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ENHANCED OVIPOSITION IN THE INSIDIOUS FLOWER BUG, ORIUS INSIDIOSUS (HEMIPTERA: ANTHOCORIDAE) WITH A PARTIALLY PURIFIED NUTRITIONAL FACTOR FROM PREY EGGS

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ABSTRACT

The insidious flower bug, *Orius insidiosus* (Say), can be maintained on a minimal artificial diet composed of brewers yeast, soy protein hydrolysate and chicken yolk. However, egg production is poor even though the level of protein in the diet exceeds the amount consumed by adults that are fed insect eggs and have higher levels of egg production. We therefore fractionated eggs of the almond moth, *Ephestia kuehniella* Zeller by preparative isoelectric focusing and bioassayed the resultant fractions in test diets. Ovipositional rates were evaluated using a short 1-week bioassay. Adult predators were placed on the diets the third day after eclosion, allowed to feed for six days, and then provided with an oviposition substrate for 24 h on day seven. Egg production significantly increased only in a fraction with an isoelectric point of pH 5. SDS-PAGE revealed the presence of several Commassie blue-stained bands; however, the nature of the factor is unknown. These results point to a fecundity factor required by females of *O. insidiosus* for egg laying that potentially may be used to supplement artificial diets for *Orius* species by commercial producers of beneficial insects.

Key Words *Orius insidiosus*, *Ephestia kuehniella*, predator, artificial diet, oviposition, prey eggs, proteins

RESUMEN

El chinche insidiador de flores, *Orius insidiosus* (Say), puede ser mantenido sobre una dieta artificial mínima compuesta de levadura de cerveza, hidrolisado de proteína de soya e yema de huevo de gallina. Sin embargo, la producción de huevos es pobre a pesar de que el nivel de proteína en la dieta excede la cantidad consumida por los adultos alimentados con huevos de insectos y con un nivel de producción de huevos mas alto. Por eso, nosotros fraccionamos los huevos de la polilla de almendra, *Ephestia kuehniella* Zeller utilizando el enfoque preparativo isoelectrico y por el bioensayo de las fracciones resultantes de las dietas probadas. Las tasas de oviposición fueron evaluadas utilizando un bioensayo corto de una semana. Los adultos depredadores fueron sujetos a las dietas el tercer dia después de eclosionar, se los permitio la alimentación por seis dias y en el septimo dia fueron proveidos con un substrato para la oviposición por 24 h. La producción de huevos aumento significativamente una fracción solamente con un punto isoelectrio de pH 5. La PAGINA-SDS reveló la presencia de varias bandas de 'Commassie' de tinte azul; sin embargo, la naturaleza del factor es desconocida. Estos resultados indican que hay un factor de fecundidad requerido por las hembras de *O. insidiosus* para la oviposición de los huevos que potencialmente puede ser usados para suplementar las dietas artificiales para las especies de *Orius* por los productores comerciales de insectos beneficos.

The insidious flower bug, *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) is a generalist feeder of thrips, aphids, mites and whiteflies, and eggs of other insects in the field (Barber 1936; McCaffrey & Horsburgh 1986; van der Veire & Degheele 1992; van Lenteren et al. 1997; Funderburk et al. 2000). This predator has been reared in the laboratory on an artificial diet devoid of any insect host components (Weiru & Ren 1989). However, predators reared on this artificial diet had reduced fecundity (Ferkovich & Shapiro 2004a). A general problem associated with a number of other species of predators reared on artificial diets has been a reduction in reproductive rate (De Clercq & Degheele 1992, 1993a & b; De Clercq et al. 1998; Wittmeyer & Coudron 2001). The reason for the reduced fecundity observed in predators fed artificial diets is not clear.

