous rearing were obtained only when the larvae were fed on the D-OO diet. However, compared to the host-plant cowpea, the pupal survival, egg production, and egg hatching were lower and the developmental duration of the immature stage was longer on the D-OO diet. The diet was improved by changing the nutritional composition, shown in Table 1. Expensive components, such as cowpea flower powder,

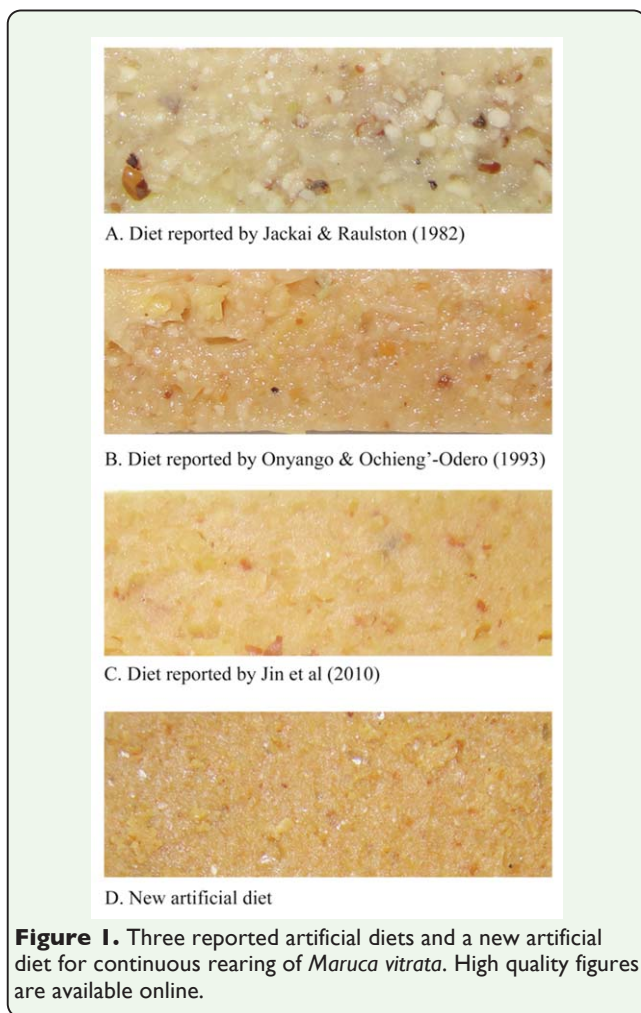
**Table 1.** Formula of 3 reported artificial diets and a new artificial diet for continuous rearing of *Maruca vitrata*.

Ingredients (g or mL)	D-JR	D-OO	D-J	D-New
Distilled water	3,600.00	3,200.00	200	1,500.00
Agar	59.2	50	40	60
Cowpea flour	400	—	10	—
Soybean flour	—	350	15	400
Cowpea leaf factor	100	—	—	—
Cowpea flower powder	—	50	—	—
Wheat germ	127.2	—	10	130
Brewers' yeast	—	40	10	25
Sugar (glucose or sucrose)	—	—	6	40
Ascorbic acid (Vitamin C)	16.8	11	—	10
Methyl-parahydroxybenzoate	6.32	5	0.63	3.5
Sorbic acid	3.84	2.5	0.25	3.5
Aureomycin	10.4	—	—	—
Formaldehyde	16	1	—	2
Wesson salt mix <sup>a</sup>	42.4	—	4.5	43
Choline chloride	29.6	—	—	4.5
Cellulose	—	—	1.5	—
KOH (4M)	21	—	—	—
Acetic acid (25%)	40	—	2.3	—
Vitamin suspension	20	—	—	—
Vitamin E calculus	—	1.5	—	—
Vitamins and antibiotic mixture	—	—	3	—
Vitamin mixture <sup>b</sup>	—	—	—	1.5

<sup>a</sup> Ingredients (mg) in 1 g of Wesson salt mix (g): CaCO<sub>3</sub> (210), CuSO<sub>4</sub>·5H<sub>2</sub>O (0.39), FePO<sub>4</sub>·4H<sub>2</sub>O (14.7), MnSO<sub>4</sub> (0.20), MgSO<sub>4</sub> (90), K<sub>2</sub>Al<sub>2</sub>(SO<sub>4</sub>)<sub>4</sub>·24H<sub>2</sub>O (0.09), KCl (120), KH<sub>2</sub>PO<sub>4</sub> (310), KI (0.05), NaCl (105), Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> (149).

