EFFECTS OF LARVAL DIETS ON GROWTH AND DEVELOPMENT OF *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)

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ABSTRACT

The fall armyworm, *Spodoptera frugiperda* (*J.E.* Smith) (FAW) is an invasive pest of corn worldwide. Due to alarming spread and continuous damage in newer geographical regions in Africa and in Asia including the Philippines, it becomes imperative to understand growth and development of this invasive species in selected crops. In this study we investigated the effect of artificial diet and corn leaf on growth and development of *S. frugiperda* larvae and generated information on development time, larval survival and pupation, weight of pupa, reproduction (fecundity) and adult longevity. The artificial diet ingredients consisted of soybean flour, wheat germ, mineral salt, sucrose, vitamin mix, agar, methyl paraben, sorbic acid, aureomycin, and calcium propionate; while young leaves of corn (10-15 days) were used as natural food Larvae fed with the artificial diet showed a significant shorter larval duration and produced heavier pupae than those fed with leaves of corn seedlings. Fecundity of the female adults averaging 1,471.6 ± 365.48 eggs/female from larvae reared on artificial diet was not significantly different to the natural food (X=1,534.36 ± 742.50 eggs per female). Moreover, larval survival (87.33 %), pupation rate (86.67 %), adult emergence (100 %) was also high in the artificial diet. These results suggest that the pupal weights, fecundity, and survivability of *S. frugiperda* is significantly affected by larval diets. In addition, the artificial diet as a substitute for the natural food of *S. frugiperda* is suitable for rearing this pest successfully under laboratory conditions. Information from this study was discussed in terms of biological attributes of fall armyworm and why larval growth and development are important in colony maintenance under laboratory conditions.

Key words: fall armyworm, biological control, invasive pest, artificial diet, rearing technique

INTRODUCTION

The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a polyphagous migratory pest native to the North and South America (Montezano et al. 2018; Igyuve et al. 2018). It is recently considered as the most important lepidopterous pest of corn (*Zea mays* L.) in the Philippines (Navasero et al. 2019; IPPC 2019) and since its detection in 2019, FAW has been monitored across the country damaging corn, sugarcane and rice. Research showed that control of *S. frugiperda* in corn mainly used chemical insecticides, but continuous use of these products may lead to development of insect resistance, negative impacts to beneficial insects, accumulation of residues in food, water and soil and air pollution. To reduce such effects, but at the same time, mitigate *S. frugiperda*, strategies as biological control, using entomopathogenic fungi and viruses have been explored (Navasero et al. 2020, 2021; Montecalvo and Navasero 2021).

Spodoptera frugiperda is adaptable to climate conditions in the Philippines and highly suitable for FAW population establishment all year round. It has high reproductive potential, short life cycle, and easy to handle. The biology of *S. frugiperda* had been reviewed by Sparks (1979) in the United States, as follows: it has no diapause mechanism and overwinters in south Florida and Texas where hosts are continually available and temperatures below 50 ^{0}F are rare; eggs are laid in clusters and densely covered with scales containing from a few to hundreds of eggs which hatched in 2-4 days; as larvae hatched from the eggs, they eat the chorions, and then continue to feed on leaves until they completed six instars and pupated, mostly in the soil. During warm weather, the life cycle requires about four weeks but longer during colder seasons. In Sub-Saharan Africa, on the other hand, the development cycle of FAW takes 25 days on average the durations of the different stages are distributed as follow: egg- 5days, larva-14d, pupa-7d and adult-16d (Tendeng et al. 2019). In the Philippines, Navasero and Navasero (2020) studied the life history and morphometry of FAW in the laboratory in native variety of corn (Lagkitan) and some natural enemies of *S.*

frugiperda population from the field, collected from Gonzaga, Cagayan. Briefly, eggs are laid in mass which hatched in 2-3 days; six larval instars in 14 days, on average; prepupa- one day; pupa-nine days; fecundity ranging 800 to 1,639 eggs per female; adult longevity of 10.18 days for the male and 9.82 days for the female. Efforts were made to mass rear this insect for efficacy testing of entomopathogenic fungi, bacteria, nematodes and viruses to establish their virulence levels against the different life stages of the pest.

