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Comparative Growth and Development *in vitro* of *Eucelatoria bryani* Sab. and *Pallexorista laxa* (Curran) (Diptera Tachinidae) Fed a Meridic Diet and a Diet of *Helicoverpa zea* (Boddie) (Lepidoptera Noctuidae) Pupae. (*)⁽¹⁾

INTRODUCTION

E. bryani and *P. laxa* are promising larval parasitoids for control of *Heliothis/Helicoverpa* spp. (Bryan *et al.*, 1972; Jackson *et al.*, 1976). Tachinids can be reared *in vitro* and the artificial diet producing adult yields of *E. bryani* contains the Nettles (1986b) medium and 1.6% chicken egg yolk (Nettles, unpublished). Using this egg yolk based diet, Bratti e Nettles (1992) developed a method for the *in vitro* rearing of *E. bryani* that produces an average of 2,000 adult flies per liter of medium. The system must be improved for mass production because adult yields were below 50% and the flies were smaller and less viable than those produced *in vivo*.

The presence of host materials in the artificial diet often strikingly improves the yield and quality of many parasitoids reared *in vitro*. Poor rearing results obtained in the absence of insect materials is strong evidence, but not proof, that at least some species of parasitoids are dependent on host chemicals (Nettles, 1990). While host components frequently have been used for successful *in vitro* rearing of hymenopteran parasitoids, they rarely have been tested on tachinids (Bratti and Nettles, 1988; Bratti and Monti, 1988; Bratti, 1989; Mellini *et al.*, 1993; Bratti and Coulibaly unpublished, Dindo and Campadelli, 1992; Mellini and Campadelli, 1994; Bratti and Coulibaly, 1995). Previous research indicated that increased host feeding by larvae of *E. bryani* boosted yields when the insects were transferred to artificial diets (Nettles *et al.*, 1980) and that *P. laxa* did not appear to develop as well as did *E. bryani* on the Nettles (1986b) artificial medium without chicken egg yolk (Nettles unpublished). Our objectives were: (1) to more thoroughly determine the potential of rearing *P. laxa* on artificial media, (2) by using all three instars to expand our study of the effects of host feeding on *E. bryani* larvae (and to inclu-

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de *P. laxa* as well), (3) to compare the response of the two species of tachinid maggots to a meridic and to a host-based artificial medium, (4) to ascertain whether other host factors in addition to free amino acids and asparagine (Nettles, 1986a; 1987a) may be involved in the *in vitro* rearing of tachinids, and (5) to re-examine the effect of dietary agar on tachinid maggots in view of the recent discovery of their blocked alimentary tracts (Gardenghi and Mellini, 1990; Gardenghi *et al.*, 1992).

MATERIALS AND METHODS

Development of the three tachinid instars on meridic diet. - Fifty adults of *E. bryani* and *P. laxa* (9-10 days old) superparasitized 10 last instar larvae of *H. zea* for 2-6 h. Maggots were obtained by dissection. First instars collected immediately after parasitization; were held a maximum of 6 h in the host; second and third instars were dissected every 12 h intervals after parasitization. Prior to dissection hosts were held at 27° C in plastic cups containing a plentiful supply of the Shaver and Raulston (1971) diet. Instars were identified by their buccopharyngeal apparatus and the form and size of the posterior spiracles.

The composition and preparation of the diet were the same for both species of parasitoids (Nettles *et al.*, 1980; Bratti and Nettles, 1992). Maggots were transferred as described by Nettles *et al.* (1980) except that the large size of the third instars required transfer by small spatulas rather than by Pasteur pipettes. The diet was preconditioned at 25° C and 50% relative humidity (RH) for the 24 h prior to the start of maggot rearing. The rearing containers were Petri dishes (55 mm diameter x 15 mm high) in which were placed a maximum of three maggots per dish. For each species of tachinid the number of larvae tested ranged from 20-30. There were four replicates for each test.

Comparison of meridic and insect based media. - Diet comparisons were made using maggots that had developed in the host for 18-24 h. At these ages *E. bryani* were about 50% first and 50% second instars; all the *P. laxa* were first instars. Maggots dissected from hosts were washed in a nutrient solution with a composition similar to the Nettles (1986b) diet and then were pipetted on to various media. *E. bryani* maggots were fed the Nettles (1986b) diet plus 1.6% egg yolk and HZPH (*Heliothis zea* pupal homogenate) prepared as described by Bratti e Monti (1988). *H. zea* pupae were homogenized after being coddled at 60° C for 10 minutes. Three parts of the homogenate was mixed with one part of a 6% (v/v) agar solution to give a final concentration of 1.5%. The homogenate also contained 0.006% gentamicin sulphate. *P. laxa* was fed the same diets as was *E. bryani*.

The maggots of both species were reared individually in 0.396 ml wells of polystyrene microtiter plates and the tests consisted of four replicates of 20-25 individuals of each tachinid species per test. The insects and rearing containers were held at 27° C and 90% r.h. except when they were removed for examination. Materials such as instruments, glassware, and meridic diet were sterilized by autoclaving for 12 min at 121° C and all operations were performed in a laminar flow hood.

