Influence of the eggs of *Ephestia kuehniella* (Lepidoptera: Pyralidae) reared on different diets on the performance of the predatory bug *Orius laevigatus* (Hemiptera: Anthocoridae)

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Abstract. The predatory bug, *Orius laevigatus* (Fieber, 1860) (Hemiptera: Anthocoridae), is an important biological control agent and widely used for augmentative biological control of the western flower thrips, *Frankliniella occidentalis* Pergande, 1895 (Thysanoptera: Thripidae) in greenhouse crops. This bug is generally reared using the Mediterranean flour moth, *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae). The aim of this study was to determine the effects of *E. kuehniella* eggs produced by adult moths reared on different larval diets on the performance of *O. laevigatus*. The diets were 95% cornmeal + 5% yeast (CY diet), 53.3% cornmeal + 26.7% wheat bran + 15% Glycerine + 5% yeast (CBGY diet) and 53.3% wheat flour + 26.7% wheat bran + 15% Glycerine + 5% yeast (WBGY diet). Laboratory studies started with newly emerged nymphs of *O. laevigatus* kept in a climate chamber at 27°C, 60% relative humidity and a 14L:10D photoperiod. Eggs of *E. kuheniella* adults that were fed on one of the three different larval diets were provided to the nymphs as a source of food and bean pods of water. Following adult emergence, all individuals were sexed, and female and male pairs were put in plastic containers (30 cc) with eggs of *E. kuehniella* and bean pods. Nymphal development time, fecundity and longevity of *O. laevigatus* were not differently affected by feeding on the eggs of *E. kuehniella* reared on the three diets. In addition, the daily and total egg consumption of adults of *O. laevigatus* were similar. Because the CY diet eggs contain more nutrients, their consumption by nymphs was lower. This study indicates that the CY diet is an adequate diet for rearing *E. kuehniella* for producing eggs for rearing *O. laevigatus.*

INTRODUCTION

Most species of Anthocoridae are predators that usually feed on small insects such as aphids, thrips, psocids, psyllids, scales, small lepidopteran larvae and the eggs of insects (Pèricart, 1972; Lattin, 1999; Ballal & Yamada, 2016). Many species of anthocorid are used as biological control agents in protected areas in European countries (EPPO, 2020). The generalist Hemipteran predator, *Orius laevigatus* (Fieber, 1860) (Hemiptera: Anthocoridae), has been widely used and commercially available in Europe since 1993 for controlling thrips infesting greenhouse crops, mainly *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) (van Lenteren, 2012).

Orius laevigatus attacks a broad spectrum of arthropods but is mainly released commercially for the augmentative biological control of thrips (Chambers et al., 1993; Lattin, 1999; Keçeci & Gürkan, 2013). *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) eggs are generally used as a food source and bean pods as an oviposition substrate for rearing predatory bugs (Castañe & Zalom, 1994; Carvalho et al., 2011; Aragón-Sánchez et al., 2018). Lepidopteran eggs can be utilized for rearing insect predators because it

is possible to mass-rear many species of Lepidoptera (Silhacek & Miller, 1972; Tunçbilek et al., 2009; Kurtuluş et al., 2020). Moreover, eggs are easy to handle and can be prevented from hatching by freezing or UV sterilization (Isenhour & Yeargan, 1981; Samsoe-Petersen et al., 1989). However, in order to facilitate the use of augmentative biological control there is a need to reduce production costs (Bonte & De Clerq, 2010a).

The use of *E. kuehniella* eggs as the main food source in mass-rearing programs of *O. laevigatus* can increase the production cost (De Clercq et al., 2005a). This has led to a search for cheaper alternatives such as brine shrimp (*Artemia* sp.) cysts and many artificial diets (Arijs & De Clercq, 2001, 2004; De Clercq et al., 2005a). However, the nutritional value of brine shrimp cysts as a source of food is not equal to that of *E. kuehniella* eggs (Arijs & De Clercq, 2001). On the other hand, developing artificial diets for parasitoids and predators can simplify their mass-rearing and make it more cost-effective. However, the nutritional values of artificial diets are often inferior to the natural prey or hosts of natural enemies (Grenier, 2009). Many studies show that using alternative diets for feeding several