Since adult females of *O. insidiosus*, as with other heteropteran predators such as *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae) (Shapiro et al. 2000), exhibited higher yolk content in developing oocytes as well as higher egg production when fed prey versus artificial diet (Shapiro & Ferkovich 2002), we surmised that natural prey may contain a specific nutritional factor needed by the predator for egg production. When adult *O. insidiosus* were fed an artificial

diet, the females exhibited poor egg production even though the level of protein in the diet exceeded the amount consumed per day by adults fed eggs of the Indian meal moth, *Plodia interpunctell*a (Hübner) (Lepidoptera: Pyralidae) When a protein extract from Plodia eggs was tested as a supplement to the *Orius* diet, it significantly increased egg production at concentrations of protein that were 8.3-, 55.7-, and 83.7-times lower than the concentrations needed for beef liver, bovine serum albumin, and chicken egg albumin, respectively (Ferkovich & Shapiro 2004a). Subsequently, Ferkovich & Shapiro (2004b) found that the egg protein extract could be replaced in the diet with cells from an embryonic cell line (PIE) derived from *P. interpunctella* eggs to enhance oviposition of *O. insidiosus*.

In view of some of the positive effects on the rate of oviposition of *O. insidiosus* fed diet containing prey egg-extracted protein (Ferkovich & Shapiro 2004a), we fractionated the proteins in prey eggs to determine if the increased rate of oviposition could be attributed to a specific fraction of proteins.

Materials and Methods

Preparation of Egg Protein Extract

Soluble egg proteins were isolated from 5 g of *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs (1.25×10^6 eggs) as described by Ferkovich & Shapiro (2004a). Briefly the eggs were homogenized in ammonium acetate buffer (pH 7.5) and the soluble proteins were separated by centrifugation. The soluble proteins were then run through a desalting column, freeze-dried, and stored at -80°C. The freeze-dried desalted powder (352 mg) was then added to 58 ml of distilled water; and the soluble protein concentration of the solution was determined to be 174 mg/total vol.

Protein Assay

The Lowry procedure (Protein Assay Kit, Sigma, St. Louis, MO) was used to assay the quantity of soluble proteins in the egg protein solution and in the fractions after isoelectric focusing.

Preparative Isoelectric Focusing

Five ml of the soluble protein solution and 3 ml of ampholyte solution (pH range 3-10, Bio-Rad, Hercules, CA) were mixed in 42 ml of 1 M urea to prevent loss of proteins due to excessive precipitation. The protein solution was run in a Rotofor Cell© isoelectric focusing unit (Bio-Rad instruction manual) for 2.5 h at 12 W constant power and 4°C. The initial conditions were 408 V and 38 mA and 668 V and 23 mA at equilibrium. Twenty fractions were collected and their volumes (approx. 2.0 ml each) and pH values measured. Fractions 12-17 were cloudy and contained precipitates. Ampholytes were used to form the pH gradient in which the egg proteins were separated. These ampholytes were then removed due to interference with the subsequent protein assay and SDS-gel electrophoresis. They were removed by adding NaCl to each fraction to a final concentration of 1 M for 15 min and dialyzing against water. Aliquots of 10 or 20µl of each fraction were analyzed for protein content. After the fractions were analyzed for protein, they were combined based on the protein profile. Fractions with low protein levels (1-8, 9-12, 18-20) were combined, and ones with higher protein concentrations (13-17) were kept as individual fractions. Each combined or individual fraction was then concentrated to 0.5 ml in a Centriprep© concentrator (10k molecular weight (MW) cutoff; Millipore, Bedford, MA) and 10 or 20µl of each fraction were used to analyze for soluble protein.

Assay of Isoelectric Focusing Fractions in Diet

The 0.5 ml-fractions obtained from the isoelectric focusing of the *Ephestia* egg proteins were each added to 0.5 ml of diet and encapsulated (20µl vol.). Artificial 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) described earlier (Carpenter & Greany 1998, Ferkovich et al. 1999). Artificial diet was prepared as described for rearing *O. sauteri* (Weiru & Ren 1989), and consisted of 0.33 g brewers yeast, 0.03 g sucrose, 0.18 g soy protein acid hydrolysate, 3.8 mg of 99% palmitic acid (all from Sigma, St. Louis, MO), 0.04 g chicken egg yolk, and 0.08 g honey in 1.0 ml of distilled water containing the concentrated fractions from isoelectric focusing. Palmitic acid was mixed with the egg yolk component before adding it to the diet.

