



**PRESENTATION OF ARTIFICIAL DIET: EFFECTS OF  
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ON EGG PRODUCTION BY ORIUS INSIDIOSUS  
(HETEROPTERA: ANTHOCORIDAE)**

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Source: Florida Entomologist, 90(3) : 502-508

Published By: Florida Entomological Society

URL: [https://doi.org/10.1653/0015-4040\(2007\)90\[502:POADEO\]2.0.CO;2](https://doi.org/10.1653/0015-4040(2007)90[502:POADEO]2.0.CO;2)

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PRESENTATION OF ARTIFICIAL DIET: EFFECTS OF COMPOSITION  
AND SIZE OF PREY AND DIET DOMES ON EGG PRODUCTION  
BY *ORIUS INSIDIOSUS* (HETEROPTERA: ANTHOCORIDAE)

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ABSTRACT

The size of prey is critical to the feeding success of any given predator, but the effects of diet packet size have not been studied. We examined the effects of size of packets (Parafilm® domes) of artificial diet and the size of prey eggs on oviposition and mortality rates of *Orius insidiosus* (Say). Artificial diet was presented to adult female *O. insidiosus* in 10-, 25- and 50- $\mu$ L domes for 6 d and rate of oviposition was measured for 24 h. Oviposition was highest after feeding on the 10- $\mu$ L domes, decreased slightly on the 25- $\mu$ L domes, and was significantly reduced on the 50- $\mu$ L domes. The effect of capsule size was negated on the 25- $\mu$ L and 50- $\mu$ L diet domes when the diet was supplemented with *E. kuehniella* egg protein. Predators were also fed eggs of 4 species of Lepidoptera, *Ephestia kuehniella* Zeller, *Plodia interpunctella* (Hübner), *Helicoverpa zea* (Boddie), *Spodoptera frugiperda* (J. E. Smith), and *Heliothis virescens* (Fabricius), ranging in volume from  $19 \pm 1.7$  nL (mean  $\pm$  SD) for *P. interpunctella* to  $108 \pm 21.3$  nL for *H. virescens* eggs. Oviposition was highest and comparable on the *E. kuehniella*, *P. interpunctella* and *H. virescens* eggs and significantly less on *S. frugiperda* and *H. virescens* eggs. Oviposition positively correlated and mortality negatively correlated with weight-specific protein contents of the eggs, but neither correlated with egg volume. When all species of eggs were extracted and combined with diet in domes of constant size and constant protein content, only extracts of *E. kuehniella* and *P. interpunctella* eggs were more active than diet alone.

Key Words: predator, artificial diet, egg size, prey egg proteins, predator, egg production, nutrition, oviposition, diet presentation, diet acceptance

RESUMEN

El tamaño de la presa es crítico para una alimentación exitosa por parte de cualquier depredador, pero los efectos por el tamaño de los paquetes de dieta no han estudiado. Examinamos los efectos del tamaño de los paquetes (domos de Parafilm®) de dieta artificial y el tamaño de los huevos de la presa sobre las tasas de oviposición y de mortalidad de *Orius insidiosus* (Say). Una dieta artificial fue presentada a hembras adultas de *O. insidiosus* en domos de 10-, 25- y 50- $\mu$ L por 6 días y la tasa de oviposición fue medida por 24 horas. La oviposición fue mas alta después de alimentar en domos de 10- $\mu$ L, en domos de dieta de 25- $\mu$ L fue menor y fue significativamente reducida en domos de dieta de 50- $\mu$ L. El efecto del tamaño de la cápsula fue anulado en domos de dieta de 25- $\mu$ L y 50- $\mu$ L cuando la dieta fue suplementada con la adición de proteína de huevos de *E. kuehniella*. Los depredadores tambien fueron alimentados con huevos de 5 especies de Lepidoptera, *Ephestia kuehniella* Zeller, *Plodia interpunctella* (Hübner), *Helicoverpa zea* (Boddie), *Spodoptera frugiperda* (J. E. Smith) y *Heliothis virescens* (Fabricius), que varían en cuanto al volumen de los huevos de  $19 \pm 1.7$  nL (mean  $\pm$  SD) para *P. interpunctella* hasta  $108 \pm 21.3$  nL para *H. virescens*. La tasa de oviposición fue mas alta y comparable en huevos de *E. kuehniella*, *P. interpunctella* y *H. virescens* y significativamente menos en huevos de *S. frugiperda* y *H. virescens*. Hubo una correlación positiva de oviposición y una correlación negativa de mortalidad con el contenido de proteína específico del peso de los huevos, pero no hubo una correlación en cuanto del volumen de huevo en ninguna de las dos. Cuando los huevos de todas las especies fueron extraídos y combinados con la dieta en los domos de tamaño constante y el contenido de proteína constante, solamente los extractos de huevos de *E. kuehniella* y *P. interpunctella* fueron mas activos que sola la dieta artificial.

