# FECUNDITY AND LONGEVITY OF *DIAPETIMORPHA INTROITA* (CRESSON) (HYMENOPTERA: ICHNEUMONIDAE) REARED ON ARTIFICIAL DIETS: EFFECTS OF A LIPID EXTRACT FROM HOST PUPAE AND CULTURE MEDIA CONDITIONED WITH AN INSECT CELL LINE

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ABSTRACT

*Diapetimorpha introita* (Cresson) (Hymenoptera: Ichneumonidae) is a native ectoparasitoid of *Spodoptera* spp. pupae. This parasitoid has been reared in the laboratory on an artificial diet devoid of any insect host components. However, wasps reared on this artificial diet had reduced fecundity. Efforts to increase fecundity included supplementing the diet with cell culture media conditioned with a cell line from ovaries of the fall armyworm, *S. frugiperda,* in one experiment and fortifying the diet with lipids extracted from pupae of *S. frugiperda* in a second experiment. In the first experiment, differences in mean oviposition and mean longevity among females reared on the artificial control diet (*artificial diet*), cell line-supplemented diet (*Sf9Cell*), and natural host (*Host*) were not significant. However, during the first 10 days of oviposition, *Sf9Cell*-reared females oviposited at a rate similar to the *Host*-reared parasitoids and at a rate faster than *artificial*-diet reared females. In the second experiment, females reared on the diet with added host lipid (*host lipid*) laid significantly more eggs than females on the *artificial diet*, however, longevity was not significantly affected by diet treatment. We conclude that total egg production by *D. introita* was improved on artificial diet supplemented with lipids from the natural host but was not increased by the addition of materials produced by an ovarial cell line derived from *S. frugiperda.* Future research efforts should focus on increasing fecundity of wasps reared on the artificial diet by identifying the lipid(s) or lipid-soluble material in the host pupal extract that is responsible for enhancing egg production in *D. introita* females.

Key Words: *Diapetimorpha introita, Spodoptera*, parasitoid, artificial diet, fecundity, host lipids, insect cell line

RESUMEN

*Diapetimorpha introita* (Cresson) (Himenóptera: Ichneumonidae) es un ectoparásito nativo en pupas de especies de *Spodoptera*. Este parásito ha sido criado en el laboratorio en una dieta artificial desprovista de componentes de insecto hospedero. Sin embargo, avispas criadas en esta dieta artificial tuvieron fecundidad reducida. Esfuerzos para incrementar la fecundidad incluyeron: suplir la dieta con medio de cultivo de células acondicionadas con una línea de células de ovarios de *S. frugiperda* en un experimento, y fortificando la dieta con lípidos extraídos de pupas de *S. frugiperda* en un secundo experimento. En el primer experimento, diferencias en oviposición promedio y longevidad promedio entre hembras criadas bajo la dieta artificial de control (*artificial diet*), la dieta complementada con línea de células (*Sf9Cell*), y hospedero natural (*Host*) no fueron significantes. Sin embargo, durante los primeros 10 días de oviposición, hembras criadas con *Sf9Cell* ovipositaron a una velocidad similar a los parásitos criados con *Host* y a una velocidad más rápida que hembras criadas con *artificial diet*. En el segundo experimento, hembras criadas con la dieta complementada con lípidos de hospedero (*host lipid*) pusieron significativamente mas huevos que hembras con *artificial diet*, sin embargo, la longevidad no fue afectada significativamente por el tratamiento de dieta. Concluimos que producción total de huevos por *D. introita* fue mejorada por la dieta artificial complementada con lípidos del hospedero natural pero no fue incrementada por la adición de materiales producidos por la línea de células de ovario derivada de *S. frugiperda*. Futuros esfuerzos de estudio deberán enfocarse en incrementar la fecundidad de avispas criadas con la dieta artificial al identificar el lípido (s) o material soluble en lípidos en el extracto pupal de hospedero que es responsable por aumentar la producción de huevos en hembras de *D. introita*.