We reviewed techniques for rearing *S. frugiperda* using artificial diets and natural food as well (Ashok et al. 2021; Lekha et al. 2019; Pinto et al. 2019; Modalon et al. 2017; Da Silva and Parra 2013; Tefera et al. 2011; Vilarinho et al. 2011; Busato et al. 2006; Chapman et al. 2000; Lynch et al. 1989; Mihn 1983; Perkins 1979; Burton and Perkins 1972; Ravelo and Raur 1967; Burton 1967; Burton and Cox 1966; and Hale 1965). However, none of them used a standardized artificial diet similar to the diet used in this study, that is easy to cook or prepare (about four minutes) and can be dispensed easily into rearing units and fed to developing FAW neonates and growing larvae. Most importantly, the diet used was very palatable to FAW neonates as they readily acclimatized and fed.

This study evaluated a standardized artificial diet as compared to its natural food (corn leaf) for laboratory rearing of *S. frugiperda* to ensure a continuous supply of homogenous and stage specific insects for efficacy testing and for colony maintenance in the laboratory. Information from this study would allow us to have a better understanding on FAW survival, reproductive behavior and performance of its offspring.

MATERIALS AND METHODS

Standard artificial diet composition and preparation. The ingredients of the commercially available standardized artificial diet (Insect Media^{R}) were soybean flour, wheat germ, mineral salt, vitamin mix, agar, methyl paraben, sorbic acid, aureomycin and calcium propionate. To prepare a liter of diet, 162g of the artificial diet was diluted with 930ml boiled distilled water and blended thoroughly for about three minutes at a high speed. While hot, the mixture was poured into sterilized plastic container (21 cm in length x 14.5 wide x 9.5 in height) and allowed to cool. The diet was refrigerated until use after solidifying at room temperature. The diet was removed from the fridge and conditioned at room temperature for 2-3h before use. The solidified diet was cut into pieces of about 2g and transferred to sterilized plastic cups (38.4mm, bottom x 47.8mm, top x 43.2mm high) for larval feeding.

Preparation of natural diet, corn. A local variety of corn, Lagkitan, was planted at high density in plots on a weekly interval as source of larval food. Excised leaves were cut into small pieces, 5cm long, disinfected in 0.5% sodium hypochlorite for 10 min, and washed two times with sterile distilled water. Finally, these were blot- dried with sterile tissue paper, prior to use.

Stock culture of Insect. Egg clusters of fall armyworm were obtained from an established colony maintained on leaves of Lagkitan as food at the National Crop Protection Center, College of Agriculture and Food Science, University of the Philippines Los Baños. Neonates were reared for one generation for acclimatization on the standardized diet and the following generation was evaluated. Neonate larvae were used in the feeding test. Neonates fed with young leaves of corn were hatched from egg clusters directly obtained from the stock culture maintained on leaves of Lagkitan.

Feeding test. Neonates hatched on the same day were individually placed in rearing cups containing 2g diet until pupation (Fig. 1). The opening of each cup was covered with sterilized tissue paper and perforated lid. Pupation occurred inside the pupal cells formed with artificial diet and frass. On the third day of pupation, the pupae were removed from the pupal cells, sex determined, weighed, and kept in the same rearing cup, cleaned off unspent diet and frass, until adult emergence. A male and a female adult emerging on the same day were paired in a cylindrical plastic cage (8.93 cm inside diameter, 11 cm high) and fed with 10% sucrose. In another set-up, larval feeding on the natural diet (corn) was prepared by placing one neonate into a plastic plate (9cm diameter x 1.5cm thickness) containing pieces of leaves taken from seedlings at 10-15 DAS (days after sowing). Daily thereafter, o fresh pieces of leaves were supplied to replace the previous ones, the plate cleaned off spent food and frass, until pupation. Fresh leaves were supplied daily since young leaves of corn dried up easily and not palatable to larvae anymore. Moreover, during the fourth larval stage, larvae consumed mostly all the pieces of leaves offered, hence the need for additional food. Pupation occurred inside the pupal cell formed with uneaten leaves or tissue lining of the plate. On the third day of pupation, pupae were removed from the pupal cells, sexed, weighed, and kept in plastic plate until adult emergence. As in the artificial diet, a male and a female adult emerging on the same day from this food source, were paired in cylindrical plastic cage, fed with 10% sucrose for recording of eggs laid (fecundity) and longevity. The number of neonates tested for the standardized diet and natural diet was 300 and 200, respectively.