Artificial diets for predators have been presented in several forms without any regard for the size of the packaged diet. Semi- or fully liquid diets have been presented within paraffin wax

capsules (1-2 mm diameter) (Hagen & Hassan 1965; Martin et al. 1978) or encapsulated in Parafilm® (Cohen & Smith 1998) for the green lacewing, *Chrysoperla rufilabris* (Stephens). Other diets for *C. carnea* have been presented on cellulose sponge (Vanderzant 1969; Hoosegow et al. 1989), and as uncovered capsules containing petroleum jelly and paraffin (Venkatesan et al. 2000). Four methods for presentation of liquid artificial diet for *C. rufilabris* (Burmeister) employed capillary tubes, cellulose sponge cubes, agarose-based jelly on coverslips, or artificial eggs (paraffin/petrolatum droplets on microscope slides) (Greenberg et al. 1994). The artificial eggs and agarose-based jelly showed the most promise. Diets for predaceous coccinellids were presented in gelled cubes (Atallah & Newsom 1966), as a powder, or as dry pellets (Smirnoff 1958; Nijima et al. 1977; Matsuka et al. 1982). Cohen (1985, 1992) and Cohen & Smith (1998) used Parafilm® to seal a semi-solid diet in the form of a sachet containing 50 mg of diet for *C. rufilabris*, and Grenier et al. (1989) used Parafilm® to seal foam cubes soaked with diet for the polyphagous predator *Macrolophus caliginosus* (Wagner). Arijis & De Clercq (2004) prepared a meat-based diet in cylindrical Parafilm® packets (1 cm long, 0.3 cm dia.).

In other studies, the form and materials used in diet presentation to predators were comparable, while size varied. With Parafilm® encapsulation, diet dome-size ranged from 50 µL/diet-dome for *Orius insidiosus* (Say) (Ferkovich & Shapiro 2004) to 40-500 µL/dome for *Podisus maculiventris* (Say) (Shapiro et al. 2001; Whittmeyer & Coudron 2001). However, none of these studies specifically controlled for the effect of capsule size. We therefore varied the size of Parafilm® diet domes to test its effect.

In nature, prey size has been reported to impact the efficiency of predators (Evans 1976; Meiracker & Sabelis 1999; Anderson et al. 2001; De Clercq et al. 2002; Obrycki et al. 2005; Roger et al. 2000). Because *O. insidiosus* preys on the egg stage of numerous Lepidoptera, we examined the effect of the size of several species of prey-egg on oviposition (and presumptive feeding). In addition, because nutritional or phagostimulatory value of prey eggs may vary and differentially affect feeding and fecundity, we determined the effect of extracts of those eggs by adding them to artificial diet contained in domes of constant controlled size.