*Diapetimorpha introita* (Cresson) (Hymenoptera: Ichneumonidae) is a native ectoparasitoid of *Spodoptera* spp. (Pair & Gross 1984) that has been reared in the laboratory on an artificial diet devoid of any insect components (Carpenter & Greany 1998; Greany & Carpenter 1996). Female parasitoids that are reared on this artificial diet are able to search for and parasitize natural hosts in the field (Carpenter and Greany 1998). However, survival rate, fecundity, and weight are less for diet-reared *D. introita* than for host-reared *D. introita*. Also, developmental time is significantly longer for wasps reared on the artificial diet than for wasps reared on host pupae (Carpenter & Greany 1998). Efforts to increase wasp weight and reduce developmental time have included the addition of commercial nutrients, the use of culture media conditioned by insect cell lines, and supplementing the diet with lipid extracts from host pupae (Ferkovich et al. 1999; Ferkovich et. al., in press). One of the cell lines, Sf, derived from ovaries of *S. frugiperda* resulted in some improvement in wasp weight (Ferkovich et al. 1999), whereas, the use of a lipid extract from *S. frugiperda* not only enhanced the average weight of the males and females but also reduced their developmental time. Other parameters such as cocoon production or adult emergence were unaltered. Molting hormone titers of diet-reared and host-reared *D. introita* were examined and it was concluded that insufficient ecdysteroid in the hemolymph during metamorphosis may contribute to the lowered emergence in wasps reared on the artificial diet (Gelman et al. 1999).

In view of some of the positive effects on growth and development of *D. introita* with dietary supplements of extracted host lipids and cell line-conditioned media (Ferkovich et al. 1999, Ferkovich et al., in press), we decided to examine their effects on fecundity and longevity of *D. introita* females.

## Materials and Methods

### Insect Rearing

Insects used in this study were obtained from laboratory colonies at the Crop Protection and Management Research Unit, Tifton, GA. *D. introita* were reared according to the methods described by Pair (1995), unless noted otherwise. *S. frugiperda* larvae were reared in plastic cups (30 ml) containing meridic diet (Burton 1969) at a photoperiod of 14:10 (L:D) h and temperature of 28 ± 1 and 25 ± 1°C, respectively.

### Diet Preparation and Encapsulation of Diet

The original artificial diet (control diet) contained ground beef liver, chicken egg yolk, and the amino acid L-glutamine (Sigma, St. Louis, MO) and was prepared according to Carpenter and Greany (1998) under aseptic conditions in a clean room as described by Ferkovich et al. (1999). All the ingredients were added to 25 ml of serum-free SF-900 II cell culture medium. The diet was encapsulated in Parafilm® using a diet encapsulation apparatus (Greany & Carpenter 1996). Diet was dispensed at 0.5 ml of diet/dome with 24 domes/sheet. Each diet sheet was covered with a modified (bottomless) Falcon® tissue culture plate (Sigma, St. Louis, MO) so that each dome (one larva/dome) was situated within a well. The entire culture plate was covered with a Plexiglas® plate to prevent escape of the larvae. Diet was changed during larval development four days after the neonates were initially placed on the diet.

### Preparation of Cell line-supplemented Diet

The Sf9 cell line was an embryonic line originally derived from ovaries of the fall armyworm, *S. frugiperda,* and purchased from ATCC, Rockville, MD. The cells were cultured in Grace's medium with 10% fetal bovine serum (FBS), 1% bovine serum albumin (BSA) and 0.33% lactalbumin enzymatic hydrolysate (Sigma, St. Louis, MO). For larger-scale culture of the cell lines, cells were grown in 250 ml magnetic spinner flasks (Bellco Glass, Vineland, NJ) at 29°C and were grown to densities of $1.3 \times 10^5$ to $2 \times 10^5$ cells/ml 10 days post inoculation. For the experiments, 25 ml of cell suspension were centrifuged at 250× g for 2 min at room temperature. The resultant cell-conditioned supernatant then was substituted for the SF-900-II medium in preparing the artificial treatment diets. Two cell line control diets were also tested to measure the effects of Grace's culture medium and the additives FBS, 1% BSA and 0.33% lactalbumin enzymatic hydrolysate, additives that were required for optimal cell growth.