Fig. 1. Set-up for rearing *Spodoptera frugiperda* on artificial (standard) diet (A), natural diet (B), and oviposition cages (C) for multiple pairs (left) and individual pair (right).

Rearing in individual containers for *S. frugiperda* is commonly used (Pinto et al. 2020; Vilarinho et al., 2011; Chapman et al. 2000; Perkins 1979; Burton and Perkins 1972; Burton and Cox 1966, among others) due to cannibalism of older larvae, although Da Silva and Parra (2013) proved that cannibalism is not obligatory.

Biological attributes of *S. frugiperda* observed on the standardized artificial and natural diets were as follows: 1) larval periods, reckoned from the day of hatching until pupation, 2) pupal periods, duration of quiescent period until adult emergence, 3) pupal weights taken at 3d old, 4) percent pupation expressed as the proportion of larvae that developed into pupae, 5) percent larval survival, the proportion of larvae that successfully pupated, 6) percent adult emergence, the proportion of adults that successfully emerged from pupae, 7) fecundity (eggs/female), 8) longevity of male and female adults, reckoned from the day of emergence from pupa until death, 9) sex ratio or male to female ratio.

The growth index (GI) was calculated using the method of Setamou et al. (1999) where:

Larval growth index= % pupation/larval period, in days; Pupal growth index= % adult emergence/pupal periods, in days; Total development index= % Survival of larva/total development of larva and pupa, in days.

The experiments were performed under laboratory conditions at 27-28^{0}C, 65% RH and 12D:12L.

Statistical analysis. The effects of artificial/standardized and natural diets on growth and development (e. g. larval period, pupal period, adult emergence, pupal weight) of *S. frugiperda* were compared and analyzed using t-test for independent samples in SAS (SAS 2018). The test was carried out using 30 samples/replication per group.

RESULTS AND DISCUSSION

Comparative effect of artificial diet and corn leaf on growth and development of FAW larva. Larvae of *S. frugiperda* reared on the standard artificial diet appeared as healthy and active as the larvae reared on leaves of their natural host, corn. The two food sources are sufficient in terms of larval development time from larva to pupa for both males and females. Larval durations were significantly shorter in the standard artificial diet than the natural diet (corn) for both male (t=-5.97; df= 210; P<0.0001) and female (t=2.48; df= 187; P=0.0140) larvae (Table 1). However, pupal duration was significantly longer in males(9.36 d in corn, 8.62d in artificial diet, t= 8.53; df=213; P<0.0001) than in females(8.26 d in corn, 7.57 in artificial diet, t=8.48; df= 205; P<0.0001). In terms of total development (Larval duration + Pupal Period), generally, the two diets were equally efficient for both males (t=2.19; df= 212; P=0.2940) and females (t=7.99; df=202; P=7.99).

Table 1. Developmental periods of larvae and pupae of *Spodoptera frugiperda* reared on standardized artificial diet and natural food, corn.

Diet	Larval Development (Days ± SD)		Pupal Development (Days ± SD)		Total Development (Days ± SD)	
	Male	Female	Male	Female	Male	Female
Standard	13.02 ± 0.69b	13.32 ± 0.70b	8.62 ± 0.73a	7.57 ± 0.70a	21.89±0.60a	19.55±1.10a
Natural	12.52± 0.55a	12.24± 0.66a	9.36 ± 0.55b	8.26 ± 0.47b	21.65±1.02a	20.50±0.61a

Means for each parameter followed by a common letter are not significantly different for comparison between treatments within each column (t-test, P<0.05).

The survival of larvae on natural diet (98.91 %) was greater than the standard diet (87.33%) and no differences were observed on sex ratio on either food source (Table 2). Percent pupation was higher when larvae were reared in the natural diet than in the standard diet. For overall assessment of the effects of larval food, we also focused on the larval growth index, pupal growth index and total development index. The growth index emphasizes the importance of both survival and developmental time in measuring food quality and suitability (Setamou et al. 1999). Higher survival rates and shorter development times yields higher values, thus indicating better food quality. No statistical analysis was done on these indices because only one value was obtained for each food source.