## MATERIALS AND METHODS

### Insect Rearing

Insects for rearing and experiments were held in a growth chamber at  $25.5 \pm 1^\circ\text{C}$ ,  $75 \pm 5\%$  RH, and a photoperiod of 15:9 (L:D). A Florida strain of *O. insidiosus* was reared on eggs of *E. kuehniella*

(Beneficial Insectary, Redding, CA). The eggs were received frozen and held at  $-80^\circ\text{C}$  until used. Insects were held in 1 pint-canning jars covered with ripstop nylon. The insects were provided water every other day as 1.2 mL of Hydrocapsules® (Analytical Research Systems, Gainesville, FL), 0.6 mL (3 mg) of *E. kuehniella* eggs, and 1 fresh green bean for oviposition. Green beans with eggs were removed every other day and placed in new jars with water beads, *E. kuehniella* eggs and 2 grains of bee pollen (purchased locally) for first-instars. Crumpled strips of wax paper were used as substrates. Following adult eclosion, the green bean was replaced and all green beans were checked for eggs every other day. Eggs in the beans were counted under a stereomicroscope, and beans with fewer than 400 eggs were added to each new jar to reduce cannibalism. A camel hair brush was used to remove the nymphs from the old green beans and to transfer the adults.

### Prey Eggs

Eggs of *E. kuehniella* Zeller were purchased from Beneficial Insectary, Redding, CA and shipped on dry ice. Eggs of *Plodia interpunctella* (Hübner) were obtained from a laboratory colony of *P. interpunctella* reared as described by Silhacek & Miller (1972). Eggs of *Helicoverpa zea* (Boddie), *Spodoptera frugiperida* (J. E. Smith), and *Heliothis virescens* (Fabricius) were reared as described by Burton (1969).

### Artificial Diet

Diet was prepared under aseptic conditions in a clean room and encapsulated in stretched Parafilm® with a diet encapsulation apparatus (Analytical Research Systems, Gainesville, FL) as described by Ferkovich & Shapiro (2004). We refer to resulting capsules as domes, because they are hemispherical in shape. The original diet was prepared as described by Weiru & Ren (1989) for rearing *Orius sauteri* (Poppius) and consisted of 397 mg brewers yeast, 39.7 mg sucrose, 208 mg soy protein acid hydrolysate, 5.0 mg of 99% palmitic acid (all from Sigma, St. Louis, MO), 49.5 mg chicken egg yolk, and 99.2 mg honey brought to 1.2 mL with distilled water. Palmitic acid was mixed with the egg yolk component before adding it to the diet. The resulting diet contained 273 mg protein/ml of diet as calculated from protein composition of the components (Souci et al. 1989).

### Preparation of Egg Protein Extract

Soluble proteins were isolated from eggs of the 4 species of insects as described by Ferkovich & Shapiro (2004). Briefly, the eggs were homogenized in ammonium acetate buffer (pH 7.5), centrifuged at 20,200g, and the soluble fraction be-

tween the upper lipid layer and pelleted debris was collected. This fraction was then run through a centrifugal desalting column to remove compounds <math><5,000\text{ MW}</math>, freeze-dried, and stored at  $-80^{\circ}\text{C}$ . The freeze-dried desalted powder was then added to distilled water and analyzed for the quantity of soluble protein by the Lowry procedure (Protein Assay Kit, Sigma, St. Louis, MO) before mixing it into diet.

#### Bioassay of Diets

Adults that had eclosed within 24 h were selected from the colony for use in the bioassay. Each replicate consisted of 6 females and 4 males in a 100-mL plant tissue culture jar (Sigma, St. Louis, MO), with 4 replicates per treatment. In the first experiment, each jar contained 0.6 mL of Hydrocapsules®, either 3 mg of *E. kuehniella* eggs or two domes each containing 10, 25, or 50  $\mu\text{L}$  of artificial diet or diet plus *E. kuehniella* egg extract (36 mg protein/mL), and 3 crumpled strips of wax paper (5 mm  $\times$  80 mm) as substrates. *E. kuehniella* eggs, Hydrocapsules, and treatment diets (artificial or insect eggs) were replaced daily and mortality was recorded. At the end of 6 d, one 7-cm section of green bean pod was placed in each jar for 24 h as an oviposition substrate. The number of females alive at the end of the oviposition period was recorded, eggs deposited in the green beans were counted under a stereomicroscope, and the number of eggs oviposited per 5 females was calculated. In the second experiment, the setup was the same, except that the treatment diets were made up of 3 mg of eggs of each species of prey, *E. kuehniella*, *P. interpunctella*, *H. zea*, *S. frugiperda*, and *H. virescens* added to each of the jars. The setup for the third experiment was identical except that each treatment diet consisted of protein (18 or 36 mg protein/mL of diet) extracted from one of 5 species of prey eggs and added to artificial diet in two 25- $\mu\text{L}$  domes per jar.

