Effectiveness of a modified diet for laboratory rearing of the cowpea pod borer *Maruca vitrata* Fabricius (Lepidoptera: Crambidae)

Fousséni Traoré[1], Antoine Waongo[2], Niango Malick Ba[3], Clémentine Dabiré-Binso[2], Mumuni Abudulai[4], Antoine Sanon Poster[5]

Abstract

In order to mass rear *Maruca vitrata* under laboratory condition, a study was carried out and aimed to modify the artificial diet commonly used to rear *Ostrinia nubilalis*. The modification consisted of the addition of ingredients, and/or the reduction of their quantities. Four diets including three artificial formulations named artificial diet 1 (AD1), artificial diet 2 (AD2), artificial diet 3 (AD3) and cowpea flowers, natural diet were tested. To prepare one liter of food substrate, diet, cowpea flour, aureomycin (14.1 %) and agar were weighted and dissolved in water to obtain a solution. The different ingredients of the three diets were as followed: Artificial diet 1 (AD1) (Diet: 125.70 g; Agar agar: 17.2 mg ; Water : 910 ml); Artificial diet 2 (AD2) (Diet : 99.74 g ; Agar agar : 14.8 mg ; Water : 1000 ml) and Artificial diet 3 (AD3) (Diet : 99.74 g ; Cowpea flours : 13.16 g ; Aureomycin (14 % active ingredient) : 5mg ; Agar agar : 14.8 mg ; Water : 1000 ml). The evaluation of the artificial diet quality consisted of the determination of biological parameters of *M. vitrata* reared on artificial diet compared with those of the same species reared on natural diet of cowpea flowers. Results showed that the larval mortality was lower and similar on the natural diet and AD3 than on AD1 and AD2. Adults' emergence rates were also higher and similar on the natural diet and AD3 than on AD1 and AD2. In addition, the larval development time, the size of pupae, the fertility and the longevity of males and females were similar on AD3 and the natural diet. However, pupal weight and the fecundity of females were higher on the natural diet than those obtained on AD3. The AD3 showed comparable performance to the natural diet and therefore can be used for *M. vitrata* continuous rearing under laboratory conditions.

Keywords: *Maruca vitrata*, diet, ingredients, mass rearing, Burkina Faso

Résumé

Dans le but d'élever en masse *Maruca vitrata* dans les conditions de laboratoire, une étude a été entreprise pour améliorer le substrat artificiel habituellement utilisé pour élever *Ostrinia nubilalis* Hübner. La modification a consisté en l'adjonction d'ingrédients, et la réduction de la quantité de certains ingrédients. Quatre milieux de culture dont trois artificiels nommés substrat artificiel 1 (AD1), substrat artificiel 2 (AD2), substrat artificiel 3 (AD3) et les fleurs de niébé, substrat naturel ont été testés. Pour préparer un litre de substrat, la diète, la farine de niébé, l'auréomycine (14.1%) et l'agar ont été pesé et dissout dans l'eau pour obtenir une solution. Les differents ingrédients des trois substrats artificiels ont été les suivants : AD1 (Diet : 125,70 g ; Agar agar : 17,2 mg ; eau : 910 ml) ; AD2 (Diet : 99,74 g ; Agar agar : 14,8 mg ; eau : 1000 ml) et AD3 (Diet : 99,74 g ; farine de niébé : 13,16 g ; auréomycine (14% matière active) : 5mg ; Agar agar : 14,8 mg ; eau : 1000 ml). L'évaluation de la qualité de l'alimentation artificielle a consisté en la détermination des paramètres biologiques de *M. vitrata* élevé sur le régime alimentaire artificiel par rapport à ceux de la même espèce élevée sur le régime alimentaire naturel. Les résultats obtenus ont montré que le taux de mortalité larvaire était faible et similaire sur le substrat naturel et le substrat AD3 que sur les substrats AD1 et AD2. Les taux d'émergence étaient plus élevés et similaires sur les substrats naturel et AD3 que sur les substrats AD1 et AD2. La durée de développement larvaire, la taille des chrysalides, la fertilité et la longévité des mâles et des femelles n'ont montré aucune différence significative tandis que le poids des chrysalides et la fécondité des femelles étaient plus élevés sur le substrat naturel que ceux des insectes élevés sur le substrat AD3. Le résultat obtenu avec le substrat AD3 a montré une performance comparable à celle du substrat naturel ; de ce fait, peut être utilisé pour un élevage continu de *M. vitrata* en condition de laboratoire.