generations of *Orius* spp. results in a reduction in the fitness of these predatory bugs, which affects their effectiveness as biological control agents (Arijs & De Clerq, 2004; Ferkovich et al., 2007; Venkatesan et al., 2008; Bonte & De Clerq, 2008, 2010a). Inspite of this, lepidopteran eggs have been used as the standard food in the commercial mass-rearing of *Orius* spp. since the early 1990s (Alauzet et al., 1994; van Lenteren & Tommasini, 2003). Moreover, there is no evidence that the performance of predatory bugs used in biological control programs has been affected by their rearing diet (Bonte & De Clerq, 2010b).

Bonte & De Clercq (2008, 2010a) report that the reproductive fitness of *O. laevigatus* is greater when reared on *E. kuehniella* eggs than on other artificial diets. Many studies have shown that *E. kuehniella* is a nutritionally superior food for natural enemies (Cocuzza et al., 1997; Specty et al., 2003; De Clerq et al., 2005b; Song et al., 2019; Ricupero et al., 2020). However, there are virtually no studies on the effects of different artificial diets on the nutritional quality of *E. kuehniella* eggs as a food source for species of *Orius*. Therefore, determining the effects of *E. kuehniella* eggs, which are obtained by rearing this moth on different larval diets, on the fitness of *O. laevigatus* is important for optimizing the mass rearing of this predator specifically for biological control purposes. Therefore, the nutritional quality of *E. kuehniella* eggs in terms of the biological parameters and consumption by *O. laevigatus* were determined in this study.

MATERIALS AND METHODS

Insect rearing

Ephestia kuehniella

The initial colony of the Mediterranean flour moth, *E. kuehniella* was obtained from the Biyolojik Tarım Company, Erzin, Hatay, Turkey. The moths were maintained for six months in the Laboratory of Entomology (hereafter referred as the laboratory), Department of Plant Protection, Çukurova University, Adana, Turkey before they were used in the experiments. Three different diets consisting, respectively, of 95% cornmeal + 5% yeast (CY diet), 53.3% cornmeal + 26.7% wheat bran + 15% Glycerine + 5% yeast (CBGY diet) or 53.3% wheat flour + 26.7% wheat bran + 15% Glycerine + 5% yeast (WBGY diet) were used to rear the larvae of *E. kuehniella* in a climate chamber at $25 \pm 1°C$, $60 \pm 10\%$ relative humidity and a 14L : 10D photoperiod. One kg amounts of the three diets were prepared and each placed in a plastic container ($34 \times 23 \times 7$ cm) to which 10 eggs were added per 1 g of food. The CY diet (I) consisted of 950 g cornmeal and 50 g yeast; the CBGY diet (II): 533 g cornmeal, 267 g wheat bran, 150 ml glycerine and 50 g yeast and WBGY diet (III): 533 g wheat flour, 267 g wheat bran, 150 ml glycerine and 50 g yeast. In each case the dry components were mixed first and then glycerine was added to the mixture. Corrugated cardboard plates were placed inside the containers to provide a pupation site and then containers were covered with fine muslin cloth. Each moth colony was reared on one of the diets for one generation before the trials. Following adult emergence, the containers were checked three times a week when any adults of *E. kuehniella* were collected with the help of an electric suction aspirator and transferred to egg laying containers (1 liter in volume). Eggs were collected from these containers every two days. Some of these eggs were used for maintaining the *E. kuehniella* colony, while others were

frozen and kept for rearing *O. laevigatus*. All diets used in the experiments in the current study were used in a previous study by Kurtuluş et al. (2020) on the most promising ways of rearing *E. kuehniella* in the laboratory.