<sup>b</sup> Ingredients (mg) in 1 g of Vitamin mixture (g): Nicotinic acid (306.99), Calcium pantothenate (306.99), Riboflavin (153.50), Aneurine hydrochloride (76.78), Pyridoxine hydrochloride (76.78), Folic acid (76.78), D-biotin (0.63), Cyanocobalamin (0.63).





cowpea leaf factor, and cowpea flour were eliminated. A new artificial diet (D-New) was obtained after a series of preliminary experiments (Table 1, Figure 1D).

### Preparation of the new artificial diet

The new artificial diet was prepared in 5 steps as follows: (1) soybeans were baked in an oven at 100° C for 6 hr and ground to a fine powder in a 250 mL stainless steel blender; (2) agar was combined with 1/4 of the final volume of water; (3) the major nutritional and antimicrobial ingredients, soybean flour, wheat germ, sucrose, brewer's yeast, sorbic acid, and methyl-parahydroxybenzoate, were combined and added to the remaining volume of the water; (4) the first 2 parts (2 and 3) of the diet were blended and stirred for 3 min

separately, then autoclaved at 121° C and 15 psi for 15 min together. After autoclaving, the 2 parts were blended for 5–6 min in the blender until cooled to approximately 60° C; then, the third part of the diet, including Wesson salt mix, ascorbic acid, choline chloride, a vitamin mixture, and approximately 50 mL of distilled water, was added to the mixture. Finally, (5) formaldehyde was added to the ingredients and blended for 2 min. After mixing all the ingredients and cooling to room temperature, the paste-like diet was dispensed into appropriate containers and stored at 4° C.

### Comparison of the efficiency of continuous rearing

The suitability of D-New for continuous rearing was estimated by comparing its effectiveness with those reared on cowpea. Life parameters were measured in terms of survival rates, developmental durations of the larvae and pupae, longevity, egg production, egg hatching, and egg developmental duration. Twenty larvae were reared in 1 box, as described by Liu and Hwang (2006). Each diet was replicated 10 times. Eight pairs of adults for each diet were used to measure the adult longevity and egg production.

### Effect of the larval rearing density

To evaluate the effect of larval rearing density on the survival rate, developmental duration, and pupal weight of *M. vitrata*, 5 densities (10, 15, 20, 25, and 30 larvae per box, 10 cm diameter) were used to determine the optimum larval density for continuous rearing. Insect rearing methods were the same as described above, and 150 larvae were used in each density.

### Rearing successive generations on the diet

To evaluate the effect of D-New on the reproductive potential of *M. vitrata*, all of the life parameters were compared for 8 successive

**Table 2.** Survival and developmental duration of *Maruca vitrata* reared on different diets at 26° C with a 14:10 L:D photoperiod.

Diet	Survival (%)		Developmental duration (days)	
	Larvae	Pupae	Larval stage	Pupal stage
D-JR	16.0 ± 6.6 (10) c	44.28 ± 9.7 (10) ab	17.7 ± 1.1 (25) b	6.6 ± 0.5(10) a
D-OO	67.0 ± 9.2 (10) b	49.3 ± 3.1 (10) b	15.7 ± 1.0 (133) c	6.5 ± 0.8 (65) ab
D-J	17.0 ± 3.5 (10) c	38.2 ± 11.9 (10) c	19.3 ± 2.1(33) a	6.6 ± 1.3 (9) a
Cowpea	77.0 ± 11.6 (10) a	90.7 ± 4.9 (10) a	9.7 ± 0.5 (153) d	5.9 ± 0.4 (138) b

Values (mean ± SD) followed by an asterisk in the same row were significantly different (*t*-test). Replicates and sample numbers are given in parentheses.

generations on D-New. The methods of estimation were the same as described above, and the larval rearing density was 20 larvae per box. Ten boxes were reared for each generation.

