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ENHANCED EGG LAYING IN ADULT PREDATORS FED ARTIFICIAL DIET SUPPLEMENTED WITH AN EMBYONIC CELL LINE DERIVED FROM EGGS OF *EPHESTIA KUEHNIELLA* ZELLER (LEPIDOPTERA: PYRALIDAE)

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Suboptimal fecundity in entomophagous insects reared on artificial diet is a common problem and barrier in implementing cost-effective large-scale production of beneficial insects for augmentative biological control (Grenier et al. 1994). The artificial diets of a number of entomophagous insects can only be improved by adding insect tissues such as hemolymph to the diet (Nettles 1990). The use of insect materials, however, is not feasible because of the labor required and associated problems (e.g., melanization), and other means of improving the diets are needed. One obvious approach is to identify the compounds in insect materials that are responsible for improving fecundity, however, this is difficult and none have been identified yet (Ferkovich & Shapiro 2004a). Another approach is to use insect cells to improve artificial diets for predators. Insect cell lines have been tested on several parasitoids with promising results (Rotundo et al. 1988; Ferkovich et al. 1994; Hu et al. 1999; Heslin et al. 2005). The fecundity of the insidious flower bug, *Orius insidiosus* (Say), reared on artificial diet was improved with IPLB-PiE, a cell line derived from eggs of the Indian meal moth, *Plodia interpunctella* (Hübner) (Ferkovich & Shapiro 2004b). Because insectaries generally produce the predator on eggs of the Mediterranean flower moth, *Ephestia kuehniella* Zeller (Association of Natural Bio-control Producers), a cell line (Ek-x4V) was recently developed from eggs of *E. kuehniella* (Lynn & Ferkovich 2004). In this study, we investigated the potential of using the *Ephestia* cell line (Ek-x4V) to promote egg production of adults of *O. insidiosus* maintained on artificial diet.

The IPLB-PiE and the Ek-x4V cell lines were cultured as described earlier (Lynn 1996; Lynn & Ferkovich 2004). Briefly, the IPLBPiE cells were grown in modified TNM-FH insect medium (Sigma, St. Louis, MO) in 25-cm^2 culture flasks for 7 days. For larger-scale culture of the cell lines, the PiE cells were grown in 250 ml of medium in 500-ml magnetic spinner flasks (Bellco Glass, Vineland, NJ) at 24.9E for 14 days. The Ek-x4V cells were grown in SF900II medium (Invitrogen Corp., Grand Island, NY) in 25-cm^2 culture flasks for 14 days but could not be cultured in the larger spinner flasks because they grew as cellular aggregates and the spinning motion of the flasks interfered with their growth. Both cell lines were centrifuged (1370 *g* for 3 min) in graduated conical glass tubes to obtain a pellet of cells. The pellets were resuspended in distilled water and washed 2×. Cells from each line were then bioassayed in two tests.

The objective of the first bioassay was to determine if the Ek-x4V cells would affect the oviposition rate of females in a dose-response manner. Washed cells were centrifuged to obtain 0.25, 0.5, 0.75, and 1.0 ml of soft pellets of cells which were each homogenized with a hand-held homogenizer. Aliquots (20µ1) of the homogenates were removed and assayed for protein by the Lowry procedure (Protein Assay Kit, Sigma, St. Louis, MO). Diet ingredients (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 fresh chicken egg yolk, and 0.08 g honey) were added to each of the tubes to give a final volume of 1.2 ml. The diet was then encapsulated in Parafilm© (25µl capsules) and bioassayed as described earlier (Ferkovich & Shapiro 2004b). Adults (three days after eclosion) were fed 3 mg of *E. kuehniella* eggs (Eph Eggs), two capsules of artificial diet (AD), and two capsules of artificial diet + Ek cells (Ek Cells) for six days. Each treatment diet consisted of six females and four males, two Parafilm© capsules of water (25µl each) and two capsules (25µl each) of treatment diet with four replicates per treatment. Diets were replaced daily and mortality was recorded. At the end of the sixth day, a green bean pod was placed in each jar as an oviposition substrate and the number of eggs oviposited during a 24-h period were recorded.

The objective of the second bioassay was to compare artificial diet fortified with Ek-x4V cells (Ek Cells) against diet fortified with the IPLB-PiE cells (PiE Cells). Cells (0.74 ml, 52 mg protein) from each cell line were added to diet. Artificial diet (AD) and *Ephestia* eggs (Eph Eggs) treatments were also included in the bioassay.

