Journal of Asia-Pacific Entomology 21 (2018) 823–828

Contents lists available at ScienceDirect

Journal of Asia-Pacific Entomology

journal homepage: www.elsevier.com/locate/jape

# Fitness of Asian corn borer, *Ostrinia furnacalis* (Lepidoptera: Crambidae) reared in an artificial diet

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ARTICLE INFO

*Keywords:*
*Ostrinia furnacalis*
Mass rearing
Artificial diet
Fitness

ABSTRACT

The Asian corn borer, *Ostrinia furnacalis* (Guenée) (Lepidoptera: Crambidae) is an economically important pest of corn. Finding simple, cheap, and suitable rearing techniques of *O. furnacalis* is an urgent need to support research for management of this insect. This research aimed to determine the suitability of a read bean and rice bran-based artificial diet used for mass rearing of this insect since 2009.

The tested artificial diet was compared with the natural diet (sweet corn kernel) and each diet was tested in individual rearing method (one larva in each vial). The criteria used to justify the quality of diet and mass rearing procedure were based on the fitness of *O. furnacalis*. The degree of fitness was based on life history, growth, and development. In general, the fitness parameteres observed from *O. furnacalis* reared in the artificial diet at 25.7 ± 1.6 °C with 57.7 ± 3.8% RH, and L12:D12 were similar than those in the natural diet.

Therefore, the existing artificial diet and rearing procedure were considered suitable and qualified for *O. furnacalis*. It is important to periodically check the laboratory colony to ensure that they have similar fitness to those found in the natural population.

## Introduction

The Asian corn borer, *Ostrinia furnacalis* (Guenée) (Lepidoptera: Crambidae) is an important pest of corn in Indonesia. Beside causing damage to corn (*Zea mays*: Graminae), this insect also attacks other plants from the families of Cucurbitaceae, Malvaceae, Phytolaccaceae, Poaceae, Polygonaceae, Solanaceae, and Zingiberaceae (Nafus and Schreiner, 1991; Ishikawa et al., 1999). The larva of *O. furnacalis* feeds all parts of the corn plant at all stages of the plant growth (Nafus and Schreiner, 1987, 1991). When the larvae feed on the vegetative growth stages of corn, specific damage symptom is shown by parallel small holes in the shoot whorl. Subiadi et al., (2014) reported that single larva of *O. furnacalis* attacking the corn stem during the V10 phase would lead to yield loss of 4.94%. Furthermore, the number of egg masses laid per plant could range 7 – 9 (da Lopez et al., 2014; Subiadi et al., 2014).

Mass rearing is a process to produce a large number of insect using natural or artificial diet for different purposes, mainly as to study the biology of beneficial insects, to test insecticides, to produce biological control agents, to determine economic injury level of particular pests, to test the effect of *Bacillus thuringiensis* (Bt) toxins on the non-target pest (Singh, 1982; Subiadi et al., 2014; Pratiwi et al., 2016), and to provide a sufficient amount of insects for developing crop resistance to particular insects through conventional breeding schemes and the use of biotechnology. Previous research (Resilva et al., 2007; Muralimohan et al., 2009; Elvira et al., 2010; Pratiwi et al., 2016; Gao et al., 2017; Kim et al., 2017) reported that the mass rearing of *Bactrocera philippinensis, Pectinophora gossypiella, Spodoptera litura, O. furnacalis, Cnaphalocrocis medinalis, Drosophila suzukii*, either using artificial or natural diet could be used to improve control techniques for those pests.

A suitable artificial diet is needed in mass rearing to produce uniform insects for commercial purposes, such as companies involving in selling insects for screening insecticides, pheromones, host plant resistance, and for producing biocontrol agents or research (Cohen, 2001). Under certain circumstances, artificial diet often provide more benefits than natural diet to rear particular insects, for example *Spodoptera litura*, *O. furnacalis*, *Chilo suppressalis* (Gupta et al., 2005; Martaya, 2007; Han et al., 2012), because the natural diet is more laborious, messy, needs a large space, and could produce only a limited-number of insects. Previous study (Ojala et al., 2005; Martaya, 2007) showed that mass rearing of *O. furnacalis* using the natural diet required replacement of the natural diet every one or two days, hence it is inefficient. In addition, the fitness of insect reared by artificial diet was as good as those fed with natural diet (Wang et al., 2013). Therefore, the increasing demand for a lot of insects necessitates the development of efficient and economical methods for rearing insects in the laboratory

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https://doi.org/10.1016/j.aspen.2018.06.003
Received 11 June 2017; Received in revised form 22 March 2018; Accepted 4 June 2018
Available online 08 June 2018
1226-8615/ 

with artificial diets. The goal of large-scale mass rearing is to produce the maximum number of insects with minimum labor, space, and cost (Singh, 1982), therefore finding a simple and cheap artificial diet that produces insect with good fitness is an essential need.

Mass rearing technique of *O. nubilalis* using artificial diet was first developed by Bottger (1942). Thirteen years later, the rearing technique of *O. furnacalis* was further developed by Kamano and Inoue using derivated-artificial diet based on Beck (Gahukar, 1976; Hirai and Legacion, 1985). Song et al. (1999) reported the success of using the non-agar diet to reduce the preparation procedure steps and the cost of diet in rearing this insect. Since then, research has been intensified by availability of artificial diet. For examples in Indonesia, an artificial diet was used to produce sufficient number of larvae to determine economic injury levels of *O. furnacalis* in the three phases of the corn growth in the field and its susceptibility to toxin Cry1Ac (Ei et al., 2008; Subiadi et al., 2014).