Table 2. Percent larval survival, pupation, adult emergence, growth indices and sex ratio of *Spodoptera frugiperda* on standard and natural diets.

Parameters	Diet	
	Standard	Natural
Survival, larva (%)	87.33	98.91
Pupation (%)	86.67	91.00
Adult emergence (%)	100.00	93.91
Larval growth index	6.46	7.34
Pupal growth index	13.89	10.58
Total development index	4.03	4.43
Sex ratio (Male:Female)	1.04: 1	1.07: 1

Reports show that several diets for rearing *S. frugiperda* have been developed and used successfully: wheat germ-casein diet (Burton 1967), modified pinto diet (Burton 1969) and the wheat and-soy-blend diet (Burton and Perkins 1972). All performed equally well on *S. frugiperda* (Perkins et al. 1973). Recently, Pinto and co-workers (2019) studied a corn-based diet for *S. frugiperda* and recorded 94.7 ± 2.49 % larval survival and 89.3 ± 7.59% pupation. Lynch et al. (1989) reported that peanut and soybean meal were unsuitable as diet ingredients due to low survival rate of larvae.

The development of *S. frugiperda* in the standard artificial diet we used was shorter than that observed by Pinto et al. (2019) on their corn-based artificial diet. The latter compared their results to be similar to Giongo et al. (2015) who obtained a larval period of 17.25 days and a pupal weight of 279.51mg using the diet developed by Greene

et al. (1976). He Li et al. (2021) on the other hand obtained a shorter larval period of 14.1 days for FAW using Green's artificial diet but similar to the values we obtained. On different artificial diets, Lekha et al. (2020) recorded larval periods between 14.0 days and 18.5 days on cowpea, chickpea, black gram, green gram and soybean- based diets.

In the present study, percent larval survival and pupation in the standard artificial diet were lower than the natural diet, however, the pupae produced all emerged into adults (100%). The larval and pupal growth indices were similar, in the standard and natural diets, but the pupation index was higher in the standard diet. Sex ratio in both diets conform to the conventional 1:1. Inbreeding depression was not observed, a situation generally observed after the fourth/fifth generation in the laboratory- reared colonies of the insect culture where larval survival and pupation decreased tremendously (Gupta et al. 1998).

Comparative effect of artificial diet and corn leaf on weight of FAW pupa. Female pupal weight of *S. frugiperda* was significantly higher in standard artificial diet, 248 ± 43.84mg and 207.6 ± 20.60 mg in corn (t=- 8.94; df=190; P<0.0001). Likewise, male pupae were significantly heavier in the standard artificial diet (245.8 ± 48.94 mg) than in the natural diet (251.7 ± 18.28 mg) (t=-6.39; df=177; P<0.0001) (Fig. 2A). When evaluating different artificial diets as food for *S. frugiperda* under laboratory conditions (temperature 25± 1^0C, 70± 10% RH and 12D:12L). Truzi et al. (2021) found a reduction in pupal weight in diet with double protein, whereas intermediate levels generated heavier pupae. Giongo et al. (2015) using an artificial diet developed by Greene et al. (1976) obtained pupae weighing 279.51mg, similar but higher than those obtained in the present study.

Comparative effect of artificial diet and corn on fecundity and adult longevity of FAW. Lifetime fecundity of females reared on artificial/standard diet during their larval stages was lower, (X= 1,471.6 ± 734 eggs/female) than in natural diet (corn) (X=1,534.36 ± 742.50 eggs/female) but the difference was not statistically significant (t=0.25; df=27;P=0.8027) (Fig. 2B). Likewise, longevity of adults was shorter on standard diet (11.67 ± 1.92 days) than in corn (12.25 ± 0.45 days) (Fig. 2C) . Likewise, the difference was not statistically significant (t=1.56; df=36; P=0.1273). Thus, it shows that the artificial diet is comparable to corn, its natural food, in providing the nutritional requirements of larvae needed by adult females for oviposition and survival.