#### Egg Measurements

Measurements of the eggs were made with a stereomicroscope with a 10 $\times$  ocular micrometer calibrated with a 1-mm stage micrometer. The volumes of the eggs of *H. zea*, *S. frugiperda*, and *H. virescens* were calculated by the formula for a sphere,  $\frac{4}{3}\pi r^3$ . Egg volumes for *P. interpunctella* and *E. kuehniella* were calculated by the formula for a prolate spheroid,  $\frac{4}{3}\pi ab^2$  where  $a$  = the semi-major axis length and  $b$  = the semi-minor axis length.

### RESULTS

Oviposition was affected by feeding females on increasing sizes of diet domes, with or without added *Ephestia* egg protein (Fig. 1). Oviposition

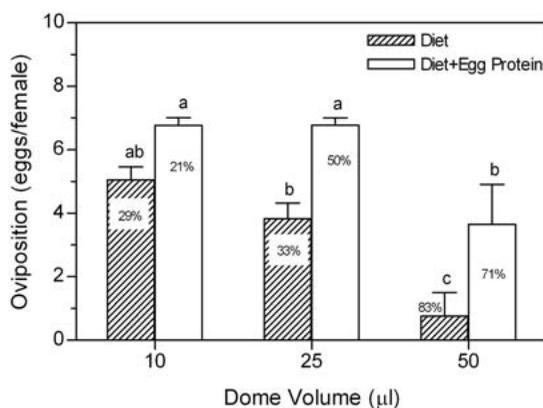


Fig. 1. Effect of size of diet domes on rate of oviposition (mean  $\pm$  SEM,  $n = 4$ ) by predators fed artificial diet with and without *E. kuehniella* egg protein extract (29 mg protein/mL diet). Percentages indicated on bars represent mortalities. Bars with the same letter are not significantly different by ANOVA and multiple range test (Newman-Keuls method,  $P > 0.05$ ).

was highest in females fed on the 10- $\mu\text{L}$  domes, 24% lower on the 25- $\mu\text{L}$  domes (N.S.), and 85% lower on the 50- $\mu\text{L}$  domes ( $P < 0.05$ ). When domes were supplemented with *E. kuehniella* egg protein, there was no decrease in oviposition between 10- and 25- $\mu\text{L}$  domes, and a 47% decrease on 50- $\mu\text{L}$  domes ( $P < 0.05$ ). Female mortalities following the 7-d bioassay on the 10-, 25- and 50- $\mu\text{L}$  diet domes containing artificial diet were 29.1%, 33.3%, and 83.3%, respectively. On 10-, 25- and 50- $\mu\text{L}$  diet domes with egg protein added to the diet, mortalities were 20.8%, 49.9%, and 70.8%, respectively. Female mortality on whole *E. kuehniella* eggs was 12.5%.

Fig. 2 shows the relative volumes of 5 species of prey eggs, protein content of prey eggs per mg of fresh weight, and resultant oviposition by *O. insidiosus* females feeding on the eggs. *Plodia interpunctella* and *E. kuehniella* eggs were the smallest,  $19 \pm 1.7$  nL (mean  $\pm$  SD) and  $26.9 \pm 3.4$  nL, respectively; *S. frugiperda* eggs were of intermediate size,  $61.3 \pm 10.0$  nL; and *H. zea* and *H. virescens* were the largest,  $102.5 \pm 10.6$  nL and  $107.6 \pm 21.3$  nL, respectively. The concentrations ( $\mu\text{g}/\text{mg}$  fresh wt) of protein in the eggs in were: *E. kuehniella*, 162; *P. interpunctella*, 122; *H. virescens*, 119; *H. zea*, 89; and *S. frugiperda*, 69. Oviposition was highest and comparable on the *E. kuehniella*, *P. interpunctella*, and *H. virescens* eggs and significantly less on *H. zea* and *S. frugiperda* eggs. Female mortality on the prey eggs was as follows: *E. kuehniella*, 8.3%; *P. interpunctella*, 8.3%; *H. virescens*, 8.3%; *H. zea*, 21%; and *S. frugiperda*, 29%. If protein extracted from *E. kuehniella* eggs was added to diet, oviposition of females fed on the 25- and 50- $\mu\text{L}$  domes significantly increased over those without additional

