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Biological Control xxx (2004) xxx–xxx
Biological Control
www.elsevier.com/locate/ybcon

Comparison of prey-derived and non-insect supplements on egg-laying of *Orius insidiosus* maintained on artificial diet as adults

S.M. Ferkovich* and J.P. Shapiro

Center for Medical, Agricultural, and Veterinary Entomology, USDA, ARS, 1700 SW 23rd Dr., P.O. Box 14565, Gainesville, FL 32604, USA

Received 7 October 2003; accepted 9 April 2004

Abstract

The effects of proteins and lipids from insect- and non-insect sources on the oviposition rate of the insidious flower bug, *Orius insidiosus* (Say) fed supplemented artificial diets were evaluated. Soluble proteins were isolated from *Plodia interpunctella* (Hübner) eggs, desalted by column chromatography, lyophilized, and tested as a diet supplement. A total lipid extract from *P. interpunctella* eggs was also evaluated as a diet supplement, and the three most abundant fatty acids (palmitic, linoleic, and oleic acid) identified in the extract were tested as a combined supplement. Non-insect supplements evaluated in diet were bovine serum albumin, chicken liver, beef liver, and chicken egg albumin. *O. insidiosus* were fed the supplemented diets, whole *P. interpunctella* eggs, or unsupplemented artificial diet, and the mean total numbers of eggs oviposited per female were recorded throughout adult life. The *Plodia* egg proteins significantly increased egg production and mean number of oviposition days at concentrations of protein that were 83-, 557-, and 837-fold lower than the concentrations needed for beef liver, bovine serum albumin, and chicken egg albumin, respectively. We conclude that soluble proteins from *P. interpunctella* eggs provided superior nutritional value for reproduction in female *O. insidiosus*. Additional information is required to explain the higher specific activity of soluble egg proteins compared to the specific activities of the non-insect proteins. However, we speculate that the quality of egg proteins or of an unknown substance bound to the proteins may be important for reproduction in this predator.

Keywords: Predator; Artificial diet; Prey egg proteins; Lipids; Fatty acids; Predator; Egg production; Non-insect proteins; Nutrition; Oviposition

1. Introduction

The insidious flower bug, *Orius insidiosus* (Say), is a polyphagous predator that can suppress populations of several pest species (Barber, 1936; Funderburk et al., 2000; McCaffrey and Horsburgh, 1986; van Lenteren et al., 1997; van der Veire and Degheele, 1992). It is produced on natural hosts by more than 33 commercial producers for augmentative biocontrol (Association of Natural Bio-control Producers, 2001). The success of the predator depends on its efficacy as a predator, its production costs, and its efficiency of reproduction (Thompson and Hagen, 1999; Vinson, 1994; Waage et al., 1985; Yazlovetsky, 1992). *Orius* spp. have been reared on artificial diets (Arijs and De Clercq, 2001a; Weiru and Ren, 1989; T.A. Coudron, personal communication), but information on egg production of predators maintained on artificial diets has not been reported.

Generally the reproductive rate of predators reared on artificial diets is reduced (Carpenter and Greany, 1998; Cohen, 1985a,b, 1992, Cohen, 2000a; Cohen and Staten, 1994; Coudron et al., 2002; De Clercq and Degheele, 1992, 1993a,b; De Clercq et al., 1998; Wittmeyer and Coudron, 2001; Rojas et al., 2000). Cohen and Smith (1998) reported significant cost savings in producing high-quality populations of the predator *Chrysoperla rufilabris* Burmeister on an insect-free artificial diet that had fecundity comparable to those reared on eggs of *Ephestia kuehniella* Zeller. Wittmeyer and Coudron (2001) emphasize the importance of good fecundity in determining the cost of rearing *P. maculiventris* on

* Corresponding author. Fax: 1-352-374-5804.
E-mail address: sferkovich@gainesville.usda.ufl.edu (S. Ferkovich).

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doi:10.1016/j.biocontrol.2004.04.005

artificial diet. Artificial diets were not cost-effective if the predators' development was prolonged and reproductive output decreased.

