



Growth of a pupal ectoparasitoid, *Diapetimorpha introita*, on an artificial diet: stimulation of growth rate by a lipid extract from host pupae

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Abstract. An artificial diet used to rear the ectoparasitoid, *Diapetimorpha introita*, was supplemented with lipids extracted from pupae of the host, *Spodoptera frugiperda* (J. E. Smith). The diet also was sequentially supplemented with four fatty acids (arachidonic, linoleic, γ -linolenic and oleic), flax oil and Lipid Concentrate[®] which is used in cell culture. Pupae were homogenized and extracted with chloroform:methanol (2:1 v/v) and after drying down the chloroform and methanol phases separately, the residues from each solvent phase were evaluated in the artificial diet. Growth-promoting activity was observed in the chloroform phase containing lipids. Diet supplemented with lipid stored at -80°C , and insects reared on diet with fresh 1 \times and 2 \times extracts developed significantly faster than those reared on the artificial diet but slower than those reared on host pupae. The fresh 1 \times and the 2 \times extracts also enhanced the average weight of the males and females, respectively. Storing the lipids at -20°C resulted in a loss of activity. A lipid extract from *Galleria mellonella* pupae increased the average weight of males and females but did not increase their developmental rate. Adult emergence was not improved by any of the dietary additives. None of the commercial lipid treatments significantly reduced developmental time; however, the γ -linolenic acid-supplemented diet significantly increased the average weight of females. TLC analyses of the lipid extract from *S. frugiperda* revealed lipids representing four classes of neutral lipids in the extract: triolein, cholesterol, diacylglycerol, and phospholipid. Data from this study indicate that optimization and successful utilization of an artificial diet to rear *D. introita* depends on identification of host factors required by the parasitoid for growth and development.

Key words: artificial diet, host, lipid, parasitoid, supplementation, *Spodoptera*

Introduction

Diapetimorpha introita is a pupal ectoparasitoid that attacks overwintering armyworms, *Spodoptera frugiperda* (J. E. Smith) and *S. exigua* (Huber) (Pair and Gross, 1984, 1989). If *D. introita* could be produced economically on an artificial diet for augmentative releases, it could be evaluated as a key agent in IPM programs for armyworms (Pair, 1995). Although an insect-free diet has been developed for rearing *D. introita*, the parasitoids have longer developmental times, are smaller in size and have reduced fecundity compared to wasps reared on host pupae (Carpenter and Greany, 1998). Clearly, required nutrients or growth factors that are not provided by commercial ingredients used in the insect-free diet are required for optimum development of the parasitoid.

In general, as more diets are developed for a wider range of species, many of the economically important parasitoids appear to be especially difficult to rear on artificial media alone because they are dependent on the addition of hemolymph or other insect materials. Examples are the low yields and quality of some species of tachinids and trichogrammatids that can be reared from egg to adult on diets without host components (Nettles, 1990; Grenier et al., 1995; Nordland et al., 1997). The addition of insect components such as hemolymph or hemolymph derived-factors or pulverized whole insects to a suboptimal diet may improve or optimize the diet so that the growth rate and reproductive capacities of the beneficial insect are increased (Nettles, 1990; Thompson, 1990; Bratti, 1993). Technical difficulties associated with hemolymph such as melanization, and cost considerations limit the utility of this approach. It is imperative that the active material(s) be identified so that the appropriate factors or nutrients can be added directly to the diets and thus bring about major advances in artificial culture of insect parasitoids (Grenier et al., 1994).

Little is known about the production, structure, and mode of action of host factors affecting entomophagous insects. Nettles (1990) summarized the evidence for the role of host factors in parasitoids and predators and emphasized the crucial need for their identification and subsequent production for use in developing artificial media for rearing beneficials. Nettles (1990) reported two parasitic species that are dependent on host factors: *Eucelatoria byani* Sabrosky needed asparagine for growth, and *Trichogramma pretiosus* Riley needed two unidentified low-molecular weight chemicals for pupation. Also, two host products produced by cell lines were required for egg development by the endoparasitoid, *M. croceipes*, in vitro (Ferkovich and Oberlander, 1991). One component induced germ-band development and was dialyzable (< 10 K); the other stimulated hatch and was nondialyzable (> 10 K). In addition, Lipid Concentrate[®] (GIBCO BRL), a defined lipid mixture for

supplementation for cell culture media, could substitute for the germ band factor. Thus, it appears that there are different types of lipids that may be required during different stages of development. Using a cell line derived from the dipteran *Ceratitis capitata* Wied, Rotundo et al. (1988) reared the aphid endoparasitoid, *Lysiphlebus fabarum* (Marshall) from first instar larva to adult *in vitro*, but did not report on the nature of the host products released by the cell line.