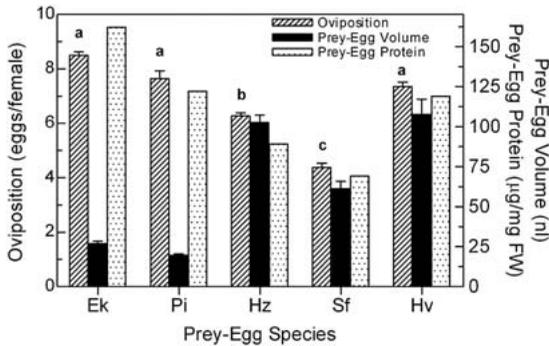


Fig. 2. Oviposition rate of *O. insidiosus* females fed whole prey eggs (mean  $\pm$  SEM;  $n = 4$ ), in relation to volumes (mean  $\pm$  SEM;  $n = 5$ ) and weight-specific protein content of prey eggs ( $\mu\text{g}/\text{mg}$  fresh weight). Prey species: *E. kuehniella* (Ek), *P. interpunctella* (Pi), *H. zea* (Hz), *S. frugiperda* (Sf), and *H. virescens* (Hv). Bars with the same letter are not significantly different (Newman-Keuls method,  $P > 0.05$ ).

protein. However, oviposition was still reduced in those fed on 50- $\mu\text{L}$  domes relative to 10- or 25- $\mu\text{L}$  domes.

When the mean oviposition and mortality were regressed against prey-egg volume and protein content of eggs, neither oviposition nor mortality correlated with egg volumes (Fig. 3A), while both strongly correlated (mortality was inversely correlated) with protein content of eggs (Fig. 3B).

Only protein extracts from the *E. kuehniella* and *P. interpunctella* eggs significantly enhanced egg production when added to artificial diet (Fig. 4). The *E. kuehniella* extract improved egg production at both protein levels tested, whereas the *P. interpunctella* extract was only effective at the higher level. Female mortality on the diets supplemented with the egg protein from each species was as follows: *E. kuehniella* whole eggs, 12.5%; artificial diet, 37%; *E. kuehniella* extract (18 mg protein), 25%; *E. kuehniella* extract (36 mg), 21%; *P. interpunctella* extract (18 mg), 37%; *P. interpunctella* extract (36 mg), 33.3%; *H. virescens* extract (18 mg), 46%; *H. virescens* extract (36 mg), 37%; *H. zea* extract (18 mg protein), 46%; *H. virescens* extract (36 mg), 33%; and *S. frugiperda* extract (36 mg), 46%.

## DISCUSSION

The mode of presentation of an artificial diet is important in determining its acceptance by a predator (Cohen & Staten 1993; Grenier et al. 1994; Cohen 2004). Important issues in diet presentation include phagostimulants, texture, liquid or semi-solid state of the ingredients, and methods of containment. Cohen and Staten (1993) found that *Geocoris punctipes* fed longer when the diet format was changed from cylindri-

