PARASITISM OF A FACTITIOUS HOST, *GALLERIA MELLONELLA* (LEPIDOPTERA: PYRALIDAE) BY AN ENDOPARASITOID: OVIPOSITION AND EMERGENCE OF *MICROPLITIS CROCEIPES* (HYMENOPTERA: BRACONIDAE)

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ABSTRACT

The effect of various diet supplements on the development of *Microplitis croceipes* in an atypical host, *Galleria mellonella* (Linnaeus), were evaluated, as were ovipositional responses to various factors. Female parasitoids were exposed to fifth instar *G. mellonella* in Petri dishes containing the following treatments either separately or in combination: a) *Helicoverpa zea (Boddie)* frass, b) 1% and 10% solutions of a host-seeking stimulant (13-methylhentriacontane), c) *H. zea* hemolymph, and d) *H. zea* hemolymph concentrated by freeze-drying. There were no significant differences be-

tween hemolymph and frass + hemolymph treatments. The host-seeking stimulant alone also stimulated oviposition. The most effective combination was host-seeking stimulant and concentrated hemolymph which induced oviposition rates comparable to that in the typical host, *H. zea*. Various diet supplements did not improve the development and emergence of *M. croceipes*. We conclude that oviposition by *M. croceipes* in the atypical host, *G. mellonella,* was significantly improved by the application of host-seeking stimulant and concentrated hemolymph, but the rate of adult parasitoid emergence was not increased by the addition of nutrient supplements to the host diet.

Key Words: Rearing, *in vivo*, host-seeking stimulant, diet supplement.

RESUMEN

Fueron evaluados el efecto de varios suplementos en la dieta sobre el desarrollo de *Microplitis croceipes* en un hospedante atípico, *Galleria mellonella* (Linnaeus), y la respuesta ovoposicional a varios factores. Las hembras del parasitoide fueron expuestas al quinto estadio de *G. mellonella* en placas de Petri conteniendo los siguientes tratamientos, separados o combinados: a) excretas de *Helicoverpa zea* (Boddie), b) soluciones de estimulante de búsqueda del hospedante (13-methylhentriacontane), c) hemolinfa de *H. zea*, y d) hemolinfa *de H. zea* concentrada mediante secado por congelación. No hubo diferencias significativas entre los tratamientos de hemolinfa y hemolinfa-excretas. El estimulante solo también aumentó la ovoposición. La combinación más efectiva fue estimulante con hemolinfa concentrada que estimuló la ovoposición hasta valores comparables a la tasa de ovoposición en el hospedante típico, *H. zea*. Varios suplementos a la dieta del hospedante no mejoraron el desarrollo y la emrgencia de *M. croceipes*. Concluimos que la ovoposición por *M. croceipes* en el hospedante atípico, *G. mellonella*, fue significativamente mejorada mediante la aplicación de estimulante de búsqueda y hemolinfa concentrada, pero la tasa de emergencia del parasitoide adulto no fue incrementada mediante la adición de nutrientes suplementarios a la dieta del hospedante.

Microplitis croceipes (Cresson) is a solitary endoparasitoid that attacks the corn earworm, *Helicoverpa zea* (Boddie) and tobacco budworm, *Heliothis virescens* (Fabricius). The mass rearing of *M. croceipes* is important because of its potential use in biological control of *Heliothis/Helicoverpa* species (Knipling & Stadelbacher 1983). However, mass rearing of *M. croceipes* is expensive because host larvae are cannibalistic and must be reared individually (Powell & Hartley 1987; Greany et al. 1989; King & Coleman 1989). A large number of insect parasitoids have been successfully cultured *in vitro* (Grenier et al. 1994), but none of the hymenopteran larval endoparasitoids have been reared from egg to adult *in vitro* (Thompson 1986; Rotundo et al. 1988; Greany, 1991; Ferkovich & Oberlander 1991; Pennachio et al. 1992; Ferkovich et al. 1994). We studied six species of Lepidoptera as potential atypical hosts for *in vivo* rearing of *M. croceipes*, but successful development of *M. croceipes* occurred only in *G. mellonella* (21% adult emergence) and to a lesser extent in the fall armyworm *Spodoptera frugiperda* (13% adult emergence) (Blumberg & Ferkovich 1994; Ferkovich & Blumberg 1994). The advantage of using *Galleria mellonella* as a factitious host for rearing is that it is often less expensive to rear than other species. For example, the cost of rearing the tachinid parasitoid *Lixophaga diatraea* was reduced 81% when reared on *G. mellonella* as compared with costs of production on the natural host, *Diatraea saccharalis* (King et al. 1979). Estimated rearing costs for diet, labor and con-