Data were analyzed by one-way ANOVA with Dunnet's test for comparison of treatment means with control and Newman-Keuls post test for multiple mean comparisons (GraphPad Software, San Diego, CA).

Females fed on the Ek Cells diet oviposited significantly more eggs at the 0.75 ml-, and 1.0- ml doses of cells per 1.2 ml of diet than those that fed on the AD ($F = 3.8, df = 4, P = 0.02$) (Fig. 1). In comparing the PiE Cells diet with the Ek Cells diet, egg production on both diets approached that of females fed Eph Eggs and both diets significantly increased the average rate of oviposition relative

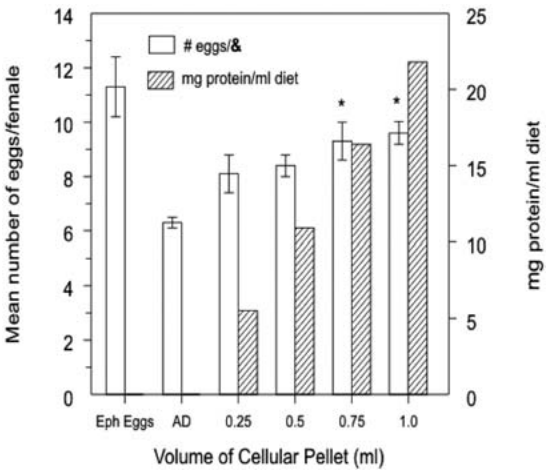

Fig. 1. Comparison of mean oviposition by females fed whole *Ephestia* eggs (Eph Eggs), artificial diet (AD) and artificial diet supplemented with aliquots of Ek-x4V h cells per 1.2 ml of diet. Dunnet's test was used to compare the treatment means against the artificial diet; asterisk indicates that the treatment means are significantly different from the artificial diet, ($P < 0.05$); error bars = standard error.

to the control AD. Neither of the cell line-supplemented diets, however, was better than the other in improving egg production (Fig. 2). The IPLB-PiE cell line is easier to culture since it grows as a suspension and lends itself to culture in spinner flasks and higher densities of cells can be achieved in less time. In contrast, the Ek-x4V line grows as aggregates of cells composed of organized vesicles that did not grow well in spinner flasks to attain the same level of cell densities as the PiE line during the 14 day culture period.

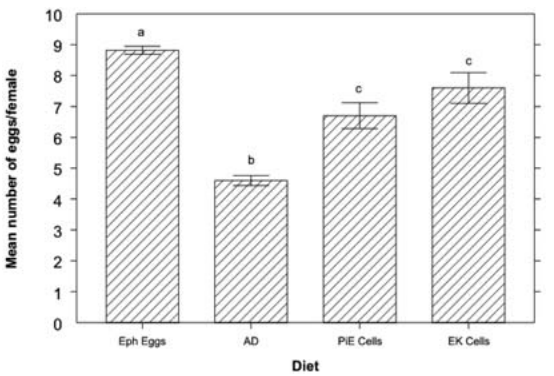

Fig. 2. Comparison of mean oviposition by females fed whole *Ephestia* eggs (Eph Eggs), artificial diet (AD), artificial diet + Pie cells (PiE Cells), and artificial diet + Ek cells (Ek Cells); bars with the same letter are not significantly different (Newman-Keuls method, $P > 0.05$); error bars = standard error.

Consequently, our present findings do not indicate that the EK-x4V cell line affords an advantage over the IPLB-PiE cell line as a diet supplement for improving the fecundity of *O. insidiosus*. The PiE cell line appears to be a better candidate for future studies directed at adapting the PiE line to grow in cheaper, serum-free cell culture medium for use in a large scale fermentation system and methods of preserving and packaging the cells for evaluation in artificial diets by insectaries.

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Summary

Artificial diet supplemented with the Ek-x4V cell line significantly enhanced the average rate of oviposition relative to the control diet but was not better than diet augmented with the IPLB-PiE cell line. The Ek-x4V line grew as aggregates of hollow vesicles of cells in contrast to the IPBL-PiE line which grew as a suspension of unattached cells and did not produce sufficient cell growth in spinner flasks for large-scale production of the cells. Therefore, the EK-x4V cell line does not afford an advantage over the IPLB-PiE cell line as a diet supplement for improving the fecunduty of *O. insidiosus*.

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