### Statistical analysis

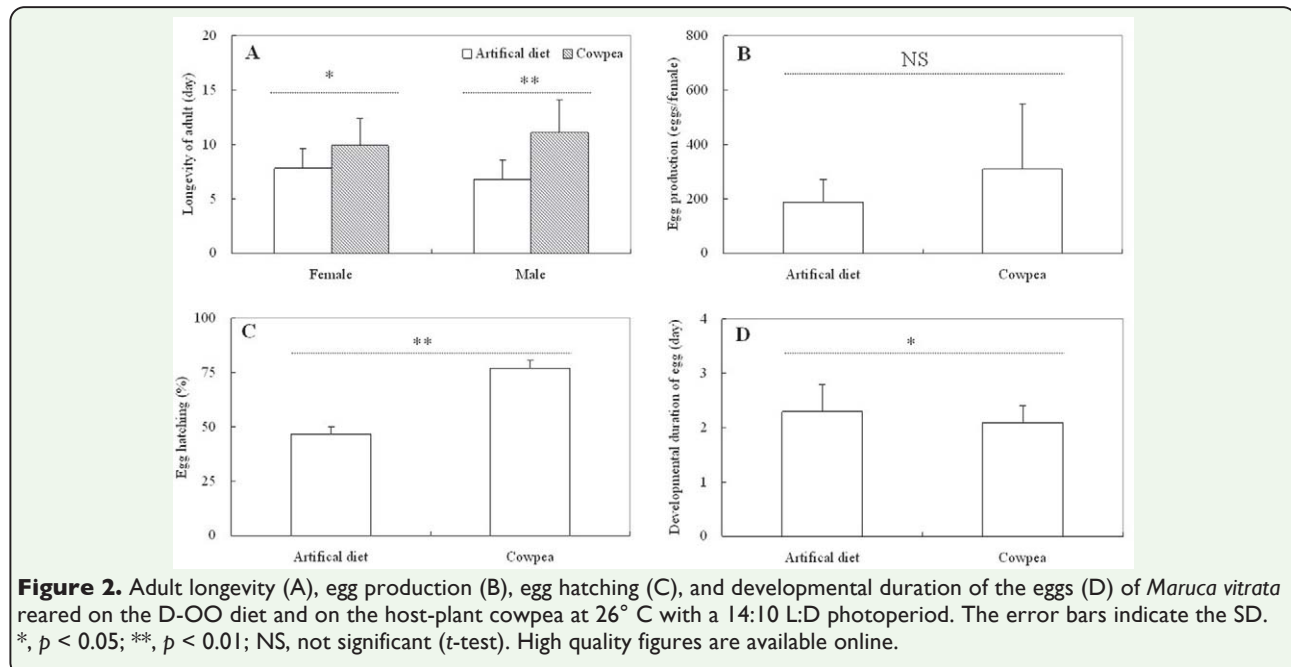
Statistical analyses were performed using SPSS 11.5 (SPSS Inc., [www-01.ibm.com/software/analytics/spss](http://www.ibm.com/software/analytics/spss)). The data for larvae and pupae survival, egg hatching, developmental duration, body weight, longevity, and fecundity were analyzed by one-way analysis of variance (ANOVA), and Tukey's HSD test ( $p < 0.05$ ) was used to compare the means. The data for all the life parameters measured on the artificial diet and the cowpea were analyzed using an independent-samples *t* test. Prior to the analysis, an arcsine square-root transformation was performed on the data

related to the egg hatching and survival of the larvae and pupae.

## Results

### Experiment 1: Re-evaluation of the reported diets

The data on the survival and developmental duration of *M. vitrata* reared on 3 reported diets and on cowpea are given in Table 2. The survival rates of *M. vitrata* reared on 3 diets were significantly lower than those on cowpea (larvae:  $F = 152.101$ ;  $df = 3, 39$ ;  $p = 0.000$ ; pupae:  $F = 84.746$ ;  $df = 3, 39$ ;  $p = 0.000$ ). The developmental duration of *M. vitrata* reared on 3 diets was significantly longer than those on cowpea (larvae:  $F = 1439.538$ ;  $df = 3, 343$ ;  $p = 0.000$ ; pupae:  $F = 13.288$ ;  $df = 3, 221$ ;  $p = 0.000$ ). For all the parameters measured in this experiment, the results of D-OO were superior to those D-JR and D-J, even though the differences were not statistically significant in some cases (e.g., for pupal developmental duration). Furthermore, enough adults for continuous rearing were obtained only for the larvae fed on D-OO or reared on cowpea. Figure 2 displays the adult longevity (Figure 2A), egg production (Figure 2B), egg hatching (Figure



**Table 3.** Comparison of life parameters of *Maruca vitrata* reared on the D-OO, D-New, and cowpea diets at 26° C with a 14:10 L:D photoperiod.