Newly emerged adults of a Florida strain of *O. insidiosus* (<24 h after eclosion) were obtained from a commercial producer of beneficial insects (Entomos, Gainesville, FL) and placed on the diets on the third day after eclosion. At the end of the sixth day, one 7-cm section of green bean pod, as a substrate for oviposition, was placed in each jar for 24 h. Eggs deposited in the green beans were then counted under a microscope. 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 treatment diets were (1) Eggs (standard)—jars contained 150 *Ephestia* eggs (approx. 3 mg) each as a reference standard; (2) Diet (control)—jars contained artificial diet with no additional substances as control diet; and (3) Diet (amended)—jars contained artificial diet supplemented with each of combined fractions 1-8, 9-12,

18-20, and individual fractions 13 through 17 as separate treatments.

Electrophoresis

Fractions resulting from the separation of the *Ephestia* egg proteins by isoelectric focusing were analyzed by SDS polyacrylamide gel electrophoresis (SDS-PAGE). Gradient SDS-PAGE (4-20%) was carried out in minivertical gels (Bio-Rad) as described by Shapiro et al. (2000).

Data Analysis

Each treatment was replicated four times with six females and four males per replicate. The egg counts were adjusted for female mortality within each treatment. Data were analyzed by ANOVA with StatMost software (Dataxiom Software Inc.). Dunnett's test was used to determine if the number of eggs oviposited per female on each of the diet treatments supplemented with the isoelectric focusing fractions was significantly greater than the number of eggs oviposited per female on the control diet. Since insectaries generally produce *O. insidiosus* on eggs of *E. kuehniella*, we used them as a reference standard but did not include the treatment in the ANOVA.

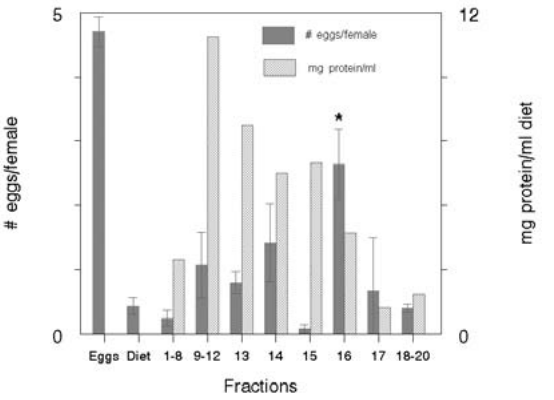

Fig. 2. Average number of eggs (±SE) oviposited by females of *O. insidiosus* after being fed artificial diet supplemented with protein in fractions from isoelectric focusing separation of *Ephestia* egg protein shown in Fig. 1. Eggs (standard)—whole eggs of *E. kuehniella*; Diet (control)—diet with no additional substances; and Diet (amended)—supplemented with each of combined fractions 1-8, 9-12 and 18-20 and individual fractions 13 through 17 in separate diet treatments. Dunnet'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) ($P < 0.05$).

Results

Figure 1 shows the protein profile versus pH of the *Ephestia* egg protein extract separated on in a pH gradient of 3-10. Average rate of eggs oviposited per female was significantly increased relative to the control Diet in only one Diet (amended) treatment, a fraction with an isoelectric point of pH 5 fractions (Fig. 2). The active fraction contained 6.8 mg or 16% of the total protein recovered in all the fractions (43.3 mg). The active fraction (#16) shown in the diet bioassay of the fractions in Fig. 2 contained one major band at 47,000 MW that also appeared to be present as a lighter band in fractions 17 and 18-20 (Fig. 3). Other faint bands at 163k, 51k, 39k, 31k, 27k, and 23k MW were present in fraction 16 (Fig. 3). Recovery of the total protein applied to the gradient was

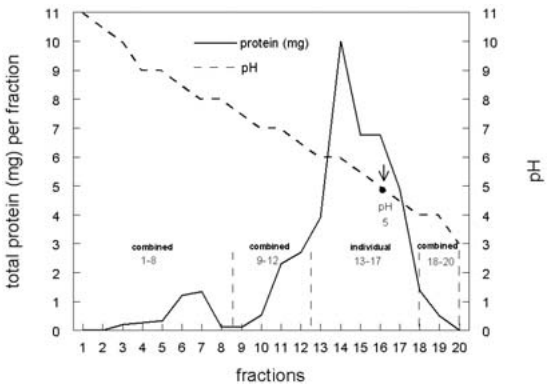

Fig. 1. Protein profile of *Ephestia kuehniella* egg protein separated by isoelectric focusing on a pH gradient of 3-10. Fractions that were combined for bioassay in artificial diet are shown by vertical dotted lines. Arrow indicates the fraction that stimulated the rate of oviposition.