tainers in our laboratory for *G. mellonella* are 3X less costly than for *H. zea*, and automated large-scale rearing of *G. mellonella* is expected to be even less expensive. However, for practical mass rearing, the rates of atypical-host parasitism and parasitoid adult eclosion from the atypical host must be improved (Gupta et al. 1996). Here, we focus on (1) improvement of the rate of oviposition, and (2) the effect of some nutritional supplements (not routinely present in the diet of *G. mellonella*) as they relate to development and emergence of *M. croceipes*.

MATERIALS AND METHODS

Parasite-Host Colony Maintenance

H. zea was reared according to Lewis & Burton (1979) at the USDA/ARS, Insect Biology and Population Management Research Laboratory, Tifton, Georgia. We received eggs by mail and allowed them to hatch on *Heliothis* Premix diet® (Stonefly Industries, Inc., Bryan, Texas). *M. croceipes* was reared as described by Ferkovich & Dillard (1986) with the following modifications. Two 3-day-old female parasitoids were added to each cup of 40-50 *H. zea* late second and early third instars for 24 h. Larvae were then removed, individually placed in 1 ounce plastic cups with 4-5 ml of semisolid diet and kept at 25°C and 60-70% relative humidity for 14 days. Parasitoid cocoons were removed and placed in an emergence cage at 26°C, 55% relative humidity and a photoperiod of 15:9 [L:D] for three weeks. Parasitoid adults were held at a sex ratio of 1:1 in Plexiglas cages streaked with honey. At 2 days, males were discarded and females kept for colony maintenance and research. *G. mellonella* was reared according to Bean & Silhacek (1989).

Experimental

Fifth instar *G. mellonella* larvae (28 mg avg. wt.) not previously exposed to parasitoids were placed in Petri dishes (9 cm diam) with 4-d-old females in the ratio of 2:1 (host:parasitoid) for 1 h. Fifth instar *G. mellonella* were used because they were similar in size to third instar *H. zea* which parasitoid females readily attacked. Fifty to 100 larvae of *G. mellonella* (5 to 10 replicates of 10 larvae each) were stung for the various treatments. Oviposition by *M. croceipes* was determined by counting the first instar parasitoids dissected from host larvae three days after parasitization. Frass and host-seeking stimulant solutions (10 μl) were smeared on the bottom of the Petri dish, leaving a coat of the material on the surface. Hemolymph was collected from early fourth instars of *H. zea* by clipping a proleg. Each *G. mellonella* larva was then rolled in a drop of hemolymph and immediately exposed to female wasps. To prevent melanization while concentrating the hemolymph, 10 μl of 5% solution of phenylthiourea in methanol was added per 500 μl of hemolymph held on ice. Hemolymph was concentrated two-fold using a lyophilizer (Virtis Research Equipment, Gardiner, New York).

Because untreated *G. mellonella* host larvae do not elicit an ovipositional response by *M. croceipes* females (Ferkovich & Blumberg 1994), they were given the following treatments just before exposing them to *M. croceipes* females: 1) *H. zea* frass at the bottom of the Petri dish; 2) *H. zea* hemolymph; 3) frass + *H. zea* hemolymph; 4) twofold concentrated freeze-dried *H. zea* hemolymph; 5) 1% host-seeking stimulant (13-methylhentriacontane); 6) 10% host-seeking stimulant; 7) 1% host-seeking stimulant plus two-fold concentrated freeze-dried *H. zea* hemolymph; 8) 10% host-seeking stimulant plus two-fold concentrated freeze-dried *H. zea* hemolymph. The host-seeking stimulant, purified from the feces and larvae of *H. zea*, triggers the short-range host

seeking response of *M. croceipes* females. Host-seeking stimulant was provided by Dr. R. E. Doolittle, USDA/ARS, Gainesville, Florida. This compound was dissolved in hexane and used at a concentration of 1% and 10% (10 μl) at the bottom of the Petri plate.

