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Laboratory Rearing of the Legume Pod Borer, *Maruca vitrata* Fabricius (Lepidoptera:Pyralidae) on Mung Bean Sprouts

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Abstract

The suitability of mung bean sprouts (*Vigna mungo* (L.) Hepper) as a diet to rear larvae of *Maruca vitrata* Fabricius was tested for the first time in laboratory. Its effect on the growth and development of the pest was also determined for two generations. Survival of larvae was high (>87%) on sprout diet in both generations (F1 and F2) but low (52.5%) when reared on fresh bean flowers and pods (F1). The average lifespan of F2 males and females from sprout diet was 11.5 days and 13.6 days respectively. The F1 female from sprout diet was more fecund (241.3 eggs/female) than that from flowers and pods (127.5 eggs/female) and the F2 female was most fecund (359.4 eggs/female). One kilogram of dry mung bean seeds can yield up to 1200 pupae.

A novel and cheap method to rear *M. vitrata* on mung bean sprouts was developed for a regular supply of eggs, larvae, pupae and adult moths for research purposes.

Key words: *Maruca vitrata*, mung bean sprout, larval diet

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1. INTRODUCTION

The legume pod borer, *Maruca vitrata* Fabricius, is an important pest of bean in Mauritius and can cause up to 57% pod damage in untreated fields (Unmole, 2007). This pest has been little studied because of the difficulty in rearing *M. vitrata* in laboratory on artificial diet.

The first procedure to rear *M. vitrata* larvae was developed by Ochieng *et al*. (1981) on cowpea flowers and pods. Subsequently, other researchers used other natural food such as, fresh runner bean, French bean and broad beans. However, their use has drawbacks. They required additional facilities (land to grow continuously beans for supply of fresh flowers and pods) and these flowers and pods rot quite rapidly thus promoting fungal attack on established colony (Ochieng *et al.*, 1981; Hassan, 2007). Rearing on artificial diet was first undertaken by Jackai and Raulston (1982; 1988) and Ochieng and Bungu (1983). They encountered many difficulties during the rearing process. There was either a decline in larval performance over successive generations or inability to obtain adequate mating pairs. In 1993, Onyango and Ochieng'Odero, developed a semi-synthetic diet that sustained good larval growth and development over successive generations. However, rearing on artificial diet requires sophisticated equipment (e.g., autoclave, cooker, blender etc.) and diet ingredients that are expensive and not readily available locally.

Maruca vitrata is a major pest of mung bean (*Vigna mungo*) (Zahid *et al.*, 2008) which is locally available as dried seeds in supermarkets. Such seeds germinate easily when soaked in water. Preliminary tests have shown that larvae of *M. vitrata* could feed on mung bean sprouts thus providing the basis for a simple and cheap rearing diet.

This paper reports on the investigation on the performance of *M. vitrata* reared on mung bean sprouts in laboratory.

2. MATERIALS AND METHODS

Rearing was conducted at ambient laboratory conditions (27 $\pm$ 2ºC temperature; 60-80% R.H and with a light regime 12L:12 D) at the Entomology Division (Agricultural Research and Extension Unit) from 2006-2009.

Source of *Maruca vitrata*

Field collected larvae were reared on young bean pods in plastic trays (32 cm x 22 cm) with a layer of sand. Recovered pupae were held in a nylon mesh cage (30 cm x 30 cm x 30 cm). Emerging moths were collected in individual plastic cups (30-mL) and sexed.

Twenty pairs of moths were set in a transparent cylindrical plastic container (15 cm wide x 20 cm long) cut at both ends and fitted with white muslin cloth as oviposition substrates. They were fed on 10% sucrose solution. Oviposition substrates were changed every 24 h. Collected substrates with eggs were placed in a plastic container for hatching to provide the first laboratory generation (F1). These larvae were used to test the effect of diets on growth and development of *M. vitrata*.

Newly hatched larvae (n=200) from F1 were reared on mung bean sprouts. Emerged moths (1-day old) were set in 5 cylindrical plastic cages (15 pairs/cage). Recovered eggs were held in plastic containers for hatching to set the second generation (F2) on sprouts. Newly hatched larvae from F2 were used to test the effect of sprouts on growth and development of *M. vitrata*.

Preparation of mung bean sprouts

Mung bean seeds (100 g) were washed with tap water, soaked in 200 mL of water in a plastic container (20 cm x 20 cm) every 24 h for two days. The sprouting seeds were then washed; water drained and allowed to sprout for another 24 h. These were either used on same day or stored in the refrigerator at 5 $\pm$ 1ºC for 4 days. One hundred gram of mung bean seeds yielded about 260 g of sprouts. Five sets of sprouts (10/set) were weighed on an electronic balance. Ten sprouts weighed on an average 3.5 grams.