Life parameter		D-OO	D-New	Cowpea
Survival or hatching (%)	Larvae	65.5 ± 11.7 (10) a	66.0 ± 13.3 (10) a	72.0 ± 12.3 (10) a
	Pupae	50.7 ± 7.7 (10) b	76.7 ± 6.6 (10) a	81.7 ± 7.2 (10) a
	Eggs	47.6 ± 1.5 (8) c	64.9 ± 2.3 (8) b	80.5 ± 4.3 (8) a
Developmental duration (days)	Larval stage	15.6 ± 1.3 (131) a	13.3 ± 1.1 (132) b	11.8 ± 0.9 (144) c
	Pupal stage	6.1 ± 0.4 (67) b	7.2 ± 0.7 (101) a	6.1 ± 0.5 (117) b
	Egg stage	2.4 ± 0.5 (183) a	2.3 ± 0.5 (196) b	2.2 ± 0.4 (200) c
Body weight (mg)	Female pupae	37.7 ± 5.5 (30) b	37.8 ± 3.9 (30) b	44.4 ± 3.8 (30) a
	Male pupae	38.7 ± 6.0 (30) b	35.7 ± 4.2 (30) b	41.8 ± 4.6 (30) a
Longevity (days)	Female adults	7.9 ± 1.7 (8) b	10.3 ± 2.0 (8) ab	11.6 ± 2.3 (8) a
	Male adults	7.3 ± 1.7 (8) a	8.0 ± 1.3 (8) a	9.6 ± 2.9 (8) a
Fecundity (eggs per female)		201.5 ± 38.8 (8) b	267.0 ± 25.9 (8) ab	351.0 ± 136.1 (8) a

Values (mean ± SD) followed by different letters in the same row were significantly different according to Tukey's HSD test ( $p < 0.05$ ). Replicates and sample numbers are given in parentheses.

2C), and developmental duration of the eggs (Figure 2D) of *M. vitrata* reared on the D-OO diet and on host-plant cowpea. Except for egg production, the measures of the three other parameters measured for the D-OO diet were not higher than the measures for the same parameters associated with rearing on the host-plant cowpea (Figure 2).

## Experiment 2: Evaluation of the new artificial diet

### Comparison of the efficiency of continuous rearing

The comparisons of all the life parameters of *M. vitrata* reared on D-OO, D-New, and host-plant cowpea are shown in Table 3. Larval survival was not significantly different for *M. vitrata* reared on these three diets. Pupal survival and egg hatching for insects reared on D-New were significantly higher than for those on D-OO, but were lower than those on host-plant cowpea (pupal survival:  $F = 50.054$ ;  $df = 2, 29$ ;  $p = 0.000$ ; egg hatching:  $F = 255.768$ ;  $df = 2, 23$ ;  $p = 0.000$ ). The developmental durations of eggs and larvae on D-New were significantly shorter than those reared on D-OO but significantly longer than those reared on host-plant cowpea (eggs:  $F = 12.403$ ;  $df = 2, 578$ ;  $p = 0.000$ ; larvae:  $F = 398.266$ ;  $df = 2, 406$ ;  $p = 0.000$ ). The developmental duration of pupae on D-New was

significantly longer than for those on the other two diets ( $F = 121.494$ ;  $df = 2, 284$ ;  $p = 0.000$ ). Pupal body weight on host-plant cowpea was significantly greater than that on D-OO and D-New (female pupae:  $F = 22.297$ ;  $df = 2, 89$ ;  $p = 0.000$ ; male pupae:  $F = 11.242$ ;  $df = 2, 89$ ;  $p = 0.000$ ). Male adult longevity for *M. vitrata* reared on these three diets changed slightly ( $F = 2.771$ ;  $df = 2, 23$ ;  $p = 0.086$ ). Female adult longevity on D-New was longer than that on D-OO and shorter than that on host-plant cowpea, but the difference was significant only between D-OO and host-plant cowpea ( $F = 7.177$ ;  $df = 2, 23$ ;  $p = 0.004$ ). The fecundity of females obtained on D-New was significantly greater than those on D-OO. Although females reared on D-New produced fewer eggs than those reared on host-plant cowpea, the difference was not statistically significant (Table 3:  $F = 6.512$ ;  $df = 2, 23$ ;  $p = 0.006$ ).

### Effect of the larval rearing density

The effects of larval rearing density on survival, the developmental duration of the larvae and pupae, and the pupal weight are shown in Table 4. Larval rearing density had no significant effect on the survival of the larvae and the pupae (Table 4; larvae:  $F = 1.893$ ;  $df = 4, 43$ ;  $p = 0.131$ ; pupae:  $F = 0.325$ ;  $df = 4, 43$ ;  $p = 0.860$ ). Pupal developmental times for the



**Table 4.** Effect of the larval rearing density on the survival, developmental duration, and pupal weight of *Maruca vitrata* reared on D-New at 26° C with a 14:10 L:D photoperiod.