### Preparation of Diet With Extracted Host Pupal Lipids

Lipids were extracted using a modified method of Folch et al. (1957) as described by Ferkovich et al. (in press). Briefly, twenty-four 4 day-old pupae of *Spodoptera frugiperda* pupae were homogenized in 12.5 ml of Ringers solution (Ephrussi & Beadle 1936); the homogenate was filtered through glass wool to remove cuticular debris and the filtrate saved. The filtrate was then extracted with a chloroform:methanol (2:1) mixture and the chloroform phase was dried down.

Twenty-five ml of diet were added to the dried chloroform extract and the flask was rotated for 5 min to dissolve the residue. The chloroform extract of freshly homogenized pupae of *S. frugiperda* was added to the artificial diet so that each diet dome contained one pupal equivalent of lipid per *D. introita* larva.

Treatment Diets

The treatment diets used in this study were as follows: 1) *Host*, *S. frugiperda* pupae; 2) *artificial diet*, original control diet; 3) *host lipid*, original diet containing chloroform-extracted lipids from freshly homogenized *S. frugiperda* pupae (prepared according to the methods described by Ferkovich et al., in press); 4) $Sf9Control_A$, artificial diet prepared with Grace's cell culture medium, 5) $Sf9Control_B$, artificial diet prepared with Grace's cell culture medium with % bovine serum albumin, 10% fetal bovine serum and lactalbumin enzymatic hydrolysate; and 6) *Sf9Cell*, diet prepared with S9 cell-conditioned Grace's medium with 1% bovine serum albumin, 10% fetal bovine serum and lactalbumin enzymatic hydrolysate.

Bioassay

First instar larvae that hatched within a 12 hour period were placed on encapsulated diet domes (one larva/dome) in individual cells of a 24 well plate. Each treatment was replicated four times. The larvae were allowed to feed and develop to adults (described below) at 29.1 ± 1°C and 70% RH. Diet was replaced four days after the neonates were initially placed on the diet domes. The third instar larvae were transferred to the new diet domes using a camel hair brush. A 24 well plate containing 24 larvae on a diet constituted one replication. As the adults emerged, they were held individually in plastic portion cups (102 cc) for 24 hrs before they were weighed.

Oviposition and Longevity Studies

Ten male and ten female wasps from each treatment were randomly selected, weighed 24 h post emergence, and paired in small (480 ml) plastic containers fitted with screened lids. Each container was maintained with a source of honey and water. A plastic cup (30 ml) containing 15 ml of soil in which a *S. frugiperda* larva had pupated was provided for each female wasp as an oviposition site. Cups were replaced daily and the number of eggs laid by each female wasp was recorded. Longevity of male and female wasps was recorded.