Mots-clés : *Maruca vitrata*, alimentation, ingrédients, élevage de masse, Burkina Faso

1. Institut de l'Environnement et de Recherches Agricoles (INERA) BURKINA FASO
2. Laboratoire Central d'Entomologie Agricole de Kamboinsé, Institut de l'Environnement et de Recherches Agricoles BURKINA FASO
3. International Crops Research Institute for the Semi-Arid Tropics, Niamey NIGER
4. CSIR-Savanna Agricultural Research Institute GHANA
5. Laboratoire d'Entomologie Fondamentale et Appliquée, UFR/SVT, Université Ouaga I Pr Joseph KI-ZERBO

INTRODUCTION

In Burkina Faso, *Maruca vitrata* Fabricius is reported as a major pest of cowpea (Jackai and Adalla, 1997; Dabiré, 2001). Damages due to this pest are variable from one season to another, but are especially high in the western region which is characterized by a higher rainfall favourable for the pest (Ba *et al*., 2009; Baoua *et al*., 2011). Damage to cowpea, by *M. vitrata* is due to the feeding of the larvae on the tender parts of the stem peduncles, flower buds, flowers and pods (Singh and Jackai, 1988). In order to develop or improve management methods against this pest, bio-ecological studies were initiated and conducted at the Laboratoire Central d'Entomologie Agricole at INERA-Farako-Bâ. These studies are essential to better understand the pest. Therefore, the establishment of an efficient rearing system for a permanent colony of insects in the laboratory is necessary.

The rearing of the pest in laboratory requires the availability of good quality food. In general, artificial substrates used for insects rearing in laboratories are produced and marketed by firms for rearing a specific pest. However, the use of these

substrates to rear another pest may sometimes be inadequate. In our work, we used an artificial diet produced and marketed by Bio-Serv, for rearing maize stem borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Pyralidae).

Apart from the unsuitability of using diet meant for another insect, rearing insects on artificial diets is an expensive process and a challenge for developing countries where sufficient research funds are unavailable (Assemi *et al*., 2012). Moreover, the availability of the natural diet such as cowpea flowers for *M. vitrata* can be costly because of the need to continuously plant and water cowpea to get flowers. These challenges can lead to poor studies of insect pests that pose economic threats to agricultural production (Ahmed 1983). Good knowledge of insect rearing procedure in the laboratory is important to study their life cycle, their behavior, their feeding habits, their susceptibility or resistance to chemical pesticides and to biological control agents (Rezapanah *et al*., 2008). This study aims to improve the artificial diet composition of the maize stem borer *O. nubilalis*, in order to massively rear *M. vitrata*. Therefore, different formulations of artificial diet were compared to identify the most suitable and nutritious diet for *Maruca* larvae production. The influence of artificial diet on the insect biodemographic parameters was examined in comparison with those of insects reared on natural diet as control.

MATERIALS AND METHODS

Origin, capture techniques and maintenance of insects in the laboratory

Insects used in this study were collected from the biotype of Farako-Ba, captured using light trap during the rainy season and reared in the laboratory. Captures were made at the research station of Farako-ba, Burkina Faso (latitude: 11811N, longitude: 048180W) during the 2010 rainy season. Burkina Faso has a unimodal rainfall pattern and a rainy season that lasts from June to October. A total rainfall of 1281 mm was recorded in 2010 at Farako-Ba.