The following agents were added to the standard diet of fifth instar *G. mellonella* immediately after parasitization by *M. croceipes*: fetal bovine serum, chicken serum, powdered Grace's medium and TC-100 powdered medium (purchased from Gibco BRL, Grand Island, New York). The following nutrient supplements were also added: 1) torula dried yeast, 5% (Rhinelander, Wisconsin); 2) powdered whole liver, 5% (Schiff, Salt Lake City, Utah); 3) *H. zea* hemolymph, 2.5%; 4) fetal bovine serum, 5%; 5) chicken serum, 5%; 6) egg yolk, 5% (Sonstegard Food Co, Sioux Falls, South Dakota); 7) powdered Grace's medium at 10, 20, and 50 mg/gm of fresh diet; 8) powdered 52-B medium at 20 and 50 mg/gm of fresh diet (JRH Biosciences, Lenexa, Kansas); 9) powdered TC-100 at 10 mg/gm; 10) 50:50 mixture of Burton's modified pinto bean diet used in rearing of *H. zea*. (Burton & Perkins 1972, Milton's Institutional Foods Inc., Oakwood, Georgia) and *G. mellonella* diet (Bean & Silhack 1989). Fetal bovine serum, chicken serum and *H. zea* hemolymph were lyophilized before they were added to the dry components of the *G. mellonella* diet. All supplements were mixed thoroughly with the dry components of the *G. mellonella* diet prior to the addition of water-honey-glycerol. Diet supplement concentrations were based on the final fresh weight of the diet.

Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA), and means were separated by the Tukey-Kramer multiple comparisons test using Instat II® (Graphpad Software, San Diego, California). All significance reported is at the $P = 0.05$ level.

RESULTS

Concentrated Hemolymph and Host-Seeking Stimulant

The combination of frass plus hemolymph did not significantly improve percent parasitism compared to frass or hemolymph tested alone (Fig. 1). One percent and 10% host-seeking stimulant applications improved parasitism over frass, hemolymph, or hemolymph plus frass, although the higher dose (10%) was not statistically significant due to the large variation among replicates. Concentrated hemolymph significantly increased parasitization compared to that induced by frass, hemolymph, or frass plus hemolymph and was comparable to the two host-seeking stimulant treatments. The 10% concentration of host-seeking stimulant was not significantly different from the 1% concentration. However, when 1 and 10% concentrations of host-seeking stimulant were applied in combination with 2× concentrated hemolymph, the rate of parasitism was greater than all the other treatments and, more importantly, these values were comparable to ovipositional rate in the typical host *H. zea*.

Nutritional Supplements

Of all the diet supplements tested, only Grace's tissue culture medium (20 mg/gm) significantly enhanced percent cocoon production relative to the control diet (22.7% vs 18.49%). None of the other diet supplements tested significantly improved the development and/or emergence of *M. croceipes* adults (Table 1).
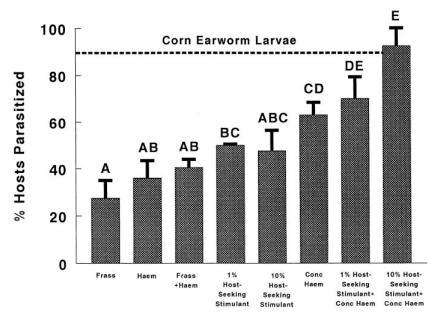
Figure 1. Effects of eight treatments on the percent parasitism of the atypical host *Galleria mellonella*, by *Microplitis croceipes*. All values are means ± SE. Mean followed by the same letter are not significantly different (P> 0.05).