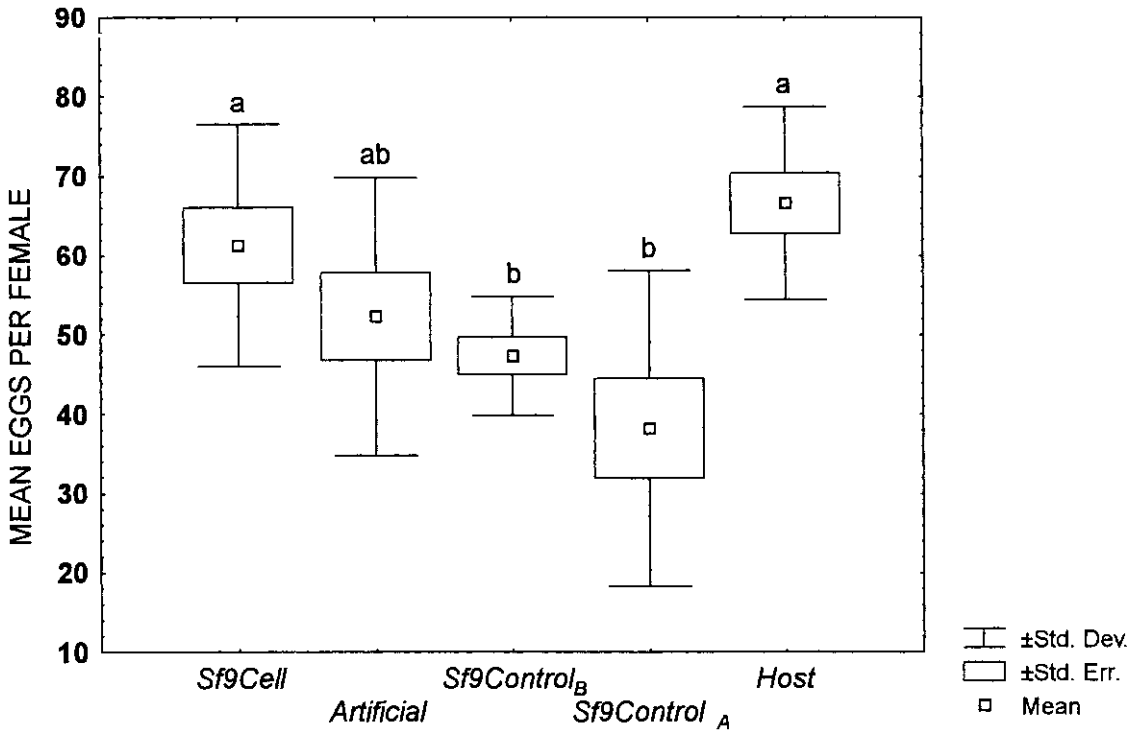


Fig. 1. Comparison of mean oviposition by female *Diapetimorpha introita* reared on: *Host* (*Spodoptera frugiperda* pupae), *artificial diet*, original control diet; $Sf9Control_A$, artificial diet prepared with Grace's cell culture medium; $Sf9Control_B$, artificial diet prepared with Grace's cell culture medium with 1% bovine serum albumin, 10% fetal bovine serum and lactalbumin enzymatic hydrolysate; and *Sf9Cell*, diet prepared with S9 cell-conditioned Grace's medium with 1% bovine serum albumin, 10% fetal bovine serum and lactalbumin enzymatic hydrolysate.