We recently reported on the effect of using two insect cell lines to provide growth-promoting materials for *D. introita* reared on an artificial diet (Ferkovich et al., 1999). One of the cell lines, Sf9, an embryonic line derived from the host, *Spodoptera frugiperda*, significantly enhanced the size of *D. introita* males. To enhance the average weight of the females, it was necessary to provide additional nutrients in the diet, along with the cell line products. However, other measured qualities such as developmental time, and percentage of cocoons and adults produced were not improved. In view of these results, we decided to examine host pupae for factors (chemical substances) that stimulate growth and development of *D. introita*.

Here we report on the growth-promoting activity of materials in a lipid extract of host pupae of *D. introita*.

Materials and methods

Insect rearing

Spodoptera frugiperda (J. E. Smith) and *D. introita* (Cresson) were reared as described by Carpenter and Greany (1998). *Galleria mellonella* was reared according to the standardized procedure of Bean and Silhacek (1989). We received four day old pupae of *S. frugiperda* and eggs of *D. introita* by mail from the Insect Biology and Population Management Research Laboratory, USDA-ARS, Tifton, GA. The eggs were placed individually in cells of a 96 well Falcon tissue culture plate at 21.1 ± 1 °C and 70% R H. First instars of *D. introita* that hatched overnight (12 hr period) were used in the bioassay.

Preparation and encapsulation of diet

Diet was prepared under aseptic conditions in a clean room (1.5 m × 2.4 m × 2.4 m) containing two HEPA filter modules (Class 100 air, 700 cfm; Celesta Cleanroom, North Syracuse, NY). Prior to using the room, an ozonator (model XL-15, Living Air, Blaine, MN) was also turned on the lowest setting for ozone output of 66 mg/hr in the room for 12 hrs.

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). 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 and Carpenter, 1998). 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.

Extraction of lipids

Lipids were extracted using a modified method of Folch et al. (1957). Twenty-four 4 day-old pupae of *Spodoptera frugiperda* or *Galleria mellonella* pupae were homogenized in 12.5 ml of Ringers solution (Ephrussi and Beadle, 1936) in with a motor-driven, Potter-Elvehjem type of homogenizer held in an ice-bath. The homogenate was filtered through glass wool to remove cuticular debris and the filtrate saved in a separatory funnel. A chloroform:methanol mixture (2:1, v:v) was added to the filtrate (12 ml) at a ratio of 1 ml filtrate to 6.5 ml chloroform:methanol (78 ml). The emulsion was stored at -80°C for 24 hrs. Distilled water (73.3 ml) was then added to break the emulsion into two phases. The methanol and chloroform extracts were each dried down to ≈ 0.5 ml at 40°C using a Rotovap[®]. Any noticeable solvent residue remaining was removed by blowing a stream of purified nitrogen into the flask. A $2\times$ concentrated chloroform extract was prepared by adding an additional chloroform extract derived from 24 *S. frugiperda* pupae to the flask and then dried down. To determine whether the lipids retained activity during short term storage, chloroform extracts of *S. frugiperda* pupae were stored at -20°C and -80°C for one week and compared with freshly extracted pupae.

In a subsequent experiment, two solvent controls were prepared by extracting Ringers's solution with the chloroform:methanol mixture as described above except without homogenized pupae.

Preparation of diet with solvent extracts of pupae

Twenty-five ml of diet was added to each of the dried extracts, including the solvent controls, and the flasks rotated for 5 min to dissolve the residues. Unless otherwise indicated, both solvent extracts of freshly homogenized *S. frugiperda* were added to the artificial diet at one pupal equivalent per *D. introita* larva.