DISCUSSION

Parasitoids often do not oviposit in atypical hosts because chemical and physical stimuli associated with their usual host are absent. However, oviposition may be increased by (1) the application of contact chemicals extracted from the host frass and (2) secretions of the host mandibular and silk glands which can be perceived by the parasitoids (Vinson 1975; Waage et al. 1985). Recently, we demonstrated the acceptance of six atypical lepidopterans as candidate hosts for rearing *M. croceipes* (Ferkovich & Blumberg 1994). These atypical hosts were acceptable for oviposition after treatment with *H. zea* hemolymph or frass or the combination of frass and hemolymph. Host-seeking stimulant, which accentuates host seeking in *M. croceipes* (Jones et al. 1971), is more effective in combination with hemolymph and improves oviposition in *G. mellonella*. Previous observations on oviposition by *M. croceipes* females indicated that they responded to a two-component ovipositional kairomone in the hemolymph of the host (Tilden & Ferkovich 1988; Eller et al. 1990; Heath et al. 1990). Our results clearly show that the application of host-seeking stimulant, in combination with two-fold concentrated *H. zea* hemolymph, stimulated oviposition by *M. croceipes* in the atypical host, *G. mellonella*, at a rate comparable to oviposition in its natural host, *H. zea*.

Achieving a rate of oviposition in a factitious host that is comparable to that in its natural host is an important factor in effective rearing of the parasitoid. In future studies, application of purified kairomone from hemolymph of *H. zea* may be possible (Heath et al. 1990; Drost & Carde 1992).

Increased oviposition does not by itself denote successful parasitization. Thus, we studied the effect of the addition of a wide range of biologically active nutrient supple-

TABLE 1. EFFECTS OF DIFFERENT DIET SUPPLEMENTS[1] ON THE DEVELOPMENT AND EMERGENCE OF *M. CROCEIPES* FROM *G. MELLONELLA*.[2]

	Treatment	% Parasitism	% Cocoon	% Emergence	% Males	% Females
1.	Control	57.6[b]	18.41[bc]	66.6[a]	50.0[a]	50.0[b]
		(49/85)	(9/49)	(6/9)	(3/6)	(3/6)
2.	Grace's	59.1[ab]	22.7[a]	63.6[a]	57.1[a]	42.9[b]
	20mg/gm	(97/164)	(22/97)	(14/22)	(8/14)	(6/14)
3.	Grace's	58.2[b]	15.3[ab]	83.3[a]	40.0[a]	60.0[b]
	50mg/gm	(78/134)	(12/78)	(10/12)	(4/10)	(6/10)
4.	52-B	69.7[a]	3.0[c]	100.0[a]	50.0[a]	50.0[a]
	20mg/gm	(62/89)	(2/62)	(2/2)	(1/2)	(1/2)
5.	52-B	61.4[ab]	9.2[c]	60.0[a]	66.7[a]	33.3[a]
	50mg/gm	(54/88)	(5/54)	(3/5)	(1/3)	(2/3)
6.	50HPM[3]:50	41.7[c]	14.2[bc]	40.0[a]	0.0[a]	100.0[b]
	GPM Diet	(35/84)	(5/35)	(2/5)	(0/2)	(2/2)
1.	Control	62.8[a]	18.4[a]	44.4[a]	25.0[a]	75.0[a]
		(49/88)	(9/49)	(4/9)	(1/4)	(3/4)
2.	Torula-	46.7[a]	11.6[a]	80.0[a]	50.0[a]	50.0[a]
	Yeast	(43/92)	(5/11)	(4/5)	(2/4)	(2/4)
3.	H. Zea Hemo-	51.1[a]	10.9[a]	80.0[a]	50.0[a]	50.0[a]
	lymph (2.5%)	(49/90)	(5/46)	(4/5)	(2/4)	(2/4)
1.	Control	77.5[a]	18.8[a]	76.9[a]	40.0[a]	60.0[a]
		(69/89)	(13/69)	(10/13)	(4/10)	(6/10)
2.	TC-100	75.6[a]	7.4[b]	60.0[a]	33.3[a]	66.7[a]
	10mg/gm	(68/90)	(5/68)	(3/5)	(1/3)	(2/3)
3.	Grace's	63.4[a]	25.0[a]	61.5[a]	50.0[a]	50.0[a]
	10mg/gm	(52/82)	(13/52)	(8/13)	(4/8)	(4/8)
1.	Control	85.4[a]	34.1[a]	28.6[a]	50.0[a]	50.0[a]
		(41/48)	(14/41)	(4/14)	(2/4)	(2/4)
2.	Whole	89.0[a]	14.3[a]	57.1[a]	25.0[a]	75.0[a]
	Liver (5%)	(49/55)	(7/49)	(4/7)	(1/4)	(3/4)
1.	Control	73.6[a]	17.9[a]	50.0[a]	33.3[a]	66.7[a]
		(67/91)	(12/67)	(6/12)	(2/6)	(4/6)