Martaya (2007) reported that a read bean and rice bran-based artificial diet was suitable for rearing one generation of the field-collected *O. furnacalis*. This artificial diet was originally developed and designed for *Helicoverpa armigera* (Budiharjo Sugiyanto, personal communication in 2001) and modified several times during 2005–2006 (Y.A. Trisyono, unpublished) before being tested for rearing of *O. furnacalis* (Martaya, 2007). To establish a laboratory-adapted population of *O. furnacalis*, sixty larvae were collected from Sleman, the Special Province of Yogyakarta, Indonesia in 2009. These collected larvae were reared using a diet and procedure as described in Martaya (2007). After four years of mass rearing in the laboratory without any additional field collection, the growth and development of *O. furnacalis* was determined to justify the fitness of the colony and the suitability of the diet. This step was considered very important to produce healthy larvae that would be used for many different entomological studies. Furthermore, this laboratory-adapted colony which had no exposure to any kind of toxicants will serve as a reference susceptible population when resistance studies are carried out.

## Materials and methods

### *Artificial diet preparation*

An artificial diet originally for *H. armigera* (Budiharjo Sugiyanto, personal communication in 2001) was modified and tested several times by Y. Andi Trisyono during the period of 2005–2006 (unpublished). After the developed diet was successful for rearing one generation of *O. furnacalis*, Martaya (2007) experimentally tested the developed artificial diet in supporting the growth and development of this insect. The final modification made by Y. Andi Trisyono in 2006 (Table 1) was reported to be suitable for rearing one generation of *O. furnacalis* of a field-collected population from the District of Sleman, the Special Province of Yogyakarta, Indonesia in 2007. Findings reported by Martaya (2007) was used as the bases to establish a laboratory-adapted population of *O. furnacalis.*

**Table 1**
The ingredients of the tested artificial diet for mass rearing of *Ostrinia furnacalis.*

| No. Ingredient | Quantity (g) |
|---|---|
| 1 Red bean | 130 |
| 2 Agar | 40 |
| 3 Rice bran | 160 |
| 4 Yeast | 62 |
| 5 Sorbic acid | 3 |
| 6 Methyl benzoate | 5 |
| 7 Tetracycline | 0.5 |
| 8 Vitamin mixture[a] | 5 |
| 9 Casein | 3 |

**Source:** Y. Andi Trisyono (2006, unpublished).
**Note:** The diet was prepared in 1000 mL aquadest.
[a] Vanderzant Product# F8045 was purchased from Bio-Serv Entomology Division (www.bio-serv.com/).

### *Ostrinia furnacalis colony*

The founding population of *O. furnacalis* (60 larvae) of the laboratory-adapted population was collected from the District of Sleman, the Special Province of Yogyakarta, Indonesia in 2009. Since the collection, *O. furnacalis* has been reared using the artificial diet as described above without any additional field population. The effect of long-term mass rearing of laboratory-adapted insects to their fitness is an important consideration when applying research to compare them (as susceptible population) to wild populations. Previous research showed that there was no reduction in average body weight, rate of growth, adult longevity or fecundity of *O. nubilalis* reared in the same artificial diet during 8 consecutive generations (Beck et al., 1968; Grayson et al., 2015). In contrast, changing in photoperiodic responses occured in the laboratory-adapted population of *Diatraea grandiosella* (Takeda and Chippendale, 1982). Considering these facts, the fitness of *O. furnacalis* was determined after being reared for four years in the laboratory using the artificial diet.

### *Experimental design*

#### *Treatments*

This experiment consisted of two treatments: the artificial diet and the sweet corn kernel (natural diet) as control. We used the kernel of the cob rather than stalk because the cob provided a faster development time (Nafus and Schreiner, 1991) and the technique was simpler. Each diet was tested by employing individual rearing method (one larva/vial) using plastic vials (4 cm in diameter and 4.7 cm in height). The experiment was carried out under the regimes of 25.7 ± 1.6 °C with 57.7 ± 3.8% RH (Termohygrometer Haar-Synt.Higro), and L12:D12.

#### *Setting the experiments and fitness observations*

The experiments were divided into three different batches to avoid the physiological stresses of *O. furnacalis* due to the observations and handlings.

#### *First batch: weight and head capsule width of newly hatched larvae*

The first batch was for measuring weight and head capsule width of newly emerged larvae. Twenty newly hatched larvae were probed carefully using a fine hair brush to ensure they were alive and active and weighed to calculate the average weight of first instars. Weighing was repeated five times using different larvae. From those twenty larvae, 16 larvae were taken randomly to measure head capsule width.

#### *Second batch: weight, head capsule width, and developmental time of the second to the fifth instars*

Five to the seven egg masses were put into a jar (14 cm in diameter and 6.2 cm in height) with a moist-tissue. The newly hatched larvae were transfered individually into each vial containing a cube of artificial diet (2 g/larva) or the kernel of the cob (regularly supplied with the fresh sweet corn kernel daily). Those larvae were reared in vials until reaching second, third, forth, and fifth instars, pupae, or adults for observation.

The second batch was intended to determine larval longevity (80 larvae, 16 samples per instar), and larval weight and larval head capsule width (64 larvae, 16 samples per instar). The weight and head capsule width of second to fifth instar came from the same samples. Sixteen larvae of first, second, third, and fourth instars which already molted were used to observe the weight and the head capsule width of second, third, fourth, and fifth instars, respectively using different larvae for each instar.