Treatment diets

(1) *artificial diet*, original control diet; (2) *host lipid* (−20 °C), original diet containing chloroform-extracted lipids from *S. frugiperda* pupae that were stored at −20 °C; (3) *host lipid* (−80 °C), diet containing chloroform-extracted lipids from *S. frugiperda* pupae stored at −80 °C; (4) *host lipid*, diet containing chloroform-extracted lipids from freshly homogenized *S. frugiperda*; (5) *host lipid* (2× fresh), diet containing two pupal equivalents of chloroform-extract of lipids from freshly homogenized *S. frugiperda* pupae per *D. introita* larva; (6) *Galleria lipid*, diet containing lipids from freshly homogenized *Galleria mellonella* pupae; (7) *host pupae*, four day old *S. frugiperda* pupae; (8) *host methanol*, diet containing methanol-extracted materials from *S. frugiperda* pupae; (9) *methanol control diet*, diet containing methanol-extracted Ringer's solution; *chloroform control diet*, diet containing chloroform-extracted Ringer's solution.

The *methanol* and *chloroform control diets* were compared against the *artificial diet* in an independent experiment to determine the effects of the each of the solvents alone on growth and development of *D. introita*.

Preparation of diet with commercial fatty acids

The following lipids were each added to the diet: free fatty acids (all from Sigma, St. Louis, MO), linoleic acid (0.3% w/v, 99% purity), γ -linolenic acid (0.3% w/v, 99% purity), oleic acid (0.3% w/v, 99% purity), and arachidonic acid (0.1% w/v, 90% purity); *flax oil* (Spectrum, Petaluma, CA) 51–57% acids, 16% omega-9 acids, and 9% mixture of stearic, lauric and palmitic acids; and a mixture of unknown fatty acids; Lipid Concentrate[®] (GIBCO BRL, Rockville, MD) (10% v/v) which contained the following: arachidonic acid (2 mg/l), cholesterol (220 mg/l), DL- α -tocopherol-acetate (70 mg/l), linoleic acid (10 mg/l), linolenic acid (10mg/l), myristic acid (10 mg/l), oleic acid (10 mg/l), palmitoleic acid (10 mg/l), palmitic acid (10 mg/l), Plutonic F-68 (100,000 mg/l), stearic acid (10 mg/l), and Tween 80. In order to disperse the fatty acids throughout the diet, the lipids were first mixed in the egg yolk portion of the diet prior to blending the ingredients together, with the yolk serving to emulsify the lipids.

Bioassay

First instar larvae that hatched within a 12 h 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 once, 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's hair brush. A 24-well plate containing 24 larvae on a diet constituted one replication. The number of days required to develop to the adult stage, and the percentage of cocoons and adults produced were recorded. The percentage of adults produced was calculated from the total number of 24 larvae originally set on the diet per replication. As the adults emerged, they were held individually in plastic portion cups (102 cc) with a source of undiluted honey for 24 hrs before they were weighed.

Thin layer chromatography

Dilutions of a chloroform extract containing lipids (0.2 pupal equivalents/l.) from *S. frugiperda* pupae were applied to 10 cm × 10 cm silica plate (HPTLC-GHLE, w/inorganic binder, UV 254, Analtech, Newark, DE). The plates were loaded with 1 µl of a 1/50 dilution or 2 µl of a 1/100 dilution of the extract and developed in a solvent system of hexane/diethyl ether/formic acid (80:20:2). A lipid standard (Sigma, St. Louis, MO) contained 99% pure equal amounts of cholesterol, cholesterol oleate (C18:1, cis-9), oleic acid (9C:18:1, cis-9), oleic acid methyl ester (C18:1, cis-9), and triolein (18:1, cis-9). Other standards, also from Sigma and individually applied, were γ -linolenate, cholesterol, cholesterol stearate, 1-monolinolenoyl glycerol, and dilinolenin. The lipid standards (1 mg/ml) were diluted 1/10 with methylene chloride and 1 µl was applied to the plate. The lipids were detected by spraying with conc. sulfuric acid then charring at 160 °C for 20 min and quantitated by scanning the plates at 400 nm, reflectance mode. Area absorbances of the resultant peaks were recorded as percentages of total absorbance.