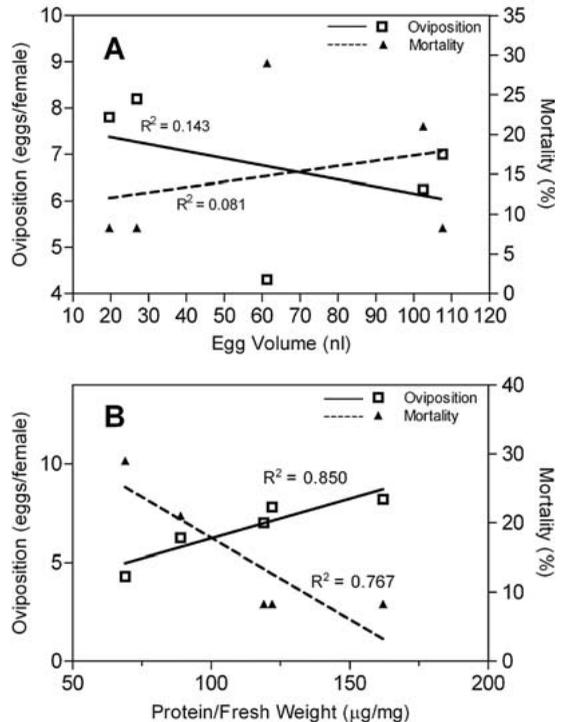


Fig. 3. Regressions on the means of oviposition and mortality vs. (A) prey-egg volume; and (B) weight-specific protein content.

cal artificial "larvae" to flattened packets made from stretched Parafilm®. Carpenter & Greany (1998) designed the encapsulation unit used in the present study, which is capable of forming diet domes of varied volumes from Parafilm®. De Clercq et al. (1998) evaluated 2 methods to present a meat-based diet to *Podisus maculiventris* (Say), and found that containing the diet in stretched Parafilm® sheets to form cylindrically shaped artificial larvae yielded better results than the gelled open form of the diet. They concluded that inferior results from the gelled form of the diet may be related to acceptability problems. Methods of diet containment have focused mainly on mechanized means of producing diet packets for mass production of predators such as *Chrysoperla rufilabris* (Cohen 2004), and little consideration has been given to the size and shape of the diet capsules.

Diet dome size clearly affected oviposition. Females that fed on 50- $\mu\text{L}$  domes produced fewer eggs than those fed on 10- or 25- $\mu\text{L}$  domes. One possible reason for this is that the digestive enzymes that the feeding predators injected into the prey eggs during feeding were diluted significantly in the larger domes. Cohen (1989) observed that *G. punctipes* varied their probing of artificial larvae while feeding, in a manner similar to prob-

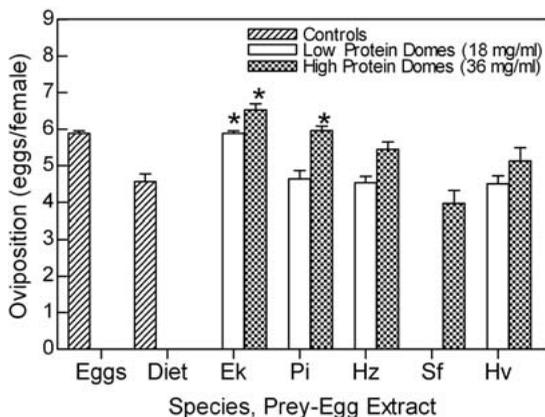


Fig. 4. Effect of supplementing diet with protein extracts from 4 species of prey eggs on oviposition rates (mean  $\pm$  SEM,  $n = 4$ ). Only the higher concentration of Sf was run. Dunnett's test was used to compare the amended treatment diets against the Diet (control); asterisk indicates that the treatment means were significantly different from Diet (control) ( $P < 0.05$ ).

ing different parts of their natural larval prey. During this feeding behavior they injected saliva containing digestive enzymes, initiating a process that he called extra oral digestion. Cohen and Smith (1998) attributed the success of their solid artificial diet to its accommodation of the extra oral digestive nature of predator feeding.

The addition of protein extract from *E. kuehniella* eggs, notably in females fed on the 50- $\mu$ L diet domes, reduced the effect of the large dome size (Fig. 1). One possibility for the reduced effect is that phagostimulants may be present in prey-egg extracts. Chemoreception is an important means by which the insect detects food resources and oviposition sites (Nation 2002; Mowery et al. 2004). De Clercq et al. (1998) stated that a lack of feeding stimulants may be more important than a lack or poor balance of nutrients in a predator's diet. There are numerous reports in the literature on chemoreception by phytophagous insects (Chapman 2002); however, little information is available on predators and prey-associated phagostimulants. Cohen & Staten (1994) found that *G. punctipes* were attracted to green beans and induced the predators to aggregate and feed and drink for extended periods. Relatedly, chemical cues derived from larval prey of *Spodoptera litura* were found to elicit prey-locating behavior by the predatory stink bug, *Eocanthecona furcellata* (Yasuda 1997).