Source of fresh bean flowers and pods

Five weeks prior to experimentation, 20 potted bean plants were set fortnightly in a greenhouse (without pesticide) to procure a regular supply of fresh flowers and pods free from pests and pesticides.

Effect of mung bean sprouts and bean flowers and pods on growth and development of *Maruca vitrata*

Performance of larvae raised on mung bean sprouts

Twenty sprouts were placed in a 200-mL paper cup lined with four layers of paper towel. Ten newly hatched larvae (<24 h) from F1 generation were placed in the cup with a clean fine camel brush. All cups were examined daily. Paper towels, when too wet, were changed. Debris (frass and decomposed diet) were removed gently and 10 sprouts was added in each cup every 2 or 3 days. The experiment was conducted in four replicates.

The same procedures were followed to evaluate the performance with newly hatched larvae from F2 generation on mung bean sprouts.

Performance of larvae raised on bean flowers and pods

The experiment was set as per method described above but fresh flowers and pods were used instead of sprouts. Twenty flowers were placed in each cup with 10 larvae (<24 h) from F1 generation. All cups were examined daily. Paper towels, when too wet, were changed. Debris was removed gently and 10 fresh flowers and five young pods were added in each cup every 2 days. The experiment was conducted in four replicates.

Newly formed pupae were collected from each diet (F1 and F2 on sprout diet and F1 on flowers and pods) cup and placed in individual plastic containers (30-mL). They were examined daily and emerging moths were recorded.

Performance of moths raised from larvae fed on mung bean sprouts and fresh bean flowers and pods

Emerging moths from each diet (F1 and F2 on sprout diet and F1 on flowers and pods) were sexed. One pair (1-day old) from each diet was placed in a plastic cage (10 cm long x 8 cm wide) fitted with white muslin cloth at its cut sides and fed on 10% sucrose solution. The pieces of muslin cloth were changed daily. Removed ones were examined under a binocular microscope (x 20) and eggs were counted. Eight such cages were set up for each diet and observed until death of the males and females.

Eggs on the muslin cloth from each diet (F_H-1st generation on fresh bean flowers and pods; F_1-1st generation on mung bean sprouts and F_2-2nd generation on mung bean sprouts) were counted under a microscope (x 20). Sets of 100 eggs from each group were kept in individual 100-mL plastic containers. Hatched larvae were recorded daily until hatching stopped. The experiment was repeated four times.

3. RESULTS

Effect of mung bean sprouts and bean flowers and pods on growth and development of *M. vitrata* larvae

Larval survival in F1 and F2 generation on sprout diet was comparatively higher (> 87%) than that (52.5%) on bean flowers and pods (Table 1). Moth emergence from pupae on both diets, irrespective of generation was above 85%. The duration of the pupal period for the F2 generation was shorter (8.5 $\pm$ 0.1) than that the F1 (10.2 $\pm$ 0.2) on the same diet and on bean flowers and pods (10.1 $\pm$ 0.2). A sex ratio (M: F) of 1:1 was observed in both diets.

About 21g of sprouts (equivalent to 60 sprouts) yielded up to 10 pupae. There was no microbial infection in any diet cup.

Adult lifespan and female fecundity

Male and female moths from the F2 generation on sprout diet lived longer than those of F1 raised on both diets (Table 1). All mated females, irrespective of diet and generation, laid eggs as from the fourth day after emergence until the thirteenth day. F2 females from sprout diet were more fecund (359.4 ± 51.2) than F1 females from same diet (241.3 ± 54.6) and F1 females from bean flower and pods (127.5 ± 30.3).

Table 1. Larval survival, egg hatchability, adult longevity and fecundity of *Maruca vitrata* on bean flowers and pods and mung bean sprouts in laboratory

Diet	Larval survival (%)	Larval period (days)	Pupal period (days)	Adult longevity (days)	Fecundity (mean no. of eggs/female)	Egg hatchability (%)
F_H	52.5	13.0 ± 0.2	10.1 ± 0.2	♀ 11.0 ± 0.7 ♂ 9.5 ± 0.7	127.5 ± 30.3	79.5 ± 2.4
F_1	87.5	12.3±0.1	10.2 ± 0.2	♀ 11.4 ± 0.9 ♂ 9.9 ± 1.0	241.3 ± 54.6	88.8 ± 3.1
F_2	90.0	13.0 ± 0.2	8.5 ± 0.1	♀ 13.6± 0.8 ♂ 11.5± 1.0	359.4 ± 51.2	90.5 ± 1.3

F_H - 1st generation on fresh bean flowers and pods; F_1 - 1st generation on mung bean sprouts and F_2 - 2nd generation on mung bean sprouts

Egg hatching occurred on the third day. It was higher in F1 and F2 generation on sprout diet than that in F1 generation on bean flowers and pods (Table 1).