Statistical analysis

The effects of the various diets on parasitic growth and development were subjected to a one-way analysis of variance (ANOVA) using Statmost (Dat@Xion Software, Los Angeles, CA). The data for per cent emergence were normalized by arcsine square root transformation before analysis. Dunnet's test compared all treatment means against the *artificial diet* treatment in one test and against the *host pupae* treatment in another test.

Table 1. Effects of supplementing artificial diet of *D. introita* with lipids extracted from host pupae of *S. frugiperda* and pupae of *G. mellonella*

Treatments	Development days \pm s.e.	Emergence % \pm s.e.	Male weight mg \pm s.e.	Female weight mg \pm s.e.
host pupae (standard)	15.9 \pm 0.09a	73.8 \pm 5.73a	23.2 \pm 0.47a	42.4 \pm 0.60a
artificial diet	18.6 \pm 0.15z	45.0 \pm 5.36z	20.2 \pm 0.44z	35.5 \pm 0.50z
artificial diet with				
host lipid (-20°C)	18.5 \pm 0.38z	19.4 \pm 6.91az	19.0 \pm 0.77z	34.2 \pm 1.19z
host lipid (-80°C)	17.1 \pm 0.10az	51.7 \pm 7.98	22.7 \pm 0.54	38.5 \pm 0.59az
host lipid (1 \times fresh)	16.9 \pm 0.10az	55.7 \pm 4.45	24.4 \pm 0.50a	37.6 \pm 0.39z
host lipid (2 \times fresh)	17.9 \pm 0.20az	53.5 \pm 2.36	20.6 \pm 0.41	40.4 \pm 1.53a
<i>Galleria</i> lipid (fresh)	18.2 \pm 0.20z	47.3 \pm 6.40	23.7 \pm 0.70a	43.5 \pm 0.61a

Means followed by the letter 'a' are significantly different ($p < 0.05$) from the *artificial diet* treatment; means followed by letter 'z' are significantly different from the *host pupae* treatment.

Results

Pupal lipid extract

Developmental time was significantly affected with certain diet treatments enriched with the chloroform phase of the pupal extraction (Table 1). High mortality was observed on the *host methanol* (1 \times fresh) diet and further tests were not conducted with this phase. Parasitoids reared on *host lipid* (-80°C), *host lipid* (1 \times fresh), *host lipid* (2 \times fresh) diets developed significantly faster than those reared on the *artificial diet*. However, all of the parasitoids reared on diets supplemented with the lipid extract took significantly longer to develop compared with those reared on *host pupae*.

The rate of emergence was not improved on any of the lipid-supplemented diets relative to the *artificial diet* (Table 1). The rates of emergence on the *artificial diet* and the *host lipid* (-20°C) diet were significantly less than those of the parasitoids reared on *host pupae*. Although the rates of emergence on the diet supplemented with *host lipid* (-80°C), *host lipid* (1 \times fresh), *host lipid* (2 \times fresh), and *Galleria lipid* (1 \times fresh) diets were lower than those reared on *host pupae*, they were not significantly different.

The average weights of male and female wasps were significantly affected by certain diet treatments. Compared with males reared on the *artificial diet*, only males reared on *host lipid* (1 \times fresh), and *Galleria* (1 \times fresh) diets produced significantly larger parasitoids (Table 1). Females reared on *host lipid* (-80°C), *host lipid* (2 \times fresh), and *Galleria* (1 \times fresh) were larger than

Table 2. Effects of supplementing artificial diet of *D. introita* with chloroform and methanol extracts of Ringers solution

Treatments	Development days \pm s.e.	Emergence % \pm s.e.	Male weight mg \pm s.e.	Female weight mg \pm s.e.
artificial diet	19.3 \pm 0.24	50.3 \pm 2.03	21.5 \pm 1.07	35.4 \pm 0.92
methanol control diet	19.7 \pm 0.29	42.8 \pm 3.73	21.6 \pm 1.31	36.9 \pm 1.34
chloroform control diet	20.5 \pm 0.25a	45.8 \pm 6.33	22.8 \pm 1.20	39.2 \pm 0.98

Means followed by the letter 'a' in a column are significantly different ($p < 0.05$) from the *artificial diet*.

females reared on the *artificial diet* and females reared on the latter two diets grew as well and as large as those reared on *host pupae*.