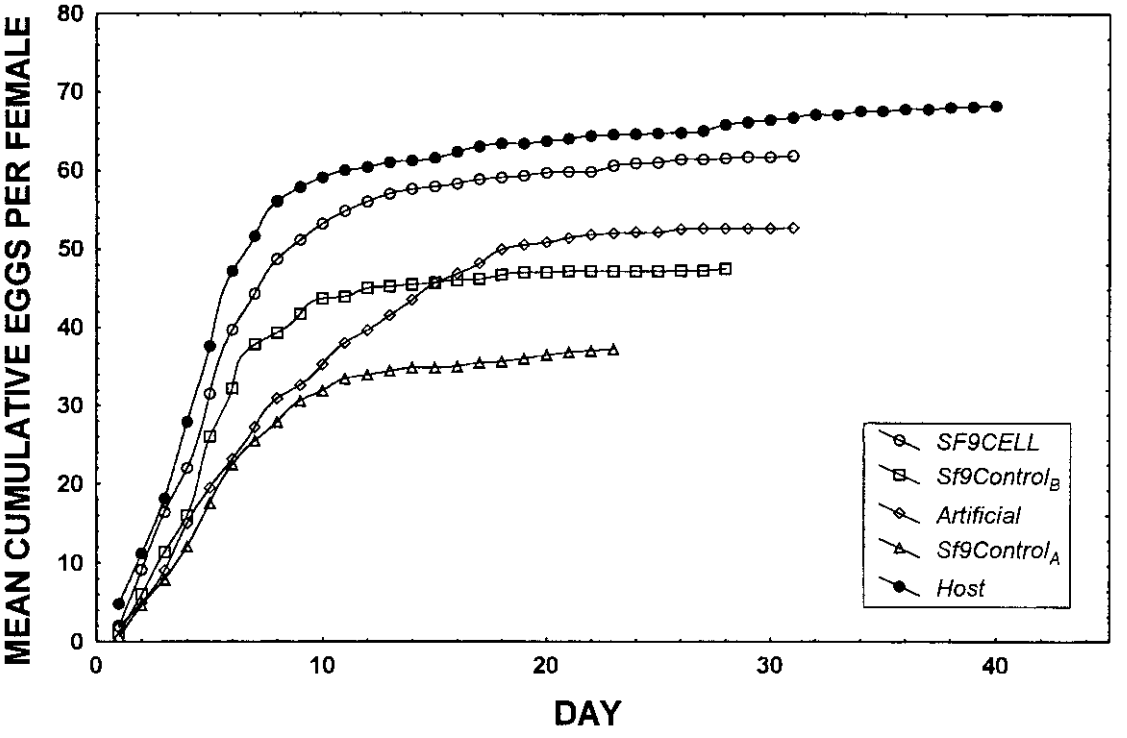


Fig. 2. Rate of oviposition by female *Diapetimorpha introita* reared on: H*ost* (*Spodoptera frugiperda* pupae), *artificial diet*, original control diet; *Sf9Control*$_A$, artificial diet prepared with Grace's cell culture medium; *Sf9Control*$_B$, artificial diet prepared with Grace's cell culture medium with 1% bovine serum albumin, 10% fetal bovine serum and lactalbumin enzymatic hydrolysate; and *Sf9Cell*, diet prepared with S9 cell-conditioned Grace's medium with 1% bovine serum albumin, 10% fetal bovine serum and lactalbumin enzymatic hydrolysate.

Statistical Analysis

The treatment means for fecundity and longevity were compared using the *t*-test (Steel & Torrie 1980). Regression analysis (StatSoft 1995) was used to examine the relationship between mean fecundity and female longevity.

## RESULTS

Diet Supplementation with Sf9 Cell Line-Conditioned Medium

Mean oviposition of females reared on the *artificial diet*, *Sf9Cell* and *Host* treatments was not significantly different, and only females reared on the *Sf9Cell* and *Host* diet treatments oviposited significantly ($P < 0.05$) more eggs than females reared on the two control diets, *Sf9Control*$_A$ and *Sf9Control*$_B$ (Fig. 1). However, during the first ten days females reared on the *Sf9Cell* diet and the *Host* oviposited at a faster rate than females reared on *Sf9Control*$_A$ diet and *artificial diet* (Fig. 2). When data from all diet treatments were combined, there was a significant ($P < 0.001$, $R^2 = 0.999$) relationship between mean oviposition and female longevity (Fig. 3). However, there were no significant differences in mean longevity of female wasps among the five treatments (*artificial diet,* 24.4d; *Sf9Cell,* 23.5d; *Host,* 18.8d; *Sf9Control*$_A$, 20.1d; and *Sf9Control*$_B$, 20.1d).

Diet Supplementation with Host Pupal Lipid Extract

Although females developing on the *host lipid* diet and the *artificial diet* demonstrated similar patterns in oviposition (Fig. 4), the mean (‡ S.D.) number of eggs laid by females reared on the *host lipid* diet (46.67 ‡ 8.7) was significantly ($t = 4.39$, $df = 8$, $P = 0.002$) more eggs than the number of eggs laid by females reared on the *artificial diet* (34.67 ‡ 10.5). The difference in longevity of females reared on the two diets was not significant (*artificial diet*, 16.86 ‡ 7.7 days and *Host Lipid*, 22.50 ‡ 7.7 days).

## DISCUSSION

The fecundity of females was increased with the addition of lipids from host pupae to the artificial diet. The concentration of lipid added to the diet was one pupal equivalent per parasitoid; this