Orius laevigatus

O. laevigatus were collected from pepper plants growing in open fields in Adana, Turkey in 2018 and maintained in the laboratory. The predatory bug was identified by using the identification key of Péricart (1972) and then reared separately in plastic jars (1 liter in volume) with a perforated lid (5 cm diameter). The jars were sealed with mesh cloth for ventilation and to prevent escape. Adults and nymphs of this predatory bug were fed (ad libitum) with U.V sterilized *E. kuehniella* eggs and provided with bean pods as an oviposition substrate and source of water. Adults and nymphs of this predatory bug were kept in separate cages to reduce cannibalism. Moreover, to avoid genetic degeneration, new individuals were collected in the field and added to the laboratory colony at least twice a year.

Effects of the different artificial diests fed to *Ephestia kuehniella* on biological parameters of *Orius laevigatus*

These experiments started with 90 newly emerged nymphs of *O. laevigatus* kept in a climate chamber at $27 \pm 1°C$, $60 \pm 10\%$ relative humidity and a 14L : 10D photoperiod. Eggs of *E. kuehniella* fed on one of the three different diets were provided as a source of food and bean pods as a source of water for the nymphs. Three times a week, the nymphs were fed 75 min U.V-sterilized (UVB light 280–315 nm) *E. kuehniella* eggs glued to pieces of cardboard with Arabic gum. The nymphs were checked twice a day and nymphal development times were determined. Following adult emergence, all individuals were sexed, and one female and one male were put in a plastic container (30 cc) along with eggs of *E. kuehniella* and bean pods and the pre-oviposition, oviposition, post-oviposition, longevity and fecundity of females recorded under the same experimental conditions as above. The containers were checked daily until the end of the pre-oviposition period and then every two days to determine the other biological parameters of *O. laevigatus*. The experiments were terminated when all individuals were dead.

Consumption by *Orius laevigatus* of the eggs of *Ephestia kuehniella* reared on different artificial diets

This experiment started in each case with 45 newly emerged nymphs of *O. laevigatus* that were fed eggs of *E. kuehniella* produced in one of the three diets and provided with fresh bean pods as a source of water. The number of eggs of *E. kuehniella* consumed by the nymphs of *O. laevigatus* were determined every two days. Following adult emergence, all individuals were sexed and one female and one male were put in a plastic container (30 cc) for mating. After four hours, the males were transferred to other containers. The consumption of *E. kuehniella* eggs by *O. laevigatus* adults (females and males) was recorded over a period of 15 days. Two hundred fresh eggs were placed in each container every two days for both nymphs and adults.

Statistical analysis

The experiments were carried out in a completely randomized design. Kolmogorov-Smirnov test and Levene's test were used to determine normality and homogeneity of variance, respectively. One-way Analysis of Variance (ANOVA) of nymphal development time, pre-oviposition, oviposition, post-oviposition, longevity and fecundity of adults of *O. laevigatus* was carried out using Tukey's HSD post hoc mean separation. The nymphal development experiments were carried out in a completely randomized design with at least 30 replicates for each diet. Data on consump-

tion (nymphs and adults) were also analyzed using one-way ANOVA followed by Tukey's post hoc test. The consumption experiments were carried out in a completely randomized design with at least 15 replicates for each diet. All analyses were done using SPSS© (ver. 25).

RESULTS

Effects of the different artificial diets on the nymphal development of *Orius laevigatus*

In this study, the development time of second nymphal stage was shorter when the eggs produced by moths reared on the CBGY diet were provided as food. But the development time of the other nymphal stages and total development time of *O. laevigatus* were not significantly affected differently by the eggs of *E. kuehniella* reared on the different larval diets ($F_{2,85} = 1.453$, $p = 0.240$; Table 1).

Effects of different artificial diets on the biological parameters of adults of *Orius laevigatus*

The biological parameters of adults of *O. laevigatus* recorded are shown in Table 2. The different artificial diets did not significantly affect the pre-oviposition ($F_{2,35} = 2.005$, $p = 0.150$), oviposition ($F_{2,35} = 0.141$, $p = 0.869$) and post-oviposition ($F_{2,35} = 0.086$, $p = 0.918$) periods. Moreover, the longevity of adults of *O. laevigatus* and their fecundity did not differ statistically when reared and fed on eggs produced by moths reared on the different artificial diets ($F_{2,35} = 0.168$, $p = 0.846$, $F_{2,35} = 0.205$, $p = 0.816$; Fig. 1, respectively).