Solvent control diets

Wasps reared on the *methanol control diet* developed at the same rate as those reared on the *artificial diet*; however, development of wasps on the *chloroform control diet* was delayed by 1.2 days compared with development of those reared on the *artificial diet* (Table 2). Adult emergence, and average weight of wasps reared on the solvent control diets were not significantly affected relative to the *artificial diet*.

Lipid supplements

Data for development of *D. introita* on diet supplemented with various commercial lipids are summarized in Table 3. None of the lipid sources significantly shortened the developmental time compared with the *artificial diet*. Similarly all the diets with added lipid sources took significantly longer to develop than *host-reared parasitoids*. Emergence on the lipid-fortified diets was not improved relative to the *artificial diet* and was lower than that of *parasitoids* reared on *host pupae* (Table 3). None of the lipid additions significantly increased the weight of males relative to those reared on the *artificial diet*. However, males reared on *arachidonic*, *linoleic* and γ -*linolenic acid* diets were not significantly smaller than *host pupae*-reared males (Table 3). The *LipidConc*, *flax oil* and *oleic acid* diets had a significant negative effect on male growth compared with the *host pupae* treatment. Female weights were significantly increased by only the γ -*linolenic acid* diet relative to the *artificial diet*, and females reared on this diet and the *arachidonic*, and *linoleic acid* diets were as large as those reared on *host pupae*. The *LipidConc*, and *flax oil* treatments had a significant negative effect on female weight relative to both the *artificial diet* and *host pupae* control treatments.

Table 3. Effects of supplementing artificial diet of *D. introita* with commercial lipids

Treatments	Development days \pm s.e.	Emergence % \pm s.e.	Male weight mg \pm s.e.	Female weight mg \pm s.e.
host pupae (standard)	15.9 \pm 0.08a	83.4 \pm 3.81a	24.4 \pm 0.99	40.8 \pm 1.23a
artificial diet	18.7 \pm 0.15z	34.4 \pm 8.27z	21.2 \pm 1.75	32.9 \pm 1.56z
artificial diet with				
Lipid Concentrate [®]	18.3 \pm 0.38z	33.7 \pm 5.11z	16.0 \pm 1.13z	25.7 \pm 1.58az
flax oil	22.6 \pm 0.52a,z	41.5 \pm 1.92z	17.3 \pm 0.88z	25.5 \pm 1.29az
arachidonic	18.2 \pm 0.42z	31.1 \pm 3.75z	23.0 \pm 4.14	36.5 \pm 2.22
linoleic	18.7 \pm 0.19z	48.9 \pm 1.10z	20.5 \pm 1.29	35.8 \pm 3.37
γ -linolenic	17.7 \pm 0.41z	52.0 \pm 2.10z	21.5 \pm 1.67	42.0 \pm 1.00a
oleic	19.0 \pm 0.52z	53.9 \pm 6.10z	18.0 \pm 1.87z	32.0 \pm 3.18z

Means followed by the letter 'a' are significantly different ($p < 0.05$) from the *artificial diet* treatment; means followed by letter 'z' are significantly different from the *host pupae* treatment.

Table 4. TLC of lipid standards and lipids extracted from *S. frugiperda* pupae

	Identity of spots	R _f	% OD ₄₀₀ + SD (n = 4)
Lipid standards	cholesterol stearate	0.78	
	oleic acid methyl ester	0.57	
	triolein	0.42	
	oleic acid	0.19	
	cholesterol	0.08	
Extracted pupal lipids	triacylglycerol	0.41	86.1 \pm 1.6
	cholesterol	0.09	5.0 \pm 0.6
	diacylglycerol	0.05	1.6 \pm 0.3
	phospholipid	0.00	7.4 \pm 1.4

R_f, mobility of separated lipids on TLC plate relative to solvent front; % OD₄₀₀, optical density of lipids at 400nm.