The trap was a wire cage (1,38 m width × 1.93 m height), which rested on metal support set 2,43 m above ground level. At the top of the cage there was a funnel-shaped opening surmounted by a hat-shaped device. This light trap utilized a 500-W mercury vapor white incandescent bulb positioned above the wire mesh cage, and powered through a source of the National Electricity Company of Burkina (110-220 volts). The light was turned on daily from 6 p.m. to 6 a.m. Adults attracted by the light fell into the trap through the funnel. Every morning, the trap was emptied, and all *M. vitrata* adults were collected and placed into plastic 30 cc vials. They were brought back to the laboratory and placed into cylindrical cages to reduce their stress and for eventual mating. After 24 hours, the females were removed and placed in vials for oviposition on the inside wall at a rate of three females per vial. The next morning, eggs were collected and incubated in the room under conditions of temperature of 22° to 26° C, a relative humidity of 70 % to 90 % RH and a 12/12h photoperiod. At hatching, larvae were maintained on germinated cowpea seeds diet.

Rearing of M. vitrata on germinated cowpea seeds

Five hundred (500) grams of cowpea seeds were weighted and soaked in 10 % of sodium hypochlorite solution for 2 to 3 hours. The seeds were then removed from the water and kept until the next day. They were finally deposited as a thin layer on the lotus placed at the bottom of boxes of 500 cm^3. The boxes containing the germinated cowpea seeds were infested using first instar larvae. The boxes were closed with table-napkin (lotus) and circular wire mesh lids to facilitate insect respiration. The freshly germinated cowpea seeds were added when the old one was exhausted. When pupae were formed, they were collected using flexible forceps and kept in boxes of 500 cc for emergence.

When emerged, adults were kept in cages (30 cm × 30 cm × 90 cm) for 72 hours for mating. Subsequently, the females were placed in vials of 30 cc for laying. The eggs were collected daily and incubated at the laboratory conditions described earlier. At hatching, the process started again and repeated until the end of our test.

Determination of the dose of the artificial diet for mass rearing

The artificial diet 1 (AD1) was the standard diet supplied by Bio-Serv, USA (Bio-Serv product No. F9478B-M) for rearing maize stem borer, and adopted for *Maruca* rearing (Table 1). The compositions of artificial 2 (AD2) and artificial 3 (AD3) were modified (Table 1), inspired by that developed by Jackai and Raulston (1988) to rear *M. vitrata* in laboratory. One hundred (100) hatching larvae were placed per box of 500 cm^3 containing 150 ml of artificial diet or 100 g of cowpea flowers. Five replications were made for each treatment. For the treatment consisting of cowpea flowers, larvae were deposited on the lotus paper placed at the bottom of the box. The bottom of the box was drilled with six small holes to drain water from sweating flowers. After infesting the diet with hatching larvae, the boxes were closed with tissue and lid. Daily, the flower diet was renewed by moving larvae into new boxes of same size and containing the same amount of food. When formed, pupae were carefully extracted using flexible tongs and larval mortality and other biological parameters including larval development time, pupal size and weight, adult emergence rate, fecundity and longevity of males and females were measured.

Preparation of artificial diet for infestation with M. vitrata hatching larvae

To prepare one liter of food substrate diet, cowpea flour and aureomycin (14.1%) were weighed (Table 1) using a sensitive balance and put together. Agar was weighed and set apart from the other ingredients. Water was measured using a graduated cylinder with a capacity of 1000 ml. The agar was then dissolved in the water to obtain a solution. The agar solution obtained was boiled for one minute and then allowed to cool to about 50°C. At this temperature, all the solid ingredients were added. The mixture was blended for 3 minutes using an electric blender. The artificial food obtained was immediately served in the rearing boxes of 500 cm^3. The

boxes were closed using lotus and box cover lid.

For the artificial diet infestation, the surface of the diet was slightly abraded using a sterilized fork. *Maruca* hatching larvae were then placed on the diet. The boxes were closed with the lotus and the lid and kept under laboratory conditions of 22 - 26 °C of temperature, 70-90% relative humidity and 12/12 hr photoperiod. Fifteen days after infestation, the pupae formed were carefully extracted using flexible tongs. Because of high larval mortality on AD1 and AD2 due to their poor quality, only the AD3 was used to study the survival and biological parameters which included larval mortality, hatchability of eggs, length of larval development, pupal weight and female fecundity, and compared with those of insects reared on the natural substrate. The larval mortality and pupation rate were the main criteria used to evaluate the nutritional quality of the artificial diets.