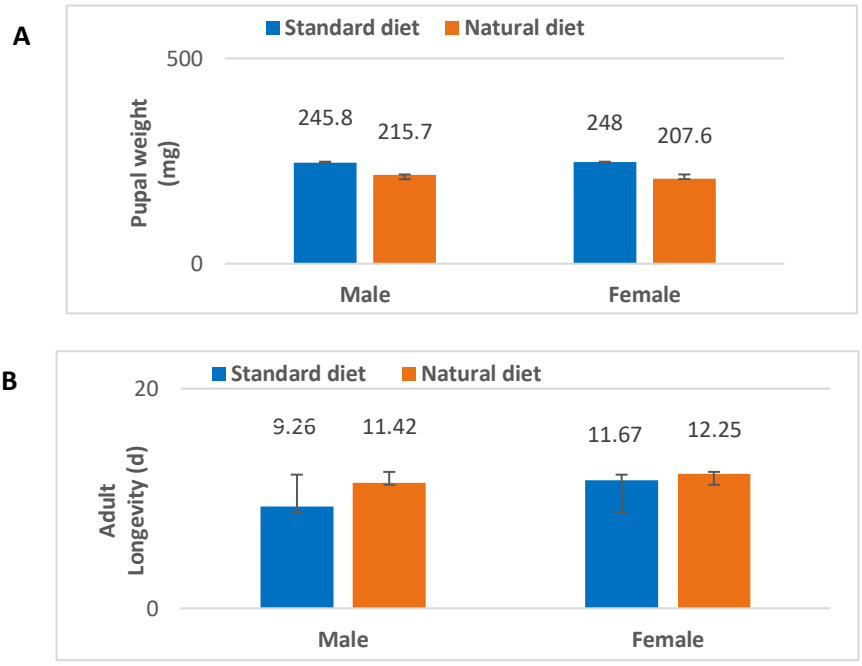

Fig. 2. (A) Pupal weight in mg (means±SD) of insects reared on artificial diet and natural food; (B) Adult longevity in days (means±SD) from adult emergence to death, of *Spodoptera frugiperda* moths obtained from larvae reared on artificial diet and natural food.

The general trend in insects is a positive relationship between pupal weight and adult fecundity, that is, heavy pupae produced bigger adults that lay more eggs (Miller et al. 1982; Bernardi et al. 2014). This relationship was not observed in the present study, since the diets offered during the larval stages did not significantly affect female fecundity and adult survival, possibly because the diets used resulted in similar pupal weight. However, the fecundities recorded in this study are within the range reported by other workers (between 1061.0 and 1850.0 eggs/per female) (Bernardi et al. 2014; Pinto et al. 2019) but higher than those reported by Truzi et al. (2021), varying from 592.9 and 667.5 eggs/female and 544.07 eggs by He Li et al. (2021). Furthermore, in this study, the fecundity of *S.*

frugiperda on the natural diet (1,534.36± 742.50 eggs/female) was higher than that reported by Murua et al. (2008), as follows: 955.05 eggs /female for population collected in maize, 555.89 in alfalfa, 519.83 in soybean, 978.10 in wheat, and 758.89 in weeds. Silva Lopez and co-workers (2008) reported a mean fecundity of 1,125 eggs/female in cassava leaves; 699 eggs/female on 2-leaf stage maize, 484 on 4 or 5 leaf stage corn, 525 on 2-leaf stage sorghum, 587 on 2-leaf stage wheat by He Li et al. (2021) for *S. frugiperda.*

Female longevities in this study are within the range reported by Truzi et al. (2021), ranging from 6.5 days to 11.5, but shorter than 17.0 days by Bernardi et al. (2014) and Pinto et al. (2019). This is possibly due to difference in rearing conditions, where room temperature was about 27-28ºC and 65% RH in our laboratory.

A diet is considered suitable for insect rearing if after undergoing evaluation biological parameters for survival, reproduction and behavior are satisfactory (Cohen 2001). Our findings suggest that the standard artificial diet evaluated supplied all the essential elements in balanced proportions needed for normal growth, development, and reproduction, allowing continuous rearing of *S. frugiperda* under laboratory conditions.

CONCLUSION

Spodoptera frugiperda reared on standard artificial diet produced healthy larvae and pupae which emerged into healthy adults that laid eggs comparable to those reared on natural food, corn. The diet was able to support growth and development of *S. frugiperda* ensuring continuous supply of homogenous stage specific insects for efficacy testing and for colony maintenance in laboratory.

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