The reason for reduced egg production in predators fed on artificial diets is not clear. Although many semi-natural and artificial diets have been successfully formulated for maintaining parasitoids, and predators, Thompson and Hagen (1999) state that the "nutritional requirements of adult entomophagous insects remain obscure." Appropriately, Cohen (1998a,b, 2000a,b) addressed this need and observed that heteropteran predators not only ingest the body fluids of their prey, but also use a "solid-to-liquid feeding" method to attack soft organs of their prey. He concluded that they require diets with highly concentrated protein (16–24% of total mass) and lipid (10–22%).

Egg production by *O. insidiosus* females may require a high quality protein meal. Adult females of *O. insidiosus* and other heteropteran predators, (e.g., *P. maculiventris*) have higher yolk content in developing oocytes as well as higher egg production when fed prey versus artificial diet (Shapiro and Ferkovich, 2002). Adams (2000a,b) observed a decrease in the number of ovarioles with vitellogenic and chorionated follicles in females of the predaceous bug *Perillus bioculatus* fed artificial diet. The requirement for a protein meal to induce egg development is well-known in mosquitoes, houseflies, and other bugs such as *Rhodnius prolixus* (Adams, 1999; Davey, 1997; Wheeler, 1996; Wigglesworth, 1972). Cohen (1989) observed a $>90\%$ digestion efficiency of protein ingested from aphid prey in *Geocoris punctipes*. Additionally, although protein in the diet is necessary, it is not always sufficient for egg development. Females of *O. insidiosus* may require a meal containing other biochemical constituents in addition to protein. The quality of protein may also be critical, and the proteins from vertebrate sources contained in many artificial diets may not fulfill this qualitative need. This accounts for the preferential use of insect eggs by insectaries to rear *Orius* spp., and the success in rearing *Orius laevigatus* (Fieber) with a high reproductive rate on shrimp eggs (Arijs and De Clercq, 2001b) and the use of insect material to correct deficiencies of artificial diets (Grenier et al., 1994).

The purpose of this study was to investigate egg production of *O. insidiosus* fed a minimal artificial diet and to determine whether supplementing the diet with insect-derived nutritional sources yielded improved egg production over non-insect sources.

2. Methods and materials

2.1. Diet preparation and constituents

Diet was prepared in a clean room to reduce contamination. Although mold was never observed in the diets between insect feedings, they cannot be considered sterile. The diet was encapsulated in stretched Parafilm using a diet encapsulation apparatus (Analytical Research Systems, Gainesville, FL) described earlier (Ferkovich et al., 2000).

2.1.1. Standard diet

Eggs of *P. interpunctella* were used as a standard diet with which the *artificial diet* and all other experimental diets were compared. Eggs were obtained from a laboratory colony of *P. interpunctella* reared on a standard diet with a controlled temperature and relative humidity as described by Silhacek and Miller (1972). The *Plodia* diet consisted of Gaines dog meal (ground), rolled oats, white cornmeal, whole wheat flour, wheat germ, brewers yeast, glycerol, and honey. Eggs were collected within 1 h after oviposition, held at 4 °C for 24 h, and then stored at −80 °C until used in our experiments.

2.1.2. Artificial diet

Artificial diet was originally developed for rearing *Orius sauteri* (Weiru and Ren, 1989) and consisted of 4.0 g brewers yeast, 0.4 g sucrose, 2.1 g soy protein acid hydrolysate, 0.045 g of 99% palmitic acid (all from Sigma, St. Louis, MO), 0.5 g chicken egg yolk, and 1.0 g honey in 12.0 ml of distilled water. Palmitic acid was mixed with the egg yolk component before adding it to the diet. The encapsulated diet was stored at −80 °C.

2.1.3. Experimental diets

The experimental diets were composed of artificial diet supplemented with either components from eggs of *P. interpunctella* or non-insect nutrients available commercially.

2.2. Plodia interpunctella egg components

2.2.1. Plodia egg homogenate

Eggs at 0.50, 0.75, and 1.0 g were each homogenized on ice in 2 ml of distilled water or 20 mM Tris–HCl, pH 7.0, in a hand-held glass homogenizer and immediately added to 12 ml of diet to give concentrations of 41.6, 62.5, and 83.3 mg of egg homogenate per ml of diet.