In analogy to the experiment on the effect of dome size on oviposition, we examined the effect of prey-egg size on oviposition. There was no correlation between prey-egg size and oviposition (Fig. 3A); however, weight-specific protein contents of prey eggs correlated directly with oviposi-

tion and inversely with mortality (Fig. 3B). These results suggest that the protein contents of eggs played critical roles in stimulating egg development and oviposition and in aiding survival. To further examine the effect of the egg protein, a constant amount from each species of egg was added to diet in domes of equal volume (Fig. 4). Only *E. kuehniella* and *P. interpunctella* egg extracts induced oviposition at a significantly higher rate than the control diet, suggesting that something nutritionally active in those eggs was lacking in other species. This relates to earlier studies in which we reported oviposition-stimulating activity in whole egg extracts of *E. kuehniella* and *P. interpunctella* (Ferkovich & Shapiro 2004a, 2005a), in embryonic cell lines from the two species (Ferkovich & Shapiro 2004b, 2005b; Ferkovich & Lynn 2005), in specific fractions from extracts of *E. kuehniella* (Ferkovich & Shapiro 2005a), and fractions from a *P. interpunctella* cell line (Ferkovich & Shapiro 2007).

Because we observed that *O. insidiosus* produced the most eggs on the smallest diet domes, we surmised that the predator would exhibit a preference for smaller prey eggs. Egg production was not only highest on the 2 smallest eggs (*E. kuehniella* and *P. interpunctella*) but also on the larger eggs of *H. virescens*. It appeared that the *H. virescens* eggs were either more attractive to the predators or better met the nutritional needs for yolk production than the other 2 species of large eggs, *H. zea* and *S. frugiperda*. Based on these results, we hypothesized that proteins extracted from *H. virescens* eggs would have activity similar to the *E. kuehniella* and *P. interpunctella* egg protein extracts when bioassayed (Ferkovich & Shapiro 2004, 2005). Bioassaying the 5 egg protein extracts at similar protein concentrations, however, revealed that the *H. virescens* protein extract did not have significant activity in the large diet domes (25  $\mu$ L) as did the *E. kuehniella* and *P. interpunctella* extracts. This indicated that either nutrients other than proteins or some other factor(s) was responsible for the increase in oviposition rate we observed with whole *H. virescens* eggs. Numerous reports indicate that feeding different prey to predators can affect their fecundity and other biological characters of predators because of differences in the nutritional values of the prey (Chyzik et al. 1995; Thompson & Hagen 1999; De Clercq et al. 1998; Roger et al. 2000; Torres et al. 2004; Venzon et al. 2001; Specky et al. 2003).

In conclusion, *O. insidiosus* oviposited at a higher rate when control diet was presented to them in small domes rather than larger domes, suggesting higher consumption rates. This effect could be overridden by adding a protein extract from either *E. kuehniella* or *P. interpunctella* eggs, likely because the extract made the diet more attractive to the predators. Alternatively, the extract may have contained enzymes that aided

digestion of the diet. Although females fed intact eggs from *H. virescens* had oviposition rates similar to those fed eggs from *E. kuehniella* and *P. interpunctella*, females fed diet with extracts from *H. virescens* eggs did not show significantly higher oviposition rates. We cannot explain this discrepancy. Identification of the active substance in the *E. kuehniella* and *P. interpunctella* extracts should provide a better understanding of why these extracts are active. Once the active components are identified they may prove to be useful in improving artificial diets for *Orius* species.

#### ACKNOWLEDGMENTS

We thank Dr. Terry Arbogast for helpful review of an earlier draft. We thank Dr. Patrick Greany for his review and valuable insight into interpretation of some of the data. We appreciate the excellent technical assistance of Delaine Miller in this study.

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