Data analysis

Data on the insects' biological parameters measured were analyzed using analyses of variance (SAS Institute, version 8.1, 2001). The mean comparisons were achieved using Student-Newman-Keuls (SNK) test at the significance level of 5 %.

RESULTS

The amount of the different ingredients used in the composition of each artificial diet is shown in Table 1.

The mean larval mortality rate which were 97.3 and 78 % on AD1 and AD2 respectively were higher than those obtained on AD3 and the natural diet which were respectively 17.3% and 17.90 (Table 2). The adults' emergence rates which were 1% for AD1 and 8.20% for AD2 were respectively lower than those obtained on AD3 and the natural diet which were respectively 80.40% and 79.70% (Table 2). The mean larval mortality rate from cowpea flowers, the control was lower and similar to those from AD3. In addition, adults' emergence rates were higher and similar on both diets.

Table 1 : Composition of artificial diet tested
Tableau 1 : Composition du substrat artificiel testé

Ingredients	Different artificials diets tested		
	Artificial diet 1 (AD1)	Artificial diet 2 (AD2)	Artificial diet 3 (AD3)
Diet quantity (g)*	125.70	99.74	99.74
Cowpea flour (g)	-	-	13.16
Aureomycin (mg) **	-	-	5
Agar agar (g)	0.0172	14.8	14.8
Water (ml)	910	1000	1000

** Diet dry mix composition is: Wheat germ, Salt Mix, Cholesterol, Methyl Paraben, Sorbic Acid, Casein, Sucrose, Linseed Oil.*
*** 14% active*

The larval developmental time of insects reared on AD3 was not significantly different from those reared-on cowpea flowers (Table 3) (F=1.91; p=0.18). Also, the size of pupae of *M. vitrata* reared on cowpea flowers were similar to those reared on AD3 (F=2.32; p=0.15). However, the weights of the pupae were higher on the natural diet than on AD3. (F=59.07, p<0.0001). Also, the number of eggs laid by females from cowpea flowers was higher than that laid by females from AD3 (F=17.31; p=0.0006). However, there were no significant differences observed between the longevity of the males and females from both diet (F=0.16; p=0.69 and F=0.64; p=0.44).

Table 2: Mortality and *M. vitrata* adult emergence rate reared on artificial diet
Tableau 2 : Taux de mortalité et émergence des adultes de *M. vitrata* élevés sur le substrat artificiel

Substrats	Parameters measured	
	Mean larval mortality rate (%)	Mean emergence rate (%)
AD 1	97.30 ± 0.26 a	1 ± 0.14 c
AD 2	78.00 ± 1.6 b	8.20 ± 0.71 b
AD 3	17.30 ± 0.47 c	80.40 ± 0.33 a
Cowpea Flowers (control)	17.90 ± 0.54 c	79.70 ± 0.21 a
Statistic	F= 2016.52; p<0.0001	F=11051; p<0.0001

Means values (±SE) within a column followed by the same letters are not significantly different (ANOVA followed by SNK test at the 5% level).

DISCUSSION

Table 3: Mean values (±SE) of different biological parameters of *M. vitrata* fed on natural and artificial diets
Tableau 3 : Valeurs moyennes ((±SE) des differents paramètres biologiques de *M. vitrata* nourris sur les substrats, naturel et artificiel

Biological paramaters	Cowpea flowers (control)	Artificial diet (AD3)	Statistic
Larval development time (days)	18.10 ± 0.27 a	18.70 ± 0.33 a	F=1.91; p=0.18
Pupal size (mm)	11.80 ± 0.25 a	11.30 ± 0.21 a	F=2.32; p=0.15
Pupal weight (n= 50) mg	25.60 ± 0.34 a	21.80 ± 0.36 b	F=59.07; p<0.0001
Emergence rate (%)	92.70 ± 3.61 a	90.83 ± 2.43 a	F=1.10; p=0.30
Fecundity (n= 50)	625.30 ± 17.34 a	510.10 ± 21.59 b	F=17.31; p=0.0006
Male longevity (days)	12.10±2.92 a	12.50±2.54 a	F=0.16; p=0.69
Females longevity (days)	13.70±2.45 a	13.11±2.43 a	F=0.64; p=0.44

Means values (±SE) within a line followed by the same letters are not significantly different (ANOVA followed by SNK test at the 5% level).