2.2.2. Plodia egg protein extract

Five grams of *P. interpunctella* eggs (1.25×10^6 eggs) were homogenized on ice using a Polytron homogenizer (1-cm dia generator, Brinkman, Westbury, NY) for 2 min at full speed in 20 ml of ammonium acetate buffer (1.16 g/L, titrated to pH 7.5 using ammonium hydroxide). The homogenates were centrifuged at 20,200*g* for 5 min. The supernatant contained three layers following centrifugation. Soluble protein in the middle layer (beneath the upper lipid layer) was collected and filtered through a Millex-HV 0.45-μm/29-mm dia. filter (Millipore, Bedford, MA). The filtrate was then applied in

1.25-ml aliquots to a D-Salt Excellulose desalting column (<5 kDa, Pierce, Rockford, IL) equilibrated in ammonium acetate buffer, eluted in 1-ml fractions, and proteins were monitored at 280 nm and collected in the void volume. These fractions were combined (37 ml), frozen in a dry ice/methanol bath and freeze-dried, resulting in a fluffy white powder. The freeze-dried desalted powder (352 mg) was added to 5 ml of distilled water; the soluble protein concentration of the solution was determined to be 17.9 mg/5 ml using the Lowry procedure for soluble proteins (Protein Assay Kit, Sigma, St. Louis, MO). Aliquots of 0.75, 1.0, and 2.0 ml of the macromolecular solution were each added to 12 ml of diet to give final concentrations of 224, 299, and 514 μg/ml of diet, respectively.

2.2.3. Plodia egg lipid extract

Lipids were extracted from 0.50, 0.75, and 1.0 g of *Plodia* eggs using a modified method of Folch et al. (1957) according to Ferkovich et al. (2000). Briefly, a chloroform:methanol mixture (2:1, v:v) was added to 12.0 ml of egg homogenate in Ringer's solution at a ratio of 1.0 ml homogenate to 6.0 ml chloroform:methanol. The emulsion was broken with distilled water (73.3 ml) and each of the two resultant chloroform and methanol phases were dried down to ≈0.5 ml at 40 °C using a Rotovap. Any noticeable solvent residue was removed by blowing purified nitrogen into the flask. Solvent controls were prepared by extracting Ringer's solution with the chloroform:methanol mixture and drying. The egg yolk component of the diet was added to the flask containing the lipid residue and then rotated for 5 min.

2.2.4. Fatty acids

One-half gram of *Plodia* eggs was analyzed for fatty acids by gas chromatography using the MIDI Sherlock Microbial Identification System (Department of Microbiology, University of Florida, Gainesville, FL). A 0.50-g quantity of *Plodia* eggs (<12 h after oviposition) was frozen, homogenized in liquid nitrogen, extracted, separated (phenylmethyl silicone fused silica capillary column) and identified according to Sasser (1997). Based on this fatty acid analysis, the three predominant fatty acids, palmitic (34.9%), linoleic (18.36%), and oleic (36.58%), all from Sigma, St. Louis, MO, were mixed in the chicken egg yolk portion of the diet and added to the diet in the appropriate concentrations of 183, 350, and 367 mg/ml, respectively. This resulted in a total concentration of 900 mg of fatty acids/ml of diet.

2.3. Non-insect supplements

2.3.1. Chicken liver

Ground chicken liver was added at 125 and 208 mg/ml of diet and based on the average digestible protein per 100 g of chicken liver (Souci et al., 1989) contained 26.7 and 45.0 mg protein/ml of diet, respectively.

2.3.2. Beef liver

Ground beef liver was added to the diet at 125 and 208 mg/ml of diet and based on the average digestible protein per 100 g of beef liver (Souci et al., 1989) contained 25.0 and 40.8 mg protein/ml of diet, respectively.

2.3.3. Chicken egg albumin

Crude dried chicken egg white albumin (Deb-EL Foods, Elizabeth, NJ) was added to the diet at 167 and 250 mg/ml.