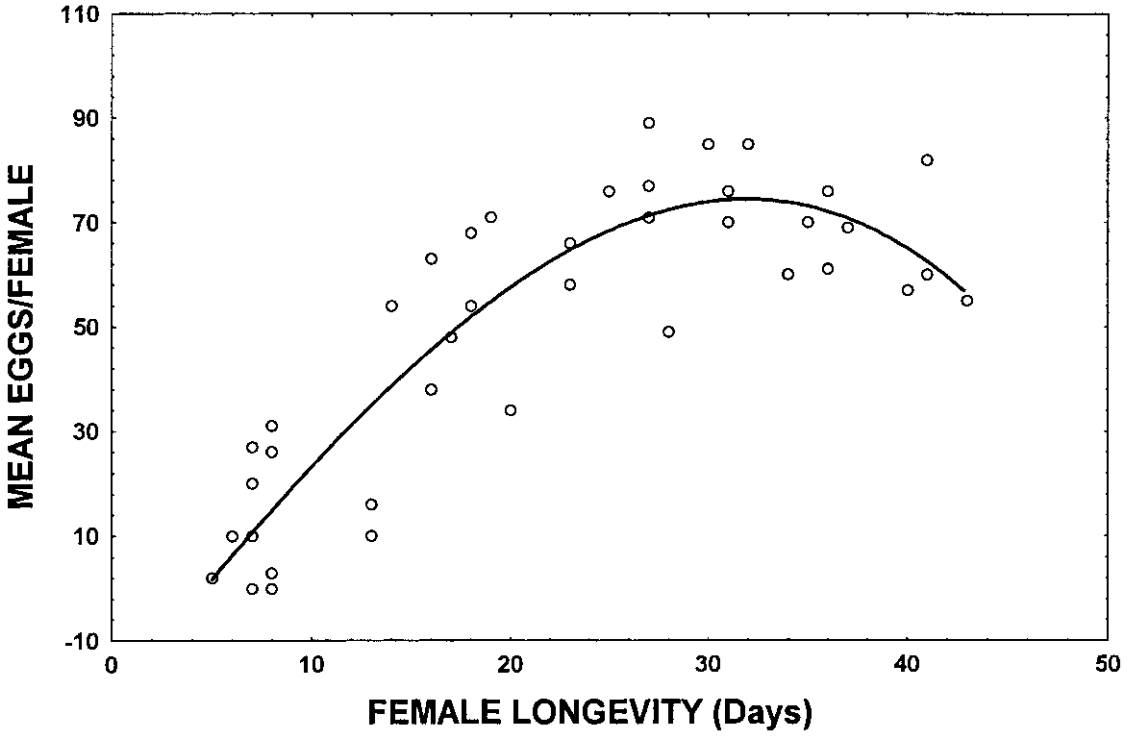


Fig. 3. Relationship between oviposition and longevity for female *Diapetimorpha introita* reared on host pupae (*Spodoptera frugiperda*) and artificial diets ($y = 21.4 + 4.74x - 0.015x^2 - 0.001x^3$, $R^2 = 0.999$, $P < 0.0001$).

concentration was selected because a single parasitoid develops on one host pupa in the wild (Pair 1995). It is possible, however, that fecundity could be improved further with the addition of a higher concentration of host lipid extract. At present, the identity of the bio-active compound active is not known. It could either be a lipid(s) or a lipid-soluble compound(s). If the material is a lipid(s), it would be difficult to speculate as on the identity of the material since the dietary lipid requirements in parasitoids vary with the species. Many species of parasitoids copy the lipid composition of their host (Thompson & Barlow 1972). Others such as *Exeristes roborator* Fab. (Thompson 1977) are able to regulate their fatty acid concentrations in the absence of the dietary lipids. Still others such as *Agria housei* (Shewell) (House 1954) and *Itoplectis conquisitro* (Say) (Yazgan 1972) can be reared on a diet without fatty acids but supplementation of fatty acids to the diet improves adult emergence and fecundity. Other parasitoids such as *Pimpla turionellae* (L) require fatty acids in their diet to produce normal looking adults (Yazgan 1981).

Reinecke (1985) stated that all insects have certain lipid dietary requirements, especially the immature stages, however very few of these lipids are essential and only the sterols are universally required. Thus, it is interesting that the lipids present in the egg yolk component of the artificial diet did not adequately support fecundity of *D. introita*. Egg yolk-based diets have been used to rear a number of parasitoids and predators (Grenier et al. 1994; Nelson 1999). The egg yolk in this artificial diet either lacked the required lipid(s) or contained the needed lipid(s) but not in levels adequate for higher fecundity. Supplementing the diet with host lipids apparently provided a better nutritional balance to the diet, allowing the *D. introita* females to oviposite at a significantly higher rate than females reared on the artificial control diet. Bracken (1969) found that sustained egg production for adults of the parasitoid, *Exeristes comstockii* was dependent on a balance of nutrients in the artificial diet.

Wheeler (1996) indicated that oogenesis is typically a nutrient-limited process and is initiated only if sufficient nourishment is taken for egg production. Nourishment for *D. introita* egg production is apparently acquired during the larval stage because the ovaries of the females are well developed at adult emergence (pers. obs.), and females are able to produce eggs throughout their adult lives by feeding only on honey and water (Pair 1995).