The rearing of *M. vitrata* was possible after the diet composition originally meant for rearing the maize stem borer *O. nubilalis* was modified, allowing continuous availability of the insect under laboratory conditions. Abbasi *et al*. (2007), Hamed and Nadeem (2008) and Assemi *et al.* (2012) have successfully reared *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) after diet modification. Diet improvement is essential for successful rearing of insects under laboratory condition (Cohen, 2003). According to the same author, this improvement of food quality must be done following four criteria: viz nutritional value, sensory qualities, availability and stability. The ingredients of the artificial diet used in insects rearing play individual roles following their quality and quantity (Assemi *et al*., 2012). In the present

study, the basic artificial substrate contained stabilizers with specific roles, which was improved with the addition of locally available cowpea flour and aureomycin.

The low mortality rate recorded on the artificial diet 3 (AD3) was probably due to its nutritional quality. In previous studies, Assemi *et al*., (2012) reported that an antibiotic such as penicillin, and agar added to 14 g per liter of diet improved the quality of diet. In our study, we added aureomycin, a similar antibiotic as penicillin and agar up to 14.8g. Indeed, from artificial diet 1 to the artificial 3, the amount of agar was increased and coupled with the addition of cowpea flour and aureomycin improved the artificial diet at two levels: (1) adding cowpea flour may have brought the quality of the artificial diet close to that of the natural diet of the insect, (2) adding aureomycin, as all of antibiotics may have prevented bacterial growth on the artificial diet facilitating the intake of food by larvae.

The larval development time was similar on artificial diet 3 and on the natural diet. This finding is in contrast with the result reported by Chi *et al.* (2004) after testing 12 artificial diets on the same insect species. Theses authors found that the larval developmental time was significantly longer in all of the artificial diets. Our result suggests that the quality of the improved artificial diet and the natural diet was similar. Further, in our study, the size of pupae was similar between artificial diet 3 and the natural diet but the weights were higher on the natural diet which is contrary to the finding of Chi *et al.,* (2004). They reported a higher pupal weight on the artificial diet than on the natural diet. This weight gain on artificial diet in their study may be related to the amount of cowpea flour added to the diet. The same authors add the flour of soybean and cowpea or Azuki in proportions of 10 to 70 % in the artificial diet. These amounts of natural ingredients were higher than the amount of cowpea flour (11 %) used in our study. Dabiré (2001) notes that poor quality of food results in decreased weight of insects. The results from our study therefore suggest that, although the artificial diet allowed the full development of the insect, it has lower nutritional quality than the flowers. Thus, the relatively short larval development time and high fertility of insects reared on the flower diet was likely the consequence of a better nutritional quality of the natural substrate. This corroborates the findings of Traoré *et al*. (2013).

Adult longevity of the insects from the artificial diets was not significantly different from that of the natural diet. However, the lower fecundity of females from the artificial diet showed that the artificial diet was of a lower quality than the natural diet. Based on the low mortality rate on AD3 comparable to the natural diet, the AD3 has been retained and currently used for *M. vitrata* mass rearing in Burkina Faso.

CONCLUSION

The results from this study showed that the artificial diet used to rear *O. nubilalis* initially unsuited for *M. vitrata* rearing can become suitable when adequately modified. The diet quality was made suitable by adding local and available ingredient, which is cowpea flour. The insects reared on the modified diet performed satisfactorily well with comparable developmental time to those reared on the natural cowpea flower diet. This study allowed the economic rearing of *M. vitrata* by reducing the quantity of the main compound in the original diet of *O. nubilalis* which is usually expensive and locally unavailable, in the modified artificial diet. With the modified diet, *M. vitrata* is presently reared in the laboratory.

ACKNOWLEDGEMENTS

The authors thank the technician Hervé BAMA for helping in the rearing of *M. vitrata* in the laboratory. The provision of the standard artificial diet used for the study by the African Agricultural Technology Foundation (AATF), is duly acknowledged.