2.3.4. BSA

Bovine serum albumin (Fraction V, minimum 96–99%, Sigma, St. Louis, MO) was added to the diet at 83 and 167 mg/ml.

2.4. Diet assay

Newly emerged adults of a Florida strain of *O. insidiosus* were obtained from a commercial producer of beneficial insects (Entomos, Gainesville, FL). The insects were reared on eggs of *E. kuehniella* before being placed on the diets on the third day after eclosion. Each diet treatment consisted of six females and four males in a 100-ml plant tissue culture jar (Sigma, St. Louis, MO) with four jars per treatment. Each jar contained one paraffin-encapsulated water dome (50 μl), diet (see below), one 7-cm section of green bean pod for oviposition, and three crumpled strips of wax paper (5 mm × 80 mm) as substrates. Green beans were removed every other day and examined under a microscope to count eggs and then replaced with a fresh green bean section and mortality was also recorded within each jar. Diet and water domes were replaced on the same days that the green beans were removed for counting eggs. The insects were held in a growth chamber at 25.5 ± 1 °C, with 75 ± 5% RH and a photoperiod of 15:9(L:D) h. The experiments were carried out for 18 days although the females were observed to intermittently oviposit for approximately 28 days when fed *Plodia* eggs.

Diet consisted of the following: *Artificial diet* jars contained artificial diet with no additional substances. *Experimental diet* jars contained artificial diet with an incorporated supplement as described. *Plodia eggs* jars contained 75 *P. interpunctella* eggs (3 mg) each. Although eggs from *E. kuehniella* Zeller are generally used by commercial producers, we used eggs from *P. interpunctella* because *O. insidiosus* feeds readily on their eggs, and we rear *P. interpunctella* in our laboratory.

2.5. Experimental design

Two tests were conducted. In the first test, *artificial diet* was compared with the experimental diets which were composed of artificial diet supplemented with

homogenized *Plodia* eggs, and protein and lipid extracts from *Plodia* eggs. Each experimental diet was compared against the control *artificial diet* in an independent experiment on different dates. In the second test, any of the statistically significant experimental diets from the first test were then simultaneously compared with the experimental diets containing non-insect supplements.

Each treatment in both tests was replicated four times with 6 ♀ and 4 ♂/replication. Each day that egg counts were made the data was adjusted for mortality and the mean number of eggs/♀/replication/day was calculated. The cumulative mean number of eggs/♀ for each of the four replications was then calculated and these means were used in the statistical analysis. In addition, the cumulative number of eggs laid/♀ on *artificial diet* and on experimental diet were calculated as a percentage of the number of eggs laid/♀ on the *Plodia* eggs (standard); thus, mean per cent of *Plodia* egg standard = [# eggs laid per ♀ on either *artificial diet* or experimental diet divided by # eggs laid per ♀ on *Plodia eggs*] × 100, $n = 4$.

Data were analyzed by ANOVA using StatMost software (Dataxiom Software, Salt Lake City, UT). Dunnet's test was used to determine if the cumulative number of eggs laid/♀ on the each of the experimental diets was significantly greater than the number of eggs laid/♀ on the *artificial diet.*

3. Results

3.1. Evaluation of P. interpunctella egg components

The *Plodia egg homogenate* did not increase the average cumulative number of eggs laid by females maintained on the diet (Table 1). At the highest concentration of homogenate, the mean number of eggs/♀ was significantly reduced compared to the *artificial diet* alone. We noticed that adding the homogenized eggs to the diet caused it to thicken and turn brownish in color (e.g., melanize) between diet replacements. This color change occurred whether the eggs were homogenized in distilled water or in Tris buffer. However, when females were fed as adults on soluble proteins extracted from a homogenate of *Plodia* eggs (*Plodia egg protein extract*, Table 1), they laid significantly more eggs than females reared on *artificial diet* alone. Melanization was not observed in this preparation. Neither the *Plodia egg lipid extract* nor the *fatty acids* supplement had a significant effect on the average number of eggs laid/♀.