Larval densities (larval/box)	Survival (%)		Developmental duration (day)		Pupal weight (mg)	
	Larvae	Pupae	Larvae	Pupae	Female	Male
10	62.7 ± 11.6 (15) a	70.1 ± 9.4 (15) a	15.5 ± 1.2 (94) a	6.4 ± 0.6 (67) a	41.7 ± 4.1 (30) a	40.1 ± 4.4 (30) a
15	62.7 ± 10.0 (10) a	71.6 ± 11.6 (10) a	15.4 ± 1.2 (94) a	6.3 ± 0.5 (66) ab	40.4 ± 4.7 (30) ab	38.3 ± 4.4 (30) a
20	65.0 ± 10.0 (8) a	74.6 ± 8.4 (8) a	15.4 ± 1.2 (104) a	6.2 ± 0.5 (77) ab	39.6 ± 4.2 (30) ab	37.6 ± 4.9 (30) a
25	60.0 ± 5.3 (6) a	72.3 ± 3.7 (6) a	15.7 ± 1.2 (91) a	6.1 ± 0.5 (66) b	38.7 ± 4.5 (20) ab	37.5 ± 5.6 (20) a
30	50.7 ± 4.3 (5) a	70.7 ± 8.7 (5) a	15.7 ± 1.0 (76) a	6.4 ± 0.6 (54) a	38.0 ± 4.9 (20) b	37.6 ± 5.1 (20) a

Values (mean ± SD) followed by different letters in the same row were significantly different according to Tukey's HSD test ( $p < 0.05$ ). Replicates and sample numbers are given in parentheses.

**Table 5.** Life parameters of *Maruca vitrata* reared on the new artificial diet in succession for 8 generations at 26° C with a 14:10 L:D photoperiod.

Life parameter		1 <sup>st</sup> generation	2 <sup>nd</sup> generation	3 <sup>rd</sup> generation	8 <sup>th</sup> generation
Survival or hatching (%)	Larvae	65.0 ± 11.5 (10) a	64.0 ± 10.7 (10) a	66.5 ± 8.8 (10) a	66.0 ± 3.2 (10) a
	Pupae	76.6 ± 6.8 (10) a	75.8 ± 5.7 (10) a	75.0 ± 5.8 (10) a	77.2 ± 4.0 (10) a
	Egg	73.0 ± 2.3 (8) a	71.6 ± 1.8 (8) a	73.2 ± 2.9 (8) a	73.7 ± 2.1 (8) a
Developmental duration (days)	Larval stage	13.0 ± 1.2 (128) b	13.9 ± 1.3 (128) a	14.0 ± 1.0 (133) a	13.9 ± 0.9 (132) a
	Pupal stage	5.7 ± 0.6 (99) c	6.1 ± 0.5 (98) b	6.4 ± 0.5 (100) a	6.2 ± 0.5 (102) ab
	Egg stage	2.2 ± 0.4 (202) a	2.2 ± 0.4 (206) a	2.2 ± 0.4 (206) a	2.2 ± 0.4 (204) a
Body weight (mg)	Female pupae	40.7 ± 5.9 (30) a	37.4 ± 5.0 (30) b	40.3 ± 4.7 (30) ab	41.0 ± 3.7 (30) a
	Male pupae	38.9 ± 6.8 (30) ab	36.8 ± 4.5 (30) b	40.0 ± 3.5 (30) a	39.1 ± 3.4 (30) ab
Longevity (days)	Female adult	9.5 ± 2.3 (8) a	9.1 ± 1.1 (8) a	9.3 ± 1.1 (8) a	9.4 ± 1.1 (8) a
	Male adult	8.0 ± 1.3 (8) a	8.5 ± 1.6 (8) a	8.8 ± 1.3 (8) a	8.9 ± 1.6 (8) a
Fecundity (eggs per female)		362.4 ± 74.3 (8) a	349.3 ± 60.5 (8) a	374.0 ± 39.0 (8) a	368.3 ± 43.7 (8) a

Values (mean ± SD) followed by different letters in the same row were significantly different according to Tukey's HSD test ( $p < 0.05$ ). Replicates and sample numbers are given in parentheses.