4. DISCUSSION

Results of this study have demonstrated the possibility of developing a simple procedure to rear *M. vitrata* at low cost for research purposes in laboratory. Locally available materials (e.g., mung bean as larval diet) and simple equipment (e.g., plastic container as rearing cage) were used as alternatives to those previously used by other researchers. Egg laying by females raised on sprout diet and on flowers and pods started on the fourth day after their emergence and similar observation was made by Ochieng and Bungu (1983) with females from artificial diet.

The sprout diet was appropriate for growth and development of *M. vitrata* larvae. This is so because the sprouts contain most of the components (proteins, fibre, vitamins and minerals) (Peavy and Peary, 1993) listed in the artificial diet of Onyango and Ochieng'Odero (1993) required for larval

development. It also contains vitamin E and C (ascorbic acid) that are not present in soy bean flour (base ingredient) but are incorporated as a separate component in the artificial diet. It was even not necessary to use preservative ingredients (sorbic acid, formaldehyde and methyl-4-hydroxybenzoate) in the sprout diet because microbial contamination was successfully avoided by proper sanitation in laboratory. Phagostimulants (e.g., cowpea flower powder used to induce feeding in artificial diet) were not required because larvae readily fed on sprouts. Overall, *M. vitrata* larvae reared on sprout diet developed better than those reared on bean flowers and pods.

In this study, larval survival on bean flowers and pods (52.5%) was better than those reported by Ochieng *et al.* (1981) on cowpea flowers (36%) and pods (27%). According to Ochieng *et al.* (1981), the low larval survival on pods could be related either to the weak mouthparts of larvae or lack of nutritional value of pods to them. On the other hand, flowers may not contain appropriate nutrients for late instars that normally feed on young or mature pods. This explains their low survival on flowers. Findings of this result supports the concept of Ochieng *et al.* (1981) that larval survival can be best obtained by rearing first and second larval instars on flowers and late instars on pods.

The survival and development time of larvae on sprouts was comparable to that on synthetic diet of Onyango and Ochieng'Odero (1993) and Liu and Hwang (2006). The duration of the pupal period from the F1 generation on sprouts and on bean flowers and pods was comparatively longer than those observed on artificial diets and cowpea flowers (Ochieng *et al.*, 1981; Onyango and Ochieng'Odero, 1993; Liu and Hwang 2006). However, it was shortened in F2 generation on the sprout diet indicating that larvae could become nutritionally more adapted to the diet.
The high fecundity of females raised on sprouts is also comparable to those raised on semi-synthetic diets by Onyango and Ochieng'Odero (1993) and Liu and Hwang (2006). The increase in egg-laying potential of F2 females on the sprout diet (Table 1) suggests that female fecundity was not adversely affected.

As a result of a high rate of survival and moth emergence (> 85%) with a sex ratio remaining at 1:1, mating pairs were easily obtained. This is crucial because the success in rearing of *M. vitrata* in laboratory relies heavily on the production of sufficient numbers of males and females. Otherwise, inadequate mating pairs hinder the rearing process. Such a problem was encountered by Jackai and Raulston (1982, 1988) during their study on soybean and cowpea diets. There had been a decline in larval performance

on chickpea diet over successive generations (Ochieng and Bungu, 1983) but not on the soybean diet with cowpea flower powder (Onyango and Ochieng'Odero, 1993). In this study, the good larval and adult performance on sprout diet in the second generation indicates that it is appropriate to rear *M. vitrata* in laboratory. However, the success of rearing over several generations remains to be elucidated.

This is the first report on the successful rearing of *M. vitrata* larvae on mung bean sprouts. Mung bean is cheap (<two US dollar/kg) and is locally available as dried seeds. One kilogram of seeds can yield up to 1200 pupae while one litre of artificial diet produces 400 pupae (Onyango and Ochieng' Odero, 1993).

A novel and cheap method to rear *M. vitrata* on mung bean sprouts was developed in laboratory. It consists of basically the following sequential steps: (1) rearing field collected larvae on sprouts in a plastic container with a layer of sand and nylon netting with regular holes (5 mm dia.) (as per Ochieng *et al.*, 1981) (2) collecting and holding pupae in a nylon mesh cage, (3) cutting small plastic transparent cylindrical plastic container on both sides and fitting with muslin cloth on cut sides as egg substrates to hold moths, (4) setting up of an adult colony, and (5) collecting and seeding eggs on sprouts.

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