3.2. Comparison of Plodia egg extract with non-insect components

In the first experiment (Table 1), the *Plodia egg protein extract* was the only insect component that increased egg production when added to the *artificial diet*. In a second experiment, we tested whether non-insect proteins would increase egg production comparable to proteins from *Plodia* eggs.

Again, as in Table 1, the *Plodia egg protein extract* significantly increased egg production at the mid-level and highest concentrations of protein tested (Table 2). Two of the non-insect protein sources, *bovine serum albumin* and *chicken egg albumin*, also significantly increased the cumulative mean number of eggs oviposited/♀ at the highest concentrations. A third non-insect protein source, beef liver, increased egg production at the mid-level, but was inhibitory at the higher concentration tested. The mid-level concentration of *Plodia egg protein extract* (299 μg protein) was 83-, 557-, and 837-fold lower than the concentration of *beef liver*, *bovine serum albumin*, and *chicken egg albumin*, respectively, that were required to significantly increase egg production. The duration of egg-laying on supplemented diets is shown in Table 3. Diets supplemented with *Plodia egg*

Table 1
Effects of supplementing *artificial diet* with components from eggs of *Plodia interpunctella* and three predominant fatty acids identified in *Plodia* eggs on rate of oviposition

Treatment[a]	Mean number of eggs/♀ ± SE[b]	% of *Plodia* egg standard[c]
Plodia egg homogenate (mg/ml)[d]		
0 (*artificial diet*, control)	10.4 ± 1.2	43.5
41.6	10.0 ± 0.9	41.8
62.5	8.8 ± 1.0	36.8
83.3	6.6 ± 0.7*	27.7
Plodia egg protein extract (μg protein/ml diet)[e]		
0 (*artificial diet*, control)	11.4 ± 0.8	43.9
299	17.4 ± 1.3*	65.1
514	15.2 ± 1.3*	56.8
Plodia egg lipid extract (egg equiv., mg/ml diet)[f]		
0 (*artificial diet*, control)	12.8 ± 2.6	34.9
41.6	12.2 ± 1.9	33.5
62.5	15.4 ± 2.5	41.3
83.3	15.9 ± 2.8	42.5
Fatty acids (mg/ml)[g]		
0 (*artificial diet*, control)	12.8 ± 2.6	34.9
900	10.5 ± 2.6	28.3

[a] Each supplement was tested on a different date in an independent experiment with the *artificial diet* and *Plodia eggs* (standard).

[b] Dunnet's test was used to compare the treatment means against the *artificial diet* (control); asterisk indicates that treatment means are significantly different from *artificial diet* (control) ($P < 0.05$).

[c] Per cent of *Plodia egg* standard = # eggs laid/♀ on treatment/# eggs laid/♀ on *Plodia* eggs standard × 100, $n = 4$.

[d] Diet supplemented with homogenates of 0.5, 0.75, and 1.0 g of *Plodia* eggs each added to12 ml of diet.

[e] Diet supplemented with *Plodia* egg protein extract; quantity of protein added to the diet was based on Lowry analysis for soluble protein in the extract.

[f] Diet supplemented with lipids extracted from 0.5, 0.75, and 1.0 g of *Plodia* eggs; each extract was added to 12 ml of diet.

[g] A mixture of linoleic (2.2 g), palmitic (4.2 g), and oleic (4.4 g) were added to 12 ml of diet which resulted in 183, 350, and 367 mg/ml, respectively, and a total concentration of 900 mg of fatty acids/ml of diet.

Table 2
Comparison of non-insect supplements with the *Plodia egg extract* that significantly improved rate of oviposition in Test 1

Treatment	Mean number of eggs/♀ ± SE[a]	% of *Plodia* egg standard[b]
Artificial diet (control)	14.8 ± 0.5	38.5
Artificial diets with:		
Plodia egg protein extract (μg/ml diet)		
224	17.8 ± 1.3	45.1
299	21.8 ± 1.9*	56.8
514	24.7 ± 0.2*	64.2
Bovine serum albumin (mg/ml diet)		
83.3	19.7 ± 1.6	51.3
167	24.9 ± 1.6*	64.9
Chicken egg albumin (mg/ml diet)		
167	20.0 ± 1.0	52.2
250	24.8 ± 0.9*	64.6
Chicken liver (mg/ml diet)[c]		
26.7	17.9 ± 1.4	46.3
45.0	18.8 ± 1.7	49.0
Beef liver (mg/ml diet)[c]		
25.0	20.9 ± 1.5*	54.7
40.8	15.9 ± 0.7	41.3

[a] Dunnet's test was used to compare the treatment means against the *artificial diet* (control); asterisk indicates that treatment means are significantly different from *artificial diet*, ($P < 0.05$).