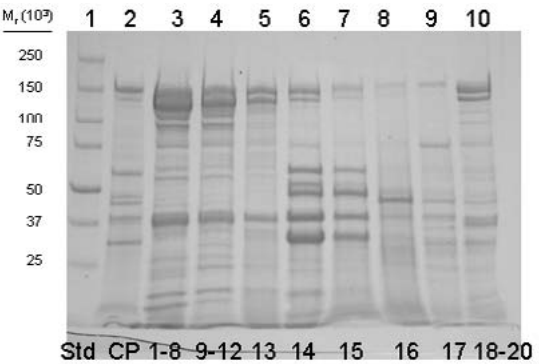

Fig. 3. SDS-PAGE analysis of fractions separated as shown in Fig. 1. Lane 1 (Std) - MW standards; lane 2 (CP)—crude protein; lane 3—combined fractions 1-8; lane 4—combined fractions 9-12; lanes 5-9—individual fractions 13-17; and lane 10—combined fractions 18-20.

24.8% (174 mg applied; 43.2 mg recovered); heavy precipitates in fractions 12-17 after isoelectric focusing resulted in a loss of protein in these fractions when the samples were dialyzed and concentrated.

Discussion

The fecundity of females was increased with the addition of a specific fraction of *Ephestia* egg proteins that were separated by isoelectric focusing. The effect was not dependent on the concentration of egg proteins in the fraction as other fractions that contained higher levels of protein did not stimulate the ovipositional rate of *Orius* females. SDS-PAGE analysis of the fractions revealed major proteins with relative molecular weights ranging between 100k and 150k and less than 40k MW which were not present in the active fraction. Although we did not identify the yolk proteins in *Ephestia kuehniella*, Shirk (1984) identified four major yolk proteins ranging in molecular weight from 33k to 150k MW in a closely related pyralid species, the Indian meal moth, *P. interpunctella*. Proteins extracted from eggs of *P. interpunctella* by the same procedure described in this study stimulated egg production in *O. insidiosus* fed artificial diet; however, a chloroform:methanol extract of the egg lipids had no effect (Ferkovich & Shapiro 2004a). Furthermore, in a separate study with *O. insidiosus*, in which total protein, RNA, and DNA were extracted from *Ephestia* eggs and bioassayed in diet, only the egg proteins stimulated the rate of oviposition when the three egg-extracted components were bioassayed in artificial diet (unpublished data).

Fecundity could be improved further with the addition of a higher concentration of the fraction that increases the rate of oviposition. Wheeler (1996) indicated that oogenesis is typically a nutrient-limited process and is initiated only if sufficient nourishment is taken for egg production. Adequate nourishment for *O. insidiosus* egg production is apparently not acquired during the nymphal stage because newly emerged adults that fed on the control Diet oviposited fewer eggs that those fed on whole *Ephestia* eggs. Only females that fed on Diet supplemented with fraction #16 oviposited significantly more eggs than the control Diet. This indicated that a specific nutrient or factor is required for egg production and is found in the protein component of the prey egg. The nature of factor, however, is unknown and awaits further purification and characterization. Protein extracts from whole eggs of *P. interpunctella* and a cell line derived from eggs of *P. interpunctella* (Ferkovich & Shapiro 2004 a & b) stimulated egg production of *O. insidiosus* females, but this is the first report of a specific protein fraction from prey eggs having oviposition-stimulating activity.

In view of a specific fraction having a positive effect on oviposition in *O. insidiosus*, we suggest that future research should focus on identifying the oviposition-enhancing material(s) from *Ephestia* eggs so that it can be more easily tested at various concentrations in the diet. Moreover, once the identity of the material is known, it may be possible to obtain it from a commercial source for supplementing the diet of *O. insidiosus*.

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