Effects of different artificial diets fed to the moths on the consumption of their eggs by *Orius laevigatus*

The consumption of eggs by the different stages of *O. laevigatus* are recorded in Table 3. The consumption of eggs by nymphs of *O. laevigatus* differed significantly on the eggs of the moths reared on the different artificial diets
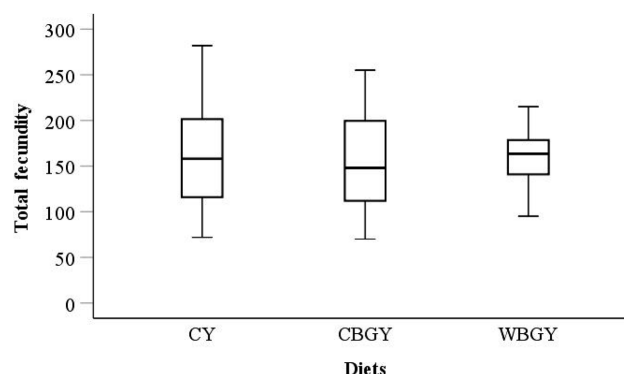

Fig. 1. The lifetime fecundity of *Orius laevigatus* fed on the eggs of *E. kuehniella* reared on the different diets.

($F_{2,42} = 5.845$, $p = 0.006$). The nymphs consumed fewer of the eggs produced by moths reared on the CY diet. However, the different artificial diets used for rearing the moths did not significantly affect the daily and total consumption of the eggs of these moths by adults of *O. laevigatus* in the first fifteen days of their adult life ($F_{2,42} = 0.155$, $p = 0.857$).

DISCUSSION

The success in rearing anthocorids depends on several factors, with the type of food being one of the most crucial (Calixto et al., 2013). *E. kuehniella* eggs are considered to be a nutritionally high quality food for natural enemies (Cocuzza et al., 1997; Specty et al., 2003; De Clercq et al., 2005b; Song et al., 2019; Ricupero et al., 2020). The nymphal development time of *O. laevigatus* fed eggs of *E. kuehniella* recorded in the literature is for different temperatures and different sources of water. Moreover, a number of previous studies used commercially available eggs of *E. kuehniella*. At 23°C, the development time is 13.0 and 11.7 days on sharp pepper seedlings (Bonte & De Clercq, 2008,

Table 1. The nymphal development times (days) of *O. laevigatus* fed on eggs of *E. kuehniella* reared on the different diets.

Diet	n	First instar nymph	2nd	3rd	4th	5th	Total
CY**	30	2.63 ± 0.09a*	1.83 ± 0.08a	1.83 ± 0.12a	2.23 ± 0.09a	1.37 ± 0.13a	9.90 ± 0.19a
CBGY	29	2.83 ± 0.09a	1.31 ± 0.08b	1.90 ± 0.09a	2.24 ± 0.11a	1.17 ± 0.07a	9.45 ± 0.18a
WBGY	29	2.55 ± 0.09a	1.86 ± 0.11a	1.79 ± 0.14a	2.24 ± 0.11a	1.24 ± 0.08a	9.69 ± 0.19a

* Means with same letter in the same column are not statistically significant according to Tukey's HSD test (P < 0.05); ** Diet names were constructed using the initials of the ingredients in each diet. C – cornmeal; W – wheat flour; B – wheat bran; G – glycerine; Y – yeast.

Table 2. The biological traits recorded in days of *O. laevigatus* fed on eggs of *E. kuehniella* reared on the different diets.

Diet	n	Pre-oviposition period (days)	Oviposition period (days)	Post-oviposition period (days)	Longevity (days)
CY	10	3.80 ± 0.36a*	18.90 ± 2.34a	2.70 ± 0.26a	25.40 ± 2.30a
CBGY	12	2.92 ± 0.34a	19.08 ± 2.58a	2.83 ± 0.34a	24.83 ± 2.50a
WBGY	16	3.25 ± 0.21a	20.31 ± 1.62a	2.88 ± 0.27a	26.44 ± 1.63a

* Means with the same letter in the same column are not statistically significant according to Tukey's HSD test at (P < 0.05).