*M. vitrata* reared in medium density (15–25 larvae per box) were shorter than for those reared in high density (30 larvae per box) and low density (10 larvae per box) (Table 4;  $F = 4.200$ ;  $df = 4, 329$ ;  $p = 0.002$ ), whereas such differences were absent at the larval stage (Table 4;  $F = 2.508$ ,  $df = 4, 458$ ;  $p = 0.041$ ). There were no significant differences among the body weights of male pupae reared at different densities (Table 4;  $F = 1.518$ ;  $df = 4, 129$ ;  $p = 0.201$ ), whereas the body weights of the female pupae decreased with an increase in larval density from 10 to 30 larvae per box (Table 4;  $F = 2.632$ ;  $df = 4, 129$ ;  $p = 0.037$ ).

### Rearing successive generations on the new artificial diet

The biological performances of the 8 successive generations fed D-New were compared by measuring survival and hatching, developmental duration, body weight, longevity, and fecundity. Except for developmental duration and body weight, no decline in the

survival rate and/or reproductive potential was found after 8 successive generations (Table 5).

### Discussion

This study demonstrated that *M. vitrata* performed excellently on D-New compared to other artificial diets, but not compared to host-plant cowpea. Some life parameters of insects reared on D-New, including immature survival, adult longevity, fecundity, and egg hatchability, were similar to those on host-plant cowpea, but the other parameters studied were significantly worse. These results imply that the new artificial diet based on soybean flour, wheat germ, brewers' yeast, and sucrose can be used as an alternative to natural hosts for rearing purposes.

Many factors in the rearing process could affect the performance of *M. vitrata* on artificial diet, e.g., proportions of different nutritional ingredients, physical conditions of the diet (including moisture levels and irregularities of



the diet surface), microbial contamination, several specific compounds, and environmental conditions in culture rooms. The main protein sources in D-OO were soybean flour and brewer's yeast. Besides soybean flour (Blanco et al. 2009) and brewer's yeast (Savopoulou-Soultani et al. 1994), wheat germ and sucrose were also important protein components of insect's artificial diets. Fat soluble vitamins and other substances found in wheat germ might also have a beneficial effect on insects (Vanderzant 1974). Sucrose was the main energy material of herbivorous insects (Wang et al. 1984), also acting as the most important feeding stimulant (Albert et al. 1982; Wang et al. 1984), particularly during early larval development (Savopoulou-Soultani et al. 1994). In addition to sucrose, ascorbic acid has been found to act as feeding stimulant (Dadd 1957; Montenegro de Sales 1972) and is a nutritional requirement (Ito 1961) for many species. The results of our study indicate that including wheat germ, sucrose, and the content of brewer's yeast and ascorbic acid can provide sufficient nutrition for normal insect growth, survival, and fecundity, and sustain completions of multiple life cycles. A high water content in a diet can cause drowning of larvae and asphyxiation of adults (Wang et al. 1984; Singh and Moore 1985). Lingappa (1987) found that low agar content in the diet increased the larval mortality. In our observation, a high mortality rate appeared in the larval stage, especially in the neonate larval stage. This result could at least partially be attributed to the inappropriate water and agar content in the D-J and D-JR.

Maintaining high-quality and contamination-free diet over a long period is an important feature of artificial diets for insects. It is a common practice to use antimicrobial compounds, separately or combined, to prevent microbial contamination of synthetic diets for

insects. The use of different antimicrobials was a breakthrough in the diet reported by Onyango and Ochieng'-Odero (1993). The addition of antimicrobial compounds in our artificial diet effectively prevented contamination by insecticidal pathogens for 8–10 days. Our study demonstrated that the bacteria were readily suppressed with the appropriate proportion of antimicrobial compounds, but that mold contamination was much more difficult to suppress, especially during the late larval development stage. *M. vitrata* could be healthily raised in the same boxes from neonate to pupae with only one diet block (4 × 2 × 0.2 cm) replacement because of the excellent preservatives, thus greatly reducing the frequency of diet changes and mechanical injury to the developing larvae.

Previous studies also showed that Wesson salt (Wang et al. 1984; Willis and Allen 1999) and choline chloride (Ichiki et al. 2009) were indispensable in insect diets, and may determine the growth and survival of insects (Wang et al. 1984). Wesson salt is a mixture of eleven different salts used in artificial diets for insects (Willis and Allen 1999), and contains all recommended minerals for insect diets (Wang et al. 1984). Choline chloride is related to reproduction, affecting egg and sperm production (Ichiki et al. 2009). Our preliminary experiments indicated that the addition of Wesson salt mix and choline chloride was helpful.