TLC of chloroform extract

Separation of the neutral lipids of *D. introita* on TLC revealed four spots (Table 4). Based on resolution of the lipid standards and sample, the four spots were identified as (1) triolein, (2) cholesterol, (3) diacylglycerol, and (4) phospholipid which remained at the origin.

Discussion

The lipids extracted from pupae of *S. frugiperda* nevertheless had a positive effect on certain developmental parameters of *D. introita*. This positive effect occurred even though the *chloroform control diet* indicated that the dried residue of the chloroform-extracted Ringer's solution had an inhibitory effect on the developmental time. It appears that the lipids compensated for the detrimental effects of the solvent and therefore their full impact on development was not observed. Compared with parasitoids reared on host pupae, the time required to develop into adult wasps was shortened on diet augmented with lipids from *S. frugiperda* pupae but the lipids had to be either freshly prepared or stored at -80°C . Storing the lipids at even -20°C resulted in a significant loss of activity. It seems likely that the loss of growth-stimulatory activity at this temperature was due not only to the loss of lipid activity but also to a greater manifestation of an inhibitor or toxin in the extract. The lipid extract also stimulated the rate of development of *D. introita* relative to parasitoids reared on the artificial diet and produced adults that were comparable in size to those reared on the host. In contrast, the lipid extract from *Galleria mellonella* pupae did not stimulate the developmental rate of *D. introita* although it did have a positive effect on the size of the adults.

The growth stimulating effect of the lipid extract was surprising since the diet already contains egg yolk which is often used to provide well-emulsified lipids such as fatty acids, cholesterol, and lecithin in artificial diets of parasitoids and predators (Grenier et al., 1994). House (1977) stated that parasitoids probably required the same nutrients required by other insects. According to Reinecke (1985), all insects have certain lipid dietary requirements, especially the immature stages, however, very few of these are essential and only the sterols are universally required. Lipids reported to enhance insect growth are dipalmitoyl lecithin, which increased the rate of larval growth in the mosquito *Culex pipens* and phospholipids of animal origin that increased emergence in the same species (Dadd and Kleinjan, 1978, 1979). Rock (1985) found that linoleic or linolenic acids caused accelerated larval development and increased body weight of pupae and emergence in the tufted apple budmoth, *Platynota idaeusalis* (Walker). In the same study, arachidonic acid showed similar activity in body weight gain and adult emergence but not in the rate of larval development. The commercial source of γ -linolenic tested in this study especially had a positive effect on size of *D. introita* females. In particular, the γ -linolenic acid-supplemented diet produced females that weighed as much as host-reared females and that were significantly larger than parasitoids reared on the control diet. However, neither γ -linolenic nor any of the other fatty acids added to the artificial diet significantly shortened the developmental time as we observed with the fresh host lipid extract.

Although the Lipid Concentrate[®] had an embryo tropic effect on development of the endoparasitoid, *Microplitis croceipes* (Ferkovich and Oberlander, 1991), it did not have a growth stimulating effect on *D. introita*. It should be noted that the formulation does not contain γ -linolenic which enhanced weight of *D. introita* females. Our results suggest that a specific lipid growth factor is present in host pupae of *D. introita* that cannot be substituted for by ingredients obtained commercially.

Nettles (1990) suggested that specific chemicals in the host which he termed 'host factors' may have become essential components of the parasitoid's diet as a result of mutations. Although these genetic changes are innocuous to the parasitoid, certain metabolites may have become essential for the parasitoid's optimal growth and development. Thus, not all parasitoids depend on insect factors for their growth and development, but certain species are dependent. Nettles (1986a, b) found a new essential nutrient required by *Eucelatoria bryani*, a tachinid parasitoid of *Heliothis* spp. Nettles (1987) also extracted an ethanol soluble pupation factor for *Trichogramma* from *Manduca sexta* hemolymph. Nakahara et al. (1997) found a water soluble pupal extract from *Galleria mellonella* containing a protein (> 100,000 daltons) that stimulates growth of *Venturia canescens*. A high molecular weight host hemolymph protein that stimulates egg development in the braconid endoparasitoid *Microplitis croceipes* was isolated and characterized (Greany et al., 1990). Partial success also was achieved in rearing *M. croceipes* from egg through the first instar *in vitro* using a fat body-derived cell line, IPL-LdFB, to produce two active water soluble products, one that induced germ band formation and one that stimulated egg hatch (Ferkovich et al., 1991; Ferkovich and Oberlander, 1991). Consequently, Ferkovich et al. (1999) investigated the potential of using insect cell lines to provide essential host factors to improve the artificial diet for *D. introita*. A cell line derived from the ovaries of host, *S. frugiperda*, increased the average weight of males. However, the parasitoids took longer to develop to the adult stage than those reared on the natural host and adult emergence was low.