Cell line-conditioned media have been used to improve the growth of two endoparasitoids, *Lysiphlebus fabarum* (Marshall) (Rotundo et al. 1988)

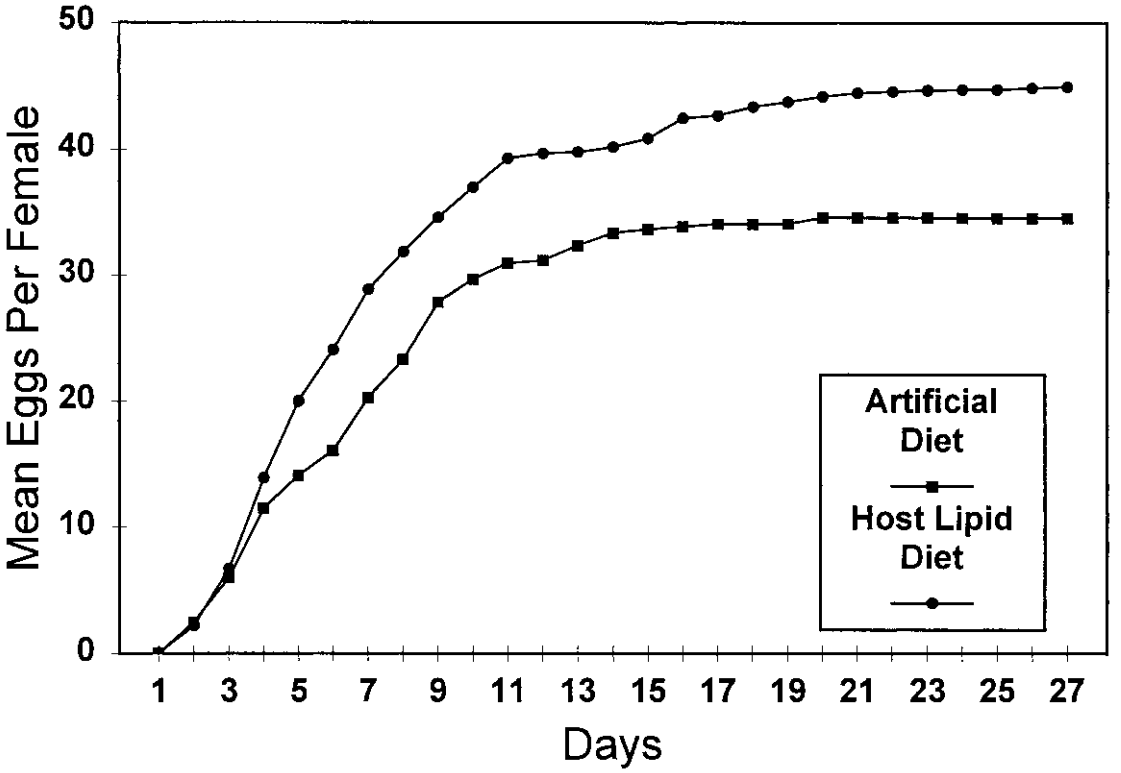


Fig. 4. Rate of oviposition by female *Diapetimorpha introita* reared on: *artificial diet*, original control diet; and *host lipid*, original diet containing chloroform-extracted lipids from freshly homogenized *Spodoptera frugiperda* pupae (prepared according to the methods described by Ferkovich et al., in press).

and *Microplitis croceipes* (Ferkovich et al. 1994), and an egg ectoparasitoid, *Edovum puttleri,* (Hu et al. 1999). However, fecundity could not be assessed in these studies because the insects either did not develop to the adult stage or only low numbers of adults emerged on the cell line-supplemented diets. In this study, we were able to examine the effects of the cell conditioned medium on fecundity because successive generations can been produced on the *artificial diet* (Carpenter & Greany 1998). The positive effect the *Sf9Cell* diet had on the increased rate of oviposition with no accompanying effect on the mean oviposition rate was interesting. It appears that the cell line produced an unknown material that induced the females to deposit their eggs in a pattern that paralleled *Host*-reared females and at a rate faster than *artificial* diet- reared females (Fig. 2).

In view of the positive effects of the pupal lipid extract on fecundity of *D. introita,* we suggest that future research should focus on identifying the fecundity-enhancing material(s) from host pupae so that it can more easily be 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.

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