[1]Diet supplement concentrations are on the basis of final fresh weight of the diet. Fifth instar *G. mellonella* were reared on these supplemented diets after parasitization. New controls were run with experiments that were run on different dates.
[2]Percentages within each column and each test group followed by the same letter are not significantly different (*P* > 0.05).
[3]HPM refers to 50% *H. zea* Premix diet and 50% *G. mellonella* diet.

TABLE 1. (CONTINUED) EFFECTS OF DIFFERENT DIET SUPPLEMENTS[1] ON THE DEVELOP-MENT AND EMERGENCE OF *M. CROCEIPES* FROM *G. MELLONELLA*.[2]

	Treatment	% Parasitism	% Cocoon	% Emergence	% Males	% Females
2.	Fetal Bovine Serum (5%)	51.4[a] (37/72)	8.1[a] (3/37)	33.3[a] (1/3)	100.0[a] (1/1)	0.0[a] (0/1)
1.	Control	70.3[a] (38/54)	31.6[a] (12/38)	75.0[a] (9/12)	44.4[a] (4/9)	55.6[a] (5/9)
2.	Chicken Serum (5%)	58.3[a] (42/72)	26.2[a] (11/42)	45.4[a] (5/11)	60.0[a] (3/5)	40.0[a] (2/5)
1.	Control	58.4[a] (45/77)	28.4[a] (13/45)	53.8[a] (7/13)	42.8[a] (3/7)	57.2[a] (4/7)
2.	Egg Yolk (5%)	56.9[a] (37/65)	29.7[a] (11/37)	45.4[a] (5/11)	40.0[a] (2/5)	60.0[a] (3/5)

[1]Diet supplement concentrations are on the basis of final fresh weight of the diet. Fifth instar *G. mellonella* were reared on these supplemented diets after parasitization. New controls were run with experiments that were run on different dates.
[2]Percentages within each column and each test group followed by the same letter are not significantly different ($P > 0.05$).
[3]HPM refers to 50% *H. zea* Premix diet and 50% *G. mellonella* diet.

ments directly to the diet of *G. mellonella* to support the development of *M. croceipes*. However, none of the supplements tested significantly affected the development and emergence of *M. croceipes*.

The possibility of mass-rearing parasitoids for use in biological control programs is associated with the parasitoids' degree of interaction with the host's physiology, as suggested by Campadelli & Dindo (1988). The nutritional and ecological considerations in propagation of entomophagous and endoparasitic insects are evaluated in detail by Thompson (1990) and Slansky (1986). In hymenopteran endoparasitoids, physiological interactions are complex, and it seems from our earlier *in vitro* studies (Ferkovich et al. 1994) and from the present *in vivo* data, that it may not be feasible to manipulate the development and emergence of *M. croceipes* by supplemental nutrients. However, recent studies by Carpenter (personal communication; see Gross et al. 1995) show that addition of torula yeast to the *G. mellonella* diet increased the weight of mature *G. mellonella* larvae and also of males and females of the tachnid parasitoid *Archytas marmoratus* reared from these *G. mellonella*.

In conclusion, these data indicate that although oviposition by *M. croceipes* in the atypical host *G. mellonella* can be significantly improved by the application of host-seeking stimulant and two-fold concentrated hemolymph. The low emergence of adult parasitoids still remains a major problem for rearing the parasitoid on this host.

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