Larval rearing density influenced the larval survival (Gindin et al. 2009). Usually, larval density is positively correlated with mortality (especially for cannibalistic insects) and with longer duration of larval developmental (Agnew et al. 2002; Gibbs et al. 2004). The results from our study indicated that the *M. vitrata* reared in a medium density condition (15–25 larvae per box, 10 cm diameter) had a shorter developmental duration time from lar-

vae to pupae, while the survival rate did not differ significantly among the larvae raised at different densities. This study also revealed that one of the causes of larval mortality was cannibalism of both young and mature larvae, mostly during molting.

Reproductive potential (e.g., fecundity and egg hatching) is influenced by many factors, such as sex ratio (Stewart and Philogène 1983), delayed female mating (Foster and Ayers 1996; Proshold 1996), ages of the virgin female and male at mating (Michereff et al. 2004), and the number times the male has previously mated (Michereff et al. 2004). However, in our study, no decline was observed after 8 successive generations of laboratory rearing, and the fecundity of the *M. vitrata* females was higher than that found in Onyango and Ochieng'-Odero's work (1993), in which the rearing density was 5 larvae per container (7.5 × 2.5 cm). Therefore, we consider that D-New and a medium rearing density (15–25 larvae per box, 10 cm diameter) are suitable for continuous rearing. In addition, our research indicated that relative humidity in the culture chamber was very important during mating. For *M. vitrata*, the relative humidity needs to be maintained at 85–90% or else adults fail to mate.

In conclusion, this study demonstrated that the new artificial diet supported a high rate of survival comparable to those reared on the host-plant (cowpea). The new artificial diet was suitable for the continuous rearing of *M. vitrata* to produce uniform insects of predictable performance without a loss of vigor or a decline in reproductive potential.

### Acknowledgements

We are grateful to anonymous referees for their valuable comments on this manuscript,

and also thank Lian Feng, Ting Fu, and Hai-Yang Wang of Huazhong Agricultural University for their technical assistance in this study. The research was supported by the grant of the Natural Science Foundation of China (30971911) and the Special Fund for Agro-scientific Research in the Public Interest (201203036).

### References

- Afun JVK, Jackai LEN, Hodgson CJ. 1991. Calendar and monitored insecticide application for the control of cowpea pests. *Crop Protection* 10(5): 363–370.
- Agnew P, Hide M, Sidobre C, Michalakis Y. 2002. A minimalist approach to the effects of density-dependent competition on insect life-history traits. *Journal of Economic Entomology* 27(4): 396–402.
- Albert PJ, Cearley C, Hanson F, Parisella S. 1982. Feeding responses of eastern spruce budworm larvae to sucrose and other carbohydrates. *Journal of Chemical Ecology* 8(1): 233–239.
- Asante SK, Tamò M, Jackai LEN. 2001. Integrated management of cowpea insect pests using elite cultivars, date of planting and minimum insecticide application. *African Crop Science Journal* 9(4): 655–665.
- Blanco CA, Portilla M, Abel CA, Winters H, Ford R, Streett D. 2009. Soybean flour and wheat germ proportions in artificial diet and their effect on the growth rates of the tobacco budworm, *Journal of Insect Science* 9:59. Available online: <http://insectscience.org/9.59>
- Chi YC, Sakamaki Y, Tsuda K, Kusigemati K. 2004. Simple artificial diets for mass-reproduction of the legume pod borer, *Maruca*

*vitrata* (Lepidoptera: Crambidae). *Japanese Journal of Applied Entomology and Zoology* 48(4): 315–320.

Cohen AC. 2004. *Insect Diets: Science and Technology*. CRC Press.

Dadd RH. 1957. Ascorbic acid and carotene in the nutrition of the desert locust, *Schistocerca gregaria* (Forsk). *Nature* 179: 427–428.

Dunkel FV, Read NR. 1991. Review of the effect of sorbic acid on insect survival in rearing diets with reference to other antimicrobials. *American Entomologist* 37(3): 172–178.

Foster SP, Ayers RH. 1996. Multiple mating and its effects in the lightbrown apple moth, *Epiphyas postvittana* (Walker). *Journal of Insect Physiology* 42(7): 657–667.

Gibbs M, Lace LA, Jones MJ, Moore AJ. 2004. Intraspecific competition in the speckled wood butterfly *Pararge aegeria*: Effect of rearing density and gender on larval life history. *Journal of Insect Science* 4:16. Available online: <http://insectscience.org/4.16>

Gindin G, Kuznetsova T, Protasov A, Yehuda SB, Mendel Z. 2009. Artificial diet for two flat-headed borers, *Capnodis* spp. (Coleoptera: Buprestidae). *European Journal of Entomology* 106(4): 573–581.