REFERENCES

Abbasi B.H., Ahmed K., Khalique F., Ayub N., Liu H.J., Kazami S.A.R. and Nauman M., 2007. Rearing the cotton bollworm, *Helicoverpa armigera,* on a tapioca-based artificial diet. *Journal of Insect Science* 7,35. Available online, http://insectscience.org/7.35.

Ahmed K., 1983**.** Development of artificial diets for mass breeding of Lepidopterous insects. M. Phil. Thesis, University of Karachi, Karachi Pakistan

Assemi H., Rezapanah M., Vafaei-Shoushtari R. and Mehrvar A., 2012. Modified artificial diet for rearing of tobacco budworm, *Helicoverpa armigera*, using the Taguchi method and Derringer's desirability function. *Journal of Insect Science* 12,100. Available online: http://www.insectscience.org/12.100

Ba, N.M., Margam V. M., Dabiré L.C., Sanon A., McNeil J. N., Murdock L.L. and Pittendrigh B.R., 2009. Seasonal and regional distribution of the cowpea pod borer *Maruca vitrata* (Lepidoptera: Crambidae) in Burkina Faso. *International Journal of Tropical Insect Science* 29, 109–113.

Baoua I., Ba N.M., Agunbiade T.A., Margam V., Dabire´-Binso C.L., Sanon A. and Pittendrigh B.R., 2011. Potential use of *Sesbania pachycarpa* (Fabaceae: Papilionoideae) as a refugia for the legume pod borer *Maruca vitrata* (Lepidoptera: Crambidae). *International Journal of Tropical Insect Science* 31, 212–218.

Chi Y., Sakamaki Y., Tsuda K. and Kusigemati K., 2004. Simple artificial diets for mass-Reproduction of the legume pod borer, *Maruca vitrata* (Lepidoptera: Crambidae). *Jpn. J. Appl. Entomol. Zool*. 48: 315–320

Cohen A.C., 2003. Insect diets*: Science and Technology*. pp. 429. CRC Press

Dabiré C., 2001. Etude de quelques paramètres biologiques et écologiques de *Clavigralla tomentosicollis* STAL., 1855 (Hemiptera : Coreidae), punaises suceuses de gousses du niébé *Vigna unguiculata* (L) WALP. Dans une perspective de lutte durable contre l'insecte au Burkina Faso. *Thèse de Doctorat d'état*, Université de Cocody, 179p.

Hamed M. and Nadeem S., 2008. Rearing of *Helicoverpa armigera* (Hub.) on artificial diets in laboratory.

Pakistan Journal of Zoology 40, 447-450.

Jackai L.E.N and Adalla C.B., 1997. Pest management practices in cowpea: a review in: B.B. Singh, D.R. Mohan Raj., K.E. Dashiell, and L.E.N. Jackai (eds): Advances in cowpea research. *Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS).* IITA, Ibadan, Nigeria, 21, 240-258.

Jackai L.E.N. and Raulston J.R., 1988. Rearing the legume pod borer, *Maruca testulalis* Geyer (Lepidoptera: Pyralidae) on artificial diet. *Tropical Pest Management* 34, 168–172.

Rezapanah M., Shojai-Estrabragh S., Jehle J.A. and Huber J., 2008. Molecular and biological characterization of new isolates of *Cydia pomonella* granulovirus (CpGV) from Iran. *Journal of Pest Science* 81, 187-191.

SAS® 8.1. 2001. Qualification Tools Users Guide. SAS Institute Inc., Cary, NC.

Singh S.R. and Jackai L.E.N., 1988. The legume pod borer, *Maruca testulalis* (Geyer): past, present and future research. *Insect Science and its Applications* 9, l–5.

Traoré F., Dabire-Binso C.L, Ba N. M., Antoine Sanon A. and Pittendrigh B.R., 2013. Feeding preferences of the legume pod borer *Maruca vitrata* (Lepidoptera: Crambidae) larvae and suitability of different flower parts for larval development. *International Journal of Tropical Insect Science* 33, 107–113.