Table 3. The consumption of nymphs and adults of *Orius laevigatus* of the eggs of *E. kuehniella* reared on the different diets.

Diet	n	Total nymphal consumption	Daily adult consumption	Total adult consumption
CY	15	137.20 ± 6.16b*	25.29 ± 2.17a	379.33 ± 32.55a
CBGY	15	148.27 ± 7.24ab	23.64 ± 2.38a	354.53 ± 35.73a
WBGY	15	169.00 ± 6.57a	24.40 ± 1.68a	365.93 ± 25.33a

* Means with the same letter in the same column are not statistically significant according to Tukey's HSD test (P < 0.05).

2010a) and 13.5 days on bean pods (Bonte & De Clercq, 2011). At 25°C and 26°C, total nymphal development is 13.2 days on geranium leaves (*Pelargonium peltatum* L.) (Alauzet et al.,1994) and 11.8 days on bean pods (Tommasini et al., 2004), respectively. In this study, total nymphal development time was shorter than that recorded in previous studies. This could be due to the different oviposition substrates used and that the temperature was higher in this study. Moreover, in the current study, the artificial diets did not significantly affect the pre-oviposition periods. Cocuzza et al. (1997) and Bonte & De Clercq (2008) report that the pre-oviposition period of *O. laevigatus* at 23°C is 3.2 days on the Spanish pepper plant (*Capsicum annuum* L. cv. 'Creta', long red) and 4.7 days, on sharp pepper seedlings, respectively. Tommasini et al. (2004) report that the pre-oviposition period of four different species of *Orius* [*Orius insidiosus* (Say, 1832), *O. laevigatus*, *Orius majusculus* (Reuter, 1879) and *Orius niger* (Wolff, 1881) (Hemiptera: Anthocoridae)] range between 3.2 to 6.7 days on bean pods at 26°C. In the current study, the pre-oviposition period is similar to that reported in previous studies even though bean pods were provided as a source of water and an oviposition substrate.

Longevity is another factor that can directly affect the fecundity of insects (Leather, 1988). Cocuzza et al. (1997) and Bonte & De Clerq (2008) report that the longevity of females of *O. laevigatus* is 41.9 and 50.2 days, respectively. However, Alauzet et al. (1994), Tavella et al. (1994) and Tommasini et al. (2004) report shorter longevities (34.5, 23.0 and 38.6 days, respectively) when reared under similar conditions. In the current study, longevity ranged between 24.83 to 26.44 days, which is lower than the average of the values reported in the previous studies. This could be due to the higher temperature used in this study. Zhang et al. (2012) and Ali et al. (2020) report that longevity decreases with increase in temperature when adults of *Orius similis* Zheng (Hemiptera: Anthocoridae) and *Orius strigicollis* (Poppius) (Hemiptera: Anthocoridae) are fed on *Tetranychus cinnabarinus* (Boisd.) (Acari: Tetranychidae) and *Pectinophora gossypiella* Saunders (Lepidoptera: Gelechiidae) eggs, respectively.

Because of their high nitrogen content, lepidopteran eggs are of higher nutritional quality than other food sources, including the predators' natural prey (Ferkovich et al., 2007). The developmental and reproductive fitness of *O. laevigatus* is greater when reared on eggs of *E. kuehniella* (Cocuzza et al., 1997; Tommasini et al., 2004; Bonte & De Clerq, 2008). Fauvel et al. (1987) and Cocuzza et al. (1997), respectively, report that *Macrolophus caliginosus* Wagner (Heteroptera: Miridae) and *O. laevigatus* perform better when reared on eggs of *E. kuehniella* than on some of their natural prey. Aragón-Sánchez et al. (2018) report that the increase in a population of *O. laevigatus* fed on *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) eggs is similar to that when naturally feeding on *E. kuehniella* and *F. occidentalis*. Furthermore their findings highlight the promising role of the eggs of *S. exigua* as an alternative food for use in laboratory tests. However,