Ichiki RT, Dung DT, Takano S-I, Nakamura S. 2009. Artificial diets for rearing the coconut hispine beetle *Brontispa longissima* and its parasitoid *Asecodes hispinarum*. *Journal of Applied Entomology* 133(7): 539–545.

Ito T. 1961. Effect of dietary ascorbic acid on the silkworm, *Bombyx mori* L. *Nature* 192: 951–952.

Jackai LEN, Daoust R. 1986. Insect pests of cowpeas. *Annual Review of Entomology* 31(1): 95–119.

Jackai LEN, Raulston JR. 1982. Rearing two maize stem borers and a legume pod-borer on artificial diet. *International Institute for Tropical Agriculture Research Briefs* 3(1): 1–6.

Jackai LEN, Raulston JR. 1988. Rearing the legume pod borer, *Maruca testulalis* Geyer (Lepidoptera: Pyralidae) on artificial diet. *Tropical Pest Management* 34(2): 168–172.

Jin JX, Zhu F, Wang XP, Lei CL. 2010. Development and reproduction of *Maruca testulalis* on an artificial diet. *Chinese Bulletin of Entomology* 47(1): 59–62.

Ke LD, Fang JL, Li ZT. 1985. Bionomics and control of the legume pod-borer *Maruca testulalis* Geyer. *Acta Entomologica Sinica* 28(1): 51–59.

Lingappa S, 1987. Importance of physical properties of artificial diet for the pink stem borer, *Sesamia inferens* Walker. *Insect Science and its Application* 8: 65–69.

Liu CY, Hwang JS. 2006. Rearing of the legume-pod borer, (*Maruca vitrata*) on an improved semi-synthetic artificial diet. *Plant Protection Bulletin (Taichung)* 48(1): 9–16.

Michereff MFF, Vilela EF, Filho MM, Nery DMS, Thiébaut JT. 2004. Effects of delayed mating and male mating history on the reproductive potential of *Leucoptera coffeella* (Lepidoptera: Lyonetiidae). *Agricultural and Forest Entomology* 6(3): 241–247.

- Montenegro de Sales FJ. 1972. Effect of dietary ascorbic acid on the development of beet armyworm, *Spodoptera exigua* (Hübner). *Ciênc. Agron. Fortaleza* 2(1): 13–17.
- Onyango FO, Ochieng’-Odero JPR. 1993. Laboratory rearing of the legume pod borer, *Maruca testulalis* Geyer (Lepidoptera: Pyralidae) on a semi-synthetic diet. *Insect Science and its Application* 14(5/6): 719–722.
- Proshold FI. 1996. Reproductive capacity of laboratory-reared gypsy moths (Lepidoptera: Lymantriidae): effect of age female at time of mating. *Journal of Economic Entomology* 89(2): 337–342.
- Sattar M, Fatima B, Ahmed N, Abro GH. 2007. Development of larval artificial diet of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). *Pakistan Journal of Zoology* 39(2): 103–107.
- Savopoulou-Soultani M, Stavridis DG, Vassiliou A, Stafilidis JE, Rakiadis I. 1994. Response of *Lobesia botrana* (Lepidoptera: Tortricidae) to levels of sugar and protein in artificial diets. *Journal of Economic Entomology* 87(1): 84–90.
- Sharma HC. 1998. Bionomics, host plant resistance, and management of the legume pod borer, *Maruca vitrata*-a review. *Crop Protection* 17(5): 373–386.
- Singh P, Moore RF. 1985. *Handbook of Insect Rearing*, Volume 1. Elsevier.
- Singh SR, Allen DR. 1980. Pests, diseases, resistance, and protection in cowpea. In: Summerfield RJ, Bunting AH, Editors. *Advances in Legume Science*. pp. 419–443. Royal Botanic Gardens.
- Stewart JG, Philogène BJR. 1983. Reproductive potential of laboratory-reared *Manduca sexta* (Lepidoptera: Sphingidae) as affected by sex ratio. *The Canadian Entomologist* 115(3): 295–298.
- Vanderzant ES. 1974. Development, significance, and application of artificial diets for insects. *Annual Review of Entomology* 19(1): 139–160.
- Wang YN, Zheng ZQ, Zhou YS. 1984. *Handbook of Insect Artificial Diets*. Shanghai Science and Technology Press.
- Willis RB, Allen PR. 1999. Measurement of amorphous ferric phosphate to assess iron bioavailability in diets and diet ingredients. *Analyst* 124(3): 425–430.