Successful optimization of the artificial diet for *D. introita* awaits isolation and identification of the growth-stimulating constituent(s) in the pupal lipid extract as well as other potential host factors in the host pupa (Nettles, 1990). Also, it is possible that the the low rate of adult emergence may be solved by supplying sufficient ecdysteroid or ecdysteroid precursors since low levels of the hormone caused high mortality in pharate adults of *D. introita* reared on artificial diet (Gelman et al., 2000).

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References

- Bean, D.W. and D.L. Silhacek, 1989. Changes in the titre of the female-predominant storage protein (81 k) during larval and pupal development of the wax moth, *Galleria mellonella*. *Arc. Insect Biochem. Physiol.* 10: 333–348.
- Bratti A, 1993. *In vitro* rearing of *Lydella thompsoni* Herting and *Archytas marmoratus* (Town.) (Dip. Tachinidae) larval stages: preliminary results. *Boll. Ist. Ent. Univ. Bologna* 48: 93–100.
- Carpenter, J.E. and P. Greany, 1998. Comparative development and performance of artificially reared vs. host-reared *Diapetimorpha introita* (Cresson) (Hymenoptera: Ichneumonidae) wasps. *Biol. Control* 11: 203–208.
- Dadd, R.H. and J.E. Kleinjan, 1978. An essential nutrient for the mosquito *Culex pipiens* associated with certain animal-derived phospholipids. *Ann. Entom. Soc. Am.* 71: 794–800.
- Dadd, R.H. and J.E. Kleinjan, 1979. Essential fatty acids for the mosquito *Culex pipiens*: arachidonic acid. *J. Insect Physiol.* 25: 295–502.
- Ephrussi, B. and G.W. Beadle, 1936. Transplantation technique. *Drosophila. Am. Nat.* 70: 218–225.
- Ferkovich, S.M., C. Dillard and H. Oberlander, 1991. Stimulation of embryonic development in *Microplitis croceipes* (Braconidae) in cell culture media preconditioned with a fat body cell line derived from a nonpermissive host, Gypsy moth, *Lymantria dispar*. *Arch. Insect Biochem. Physiol.* 18: 169–175.
- Ferkovich, S.M. and H. Oberlander, 1991. Stimulation of endoparasitoid egg development by a fat body cell line: activity and characterization of factors that induce germ band formation and hatching. In: *Proc. VIII Intern. Conf. Invert. and Fish Tiss. Culture, Tissue Culture Assoc.*, Columbia, MD, pp. 181–187.
- Ferkovich, S.M., J.A. Morales-Ramos, M.G. Rojas, H. Oberlander, J.E. Carpenter and P. Greany, 1999. Rearing of ectoparasitoid *Diapetimorpha introita* on an artificial diet: supplementation with insect cell line-derived factors. *BioControl* 44: 29–45.
- Folch, J., M. Lees and G.H.S. Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226: 497–509.
- Gelman, D.B., J.E. Carpenter and P.D. Greany, 2000. Ecdysteroid levels/profiles of the parasitic wasp, *Diapetimorpha introita*, reared on its host, *Spodoptera frugiperda* and artificial diet. *J. Insect Physiol.* 46: 457–465.
- Greany, P., W. Clark, S.M. Ferkovich, J. Law and R. Ryan, 1990. Isolation and characterization of a host hemolymph protein required for development of the eggs of the endoparasite *Microplitis croceipes*. In: H.H. Hagedorn, J.G. Hildebrand, M.G. Caldwell and J.H. Law (eds), *Molecular Insect Science*. Plenum, New York. 306 pp.
- Greany, P. and J.E. Carpenter, 1996. Culture medium for parasitic and predaceous insects. U.S. Patent 08/692,565: Docket No. 000010.96 (Application pending).