the cost of mass-rearing *S. exigua* is considerably greater than that of *E. kuehniella*. Moreover, Ferkovich & Shapiro (2005) note the presence of a specific nutritional factor, in certain lepidopteran eggs, enhances the fecundity of predators. Lifetime fecundity is often considered to be a crucial factor determining the fitness of insects reared on artificial diets (Grenier & De Clercq, 2003). In the present study, the fecundity of *O. laevigatus* did not differ on the eggs of the moths reared on the three different artificial diets (Fig. 1). Tavella et al. (1994) and Tommasini et al. (2004) report that the fecundity of *O. laevigatus* is 104.6 and 118.6 eggs/female, respectively when reared on eggs of *E. kuehniella* eggs. Other researchers report higher fecundities ranging from 141 eggs to 198.0 eggs/female (Alauzet et al., 1994; Cocuzza et al., 1997; Vacante et al., 1997; Bonte & De Clerq, 2008). In the current study, the average fecundity of *O. laevigatus* was 150 eggs/female. These differences may be due to the different temperatures and oviposition substrates used in the experiments.

The nymphs of this predator consumed fewer of the eggs produced by the moths reared on the CY diet (Table 3). Van den Meiracker (1999) report that female and male nymphs of *O. insidiosus* consume a total of 54.73 and 48.28 eggs, respectively. Moreover, total nymphal consumption of *O. niger* is 77.10 and 67.25 eggs for females and males. Tommasini et al. (2004) also report different total consumptions of eggs of *E. kuehniella* for different species of *Orius* (*O. insidiosus* 94.3 eggs, *O. majusculus* 99.4 eggs, *O. laevigatus* 174.6 eggs and *O. niger* 183.5 eggs). In the current study, total nymphal consumption of *O. laevigatus* of eggs produced by moths reared on the WBGY diet is similar to that reported by Tommasini et al. (2004). This may be related to the nutritional value of the *E. kuehniella* eggs. Moreover, it is not possible to compare this value because the ingredients of the *E. kuehniella* diets used in the other studies is unknown. However, the artificial diets did not affect the daily and total consumption by adults of *O. laevigatus* in the first 15 days of their life (Table 3). Van den Meiracker (1999) and Calixto et al. (2013) report that the daily consumption of *E. kuehniella* eggs by *O. insidiosus* is 8 eggs/female and 15.7 eggs/female, respectively. In addition, Tommasini et al. (2004) report that the daily consumption of eggs by four species of *Orius* (*O. insidiosus*, *O. laevigatus*, *O. majusculus* and *O. niger*) is 25 eggs. Bonte & De Clerq (2010b) report that the consumption of eggs of *E. kuehniella* by *O. laevigatus* is 21.5 eggs/day. The findings of the current study are similar to those reported by Tommasini et al. (2004) and Bonte & De Clerq (2010b). The consumption of the nymphal and adults stages can differ. Bonte & De Clerq (2008) report that adult diet of *O. laevigatus* had a greater effect than the nymphal diet on its fecundity, which is directly related to their consumption. Moreover, adults of *O. laevigatus* fed on eggs of *E. kuehniella* were clearly able to compensate for any deficiencies in their nymphal diet.

Understanding the role of artificial diets in the development, fecundity and egg consumption of predatory bugs could improve the success of biological control programs

by ensuring a greater efficiency of the predators used in augmentative releases. The use of the eggs of *E. kuehniella* as the main source of food in the mass rearing of *O. laevigatus* can increase production costs (De Clercq et al., 2005a). Based on the current study, the three artificial diets fed to the moth did not affect either the development or fecundity of *O. laevigatus*. In addition, the CY diet resulted in nymphs consuming fewer eggs but had no effect on the egg consumption of adults. Moreover, the CY diet has the advantage that it is cheaper, more easily produced and less likely to be contaminated due to the lack of liquid in the diet. For this reason, the CY diet could be a suitable standard diet for the rearing of *O. laevigatus*. Further research on the effects of the CY diet on some biological parameters of this bug in subsequent generations should be conducted. In addition, the efficacy of parasitoids and predators that are reared on the CY diet should be determined in the field.

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