[b] Per cent of *Plodia egg* standard = # eggs laid/♀ on treatment/# eggs laid/♀ on *Plodia* eggs standard × 100, $n = 4$.

[c] The quantity of protein in the chicken and beef liver supplements added to the diet was based on average digestible protein per 100 g as described by Souci et al. (1989).

Table 3
Duration of egg laying on supplemented diets

Treatment	Mean number of days ± SE[a]
Plodia eggs	23.7 ± 0.2
Artificial diet (control)	17.0 ± 0.5
Artificial diet with:	
Plodia egg protein extract (μg/ml diet)	
224	18.0 ± 0.6
299	19.0 ± 0.8
514	21.0 ± 0.0*
Bovine serum albumin (mg/ml diet)	
83.3	21.0 ± 0.0*
167	22.0 ± 1.2*
Chicken egg albumin (mg/ml diet)	
167	18.5 ± 0.5
250	19.5 ± 0.5
Chicken liver (mg/ml diet)[b]	
26.7	20.0 ± 1.0*
45.0	18.5 ± 0.5
Beef liver (mg/ml diet)[b]	
25.0	19.0 ± 0.8
40.8	17.5 ± 0.5

[a] Dunnet's test was used to compare the treatment means against the *artificial diet* (control); asterisk indicates that treatment means are significantly different from *artificial diet*, ($P < 0.05$).

[b] The quantity of protein in the chicken and beef liver supplements added to the diet was based on average digestible protein per 100 g as described by Souci et al. (1989).

protein extract, *bovine serum albumin*, and *chicken liver* all significantly increased the average duration of egg laying relative to the *artificial diet*. Mortality was 65% on the *Plodia egg* (standard) diet and 95% on the *artificial diet*. Mortality on the experimental diets was 72, 76, 80, 82, and 85% on *BSA*, *Plodia egg protein extract*, *chicken albumin*, *chicken liver*, and *beef liver*, respectively.

4. Discussion

In this study, we selected an artificial diet (Weiru and Ren, 1989) that contained no insect-derived protein. By maintaining *O. insidiosus* adults on this diet supplemented with nutrients from various sources, we were able to approach the level of oviposition represented by the standard diet of *P. interpunctella* eggs. The rate of ovipositon of *O. insidious* species can vary with the species of prey (Nakashima and Hirose, 1999) and the rate of oviposition we observed on *Plodia* eggs was 43.4 eggs/♀ during the 18 day bioassay we conducted. This rate of oviposition was lower than that reported for *O. insidious* reared on eggs of *Heliothis virescens* (F.) (106.4 eggs/♀) (Kiman and Yeargan, 1985) and 103.9 eggs/♀ for *Orius sauteri* (Poppius) reared on eggs of *E. kuehniella* Zeller, however, both of these studies recorded oviposition for 40.4 and 40 days, respectively. Adding a protein extract from eggs of *P. interpunctella* to the artificial diet had a significant positive effect on *Orius* egg production. A whole homogenate of *P. interpunctella* eggs had a negative effect, likely due to the release of inhibitory substances such as phenol oxidases. We were surprised to find such large differences in the concentrations of the various proteins that were required to increase egg production compared with the *Plodia protein extract*, especially because the predator is a generalist feeder and would be expected to utilize various sources of protein. The positive effect with the *Plodia egg protein extract* occurred at concentrations ca. 80- to 800-fold lower than the concentrations of non-insect components beef liver, bovine serum albumin, and chicken egg albumin required to improve egg production. It is unknown what the source of stimulatory activity is in the *Plodia egg protein extract*. However, the extract contains soluble proteins, and it is possible that *Orius* females require the quality of those proteins (e.g., amino acid profile) in the prey egg extract for their egg production.