- Grenier, S., P.D. Greany and A.C. Cohen, 1994. Potential for mass release of insect parasitoids and predators through development of artificial culture techniques. In: D. Rosen, F.D. Bennett and J.L. Capinera (eds), *Pest Management in the Subtropics: Biological Control – a Florida Perspective*. Intercept Publishers, Andover, Hampshire, UK. pp. 181–205.
- Grenier, S., H. Yang, J. Guillaud and L. Chapelle, 1995. Comparative development and biochemical analyses of *Trichogramma* (Hymenoptera: Trichogrammatidae) grown in artificial media with hemolymph or devoid of insect components. *Comp. Biochem. Physiol.* 111B: 83–90.
- House, H.L., 1977. Nutrition of natural enemies. In: R.L. Ridgway and S.B. Vinson (eds), *Biol. Control Augment. of Nat. Enemies*. Plenum, New York. pp. 151–182
- Nakahara, Y., K. Iwabuchi and J. Mitsuhashi, 1997. *In vitro* rearing of a larval endoparasitoid, *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae). III. Growth promoting ability of extract of *Galleria mellonella* pupae. *Appl. Entom. Zool.* 32: 91–99.
- Nettles, W.C., 1986a. Asparagine: a host chemical essential for the growth and development of *Eucelatoria bryani*, a tachinid parasitoid of *Heliothis* spp. *Comp. Biochem. Physiol.* 85A: 697–701.
- Nettles, W.C., 1986b. Effects of soy flour, bovine serum albumin, and three amino acid mixtures on growth and development of *Eucelatoria bryani* (Diptera: Tachinidae) reared on artificial diets. *Environ. Entomol.* 15: 1111–1115.
- Nettles, W.C., 1987. Amino acid requirements for growth and development of the tachinid *Eucelatoria bryani*. *Comp. Biochem. Physiol.* 86A: 349–354.
- Nettles, W.C., 1990. *In vitro* rearing of parasitoids: Role of host factors in nutrition. *Arch. Insect Biochem. Physiol.* 13: 167–175.
- Nordland, D.A., Z.X. Wu and S.M. Greenberg, 1997. *In vitro* rearing of *Trichogramma minutus* Riley (Hymenoptera: Trichogrammatidae) for ten generations with quality assessment comparisons of in vitro and in vivo reared adults. *Biol. Control* 9: 201–207.
- Pair, S.D. and H.R. Gross, 1984. Field mortality of pupae of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith), by predators and a newly discovered parasitoid, *Diapetimorpha introita*. *J. Georgia Entomol. Soc.* 19: 22–26.
- Pair, S.D. and H.R. Gross, 1989. Seasonal incidence of fall armyworm (Lepidoptera: Noctuidae) pupal parasitism in corn by *Diapetimorpha introita* and *Cryptus albitarsis* (Hymenoptera: Ichneumonidae). *J. Entomol. Sci.* 81: 339–343.
- Pair, S.D., 1995. Biology and rearing of *Diapetimorpha introita* (Cresson) (Hymenoptera: Ichneumonidae) on host and non-host noctuid pupae. *J. Entomol. Sci.* 3: 468–480.
- Reinecke, J.P., 1985. Nutrition: artificial diets. In: L.I. Gilbert and G.A. Kerkut (eds), *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Vol. 8. Pergamon Press, Oxford. pp. 391–419.
- Rock, G.C., 1985. The essential dietary fatty acid requirements of the tufted apple budmoth, *Platynota idaensalis*. *J. Insect Physiol.* 31: 9–13.
- Rotundo, G., R. Cavalloro and E. Tremblay, 1988. *In vitro* rearing of *Lysiphlebus fabarum* (Hymenoptera: Braconidae). *Entomophaga* 33: 264–267.
- Thompson, S.N., 1990. Nutritional considerations in propagation of entomophagous species. In: R.R. Baker and P.E. Dunn (eds), *New Directions in Biological Control: Alternatives for Suppressing Agricultural Pests and Diseases*. Vol. 112, UCLA Symposia, A.R. Liss, New York. pp. 389–404.