In related studies, lower rates of assimilation and conversion efficiency were observed in *G. punctipes* (Say) reared on artificial diet (Cohen, 1989, 1992) than in predators reared on host eggs (Cohen and Urias, 1988). When the predator *P. bioculatus* was maintained on an artificial diet, reduced egg production was observed and was attributed to the failure to form mature follicles (Adams, 2000a). The decreased egg production was thought to be due to either a reduction in the juvenile hormone titer needed for vitellogenin synthesis, or to a lack of vitellogenin precursors such

as amino acids, lipids or carbohydrates. Improved development of the predator *P. bioculatus* was attributed to the quality of dietary protein (Rojas et al., 2000). Tuna fish promoted development better than other protein sources such as beef, veal, and chicken. The authors attributed the reduction in egg production not only to a protein deficiency but also to a lack of chemical or behavioral cues that confirmed the presence of live prey.

Another observation that points to protein quality as an important factor in *O. insidiosus* egg production is the level of protein in the *artificial diet*. Considering the quantity of protein that *Orius* predators ingest on a daily basis when maintained on *Plodia* eggs, the females had more than an adequate level of protein available in the *artificial diet*. The *artificial diet* contained 34.2% protein, 2.4% lipid, 6% carbohydrate, and 67.4% water content. The concentration of protein is in keeping with reports of 15–20% protein in prey larvae and eggs (Cohen, 1992, 1998a). Moreover, *artificial diet* contained 51 μg/0.15 μl of protein which is comparable to the amount found in the average number of *Plodia* eggs consumed by *Orius* daily (5 eggs/adult contain 55 μg of protein/0.15 μl, personal observation). Consequently, the *artificial diet* contained a protein concentration that was comparable to that in prey eggs. If we assume that the insects consumed sufficient quantities of the diet, then the quality of yeast and soy protein hydrolysates in the *artificial diet* were not optimal for promoting egg production of *O. insidiosus*.

Another explanation for the stimulatory effect of the *Plodia egg protein extract* on ovipostion of *O. insidiosus* at low concentrations of protein may be that the active material in the extract was a smaller molecule bound to the proteins. These may include bound enzymes, lipids, vitamins, or minerals that would contribute to the activity (Reinecke, 1985).

Finally, toxicants or inhibitors in the *artificial diet* could have contributed to the reduced egg production of *Orius* females. Conversely, the increase in egg production we observed upon the addition of the *Plodia* and non-insect proteins to the artificial diet may have been due in part to neutralizing effects of the added proteins on inhibitors (Baker, 1982; Baker et al., 1984; Bondi and Alumot, 1987; Broadway and Duffey, 1986; Burns, 1987; Larocque and Houseman, 1990). Burgess et al. (1991) studied the effects of protein levels and protease levels on the black field cricket, *Teleogryllus commodus* (Walker), and found that the quality of protein influences the antinutritional effects of the inhibitors on growth. Higher levels of casein were found more effective than wheat germ in alleviating the inhibition. Reinecke (1985) stated that crude protein such as soybean products provide a good source of protein in insect diets. However, they may also be contaminating components that could either be toxic or act as feeding inhibitors. He stated, however, that they generally must be used in combination with other proteins to guard against imbalances in amino acids.

In conclusion, the *artificial diet* composed of chicken egg yolk, yeast, and soy protein hydrolysate did not support good egg production in adult *O. insidiosus*. However, supplementing the diet with a protein extract from eggs of *P. interpunctella* resulted in higher egg production at low concentrations relative to those of non-insect proteins. Our understanding of why the *Plodia* egg proteins stimulate the rate of oviposition of *O. insidiosus* at such low concentrations will require further purification of the *Plodia egg protein extract*. Identification of the active protein or substance associated with the proteins may prove to be useful in fortifying artificial diets for *Orius* species.

Acknowledgments

We appreciate the excellent technical assistance in this study of Delaine Miller and Jan Sasser.

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