With the exception of adult emergence, percentages were based on the number of original maggots surviving transfer to the diets. Unlike percentage adults which was based on the number of maggots, percentage adult emergence (a measure of the development of pupae to adults) was calculated using numbers of adults which developed from pupariae. The data were analyzed by using CSS:Statistica ⁽²⁾ (1991) by ANOVA (2x3 and 2x2 factorial) and Tukey's HSD test was used to determine significantly different means for percentages and weight of puparia. Arcsin transformations were made using the tables of Mosteller and Youtz (1961) and square root transformations were used for the maggot puparial weights.

RESULTS

Development of *E. bryani* and *P. laxa* on meridic diet according to maggot instar. - When first, second and third instars of *E. bryani* and *P. laxa* were transferred to the meridic diet, the interactions of species and instars (2 x 3 factorial design) were significant for all parameters (puparia weight: F= 46.9, df = 2,362, P < 5x10⁻⁶; puparia yield: F = 8.42, df = 2,18, P = 0.0026; adult yield: F = 8.97, df = 2,18, P = 0.0198; adult emergence: F = 7.06, df = 2,18, P = 0.0054).

As shown in Table 1, the instar (whether first, second or third) of *E. bryani* placed on the meridic diet had no effect on puparial weight and adult emergence from pupariae. For both puparial and adult yields there was not difference between second and third instars. However, yields of puparia and adults reared from first instars were significantly lower (by at least 56%) than those reared from second and third instars.

Table 1. Yields and weights of *E. bryani* and *P. laxa* reared from first, second and third instars on a meridic diet ^{a,b}

Instar	Percent Yield (s.e.)							
	Puparia		Adults		Adult Emergence		Puparial weight (mg, s.e)	
	<i>E. bryani</i>	<i>P. laxa</i>	<i>E. bryani</i>	<i>P. laxa</i>	<i>E. bryani</i>	<i>P. laxa</i>	<i>E. bryani</i>	<i>P. laxa</i>
First	32.8 ± 3.2 Aa	25.5 ± 5.9 Aa	24.7 ± 2.1 Aa	3.7 ± 2.5 Bb	77.9 ± 10.3 Aa	11.4 ± 7.8 Ba	11.9 ± 0.4 Aa	13.2 ± 0.5 Ba
Second	74.1 ± 4.3 Ab	55.3 ± 5.0 Ab	63.4 ± 5.4 Ab	30.4 ± 2.8 Bb	85.3 ± 3.9 Aa	57.4 ± 9.9 Ab	11.2 ± 0.2 Aa	12.9 ± 0.5 Ba
Third	83.6 ± 1.0 Ab	95.1 ± 3.5 Ac	69.2 ± 3.4 Ab	76.1 ± 9.2 Ac	82.7 ± 4.2 Aa	79.3 ± 7.3 Ab	11.4 ± 0.2 Aa	18.5 ± 0.6 Bb

a - Means followed by different capitalized letters in the same row statistically different (P ≥ 0.05) from each other, Tukey's HSD test.

b - Means followed by different lower cases in the same column statistically different (P ≥ 0.05) from each other, Tukey's HSD test.

⁽²⁾ This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or recommendation for its use by USDA.

For *P. laxa* all parameters were affected by the instar in which the insects were transferred to the meridic diet. The weights of pupariae obtained from third instars were heavier than those reared from first and second instars. There were significant differences between all three instars in puparia and adult yields: yields increased with age/instar. For adult emergence there was no significant difference between yields from second and third instars, but yields from first instars were much lower (by at least 88%) than those from second and third instars.

When reared *in vivo*, *P. laxa* obviously was larger visually (also confirmed by the unpublished weights of F.D. Brewer, personal communication) than *E. bryani* and a comparison of tachinid species reared *in vitro* reveals that *P. laxa* puparia were heavier than *E. bryani* when each of the three instars was transferred to the meridic diet. Although there was no difference in puparial yields between species for any of the three instars, first and second instars of *E. bryani* developed better to the adult stage than did the same instars of *P. laxa*. However, there was no difference between species in adult yields after third instars were transferred to the artificial diet. Adult emergence from pupariae was significantly different (85% lower in *P. laxa*) between species only when the insects were reared on the meridic diet from first instars.

Comparison of meridic and insect based diet. - The puparial weights and yields of insects reared from one-day-old maggots of *E. bryani* and *P. laxa* on the meridic diet and the diet composed of the HZPH are shown in table 2 ; the results of statistical analyses are shown in table 3.

Table 2. Yields and weights of one-day old *E.bryani* and *P.laxa* reared on meridic diet and HZPH diet.

Diet	Percent Yield (s.e.)									
	Third instar		Puparia		Adults		Adult Emergence		Puparial weight (mg, s.e)	
	<i>E.bryani</i>	<i>P.laxa</i>	<i>E.bryani</i>	<i>P.laxa</i>	<i>E.bryani</i>	<i>P.laxa</i>	<i>E.bryani</i>	<i>P.laxa</i>	<i>E.bryani</i>	<i>P.laxa</i>
Meridic	73.1 ± 4.5	56.8 ± 1.7	68.2 ± 5.9	51.5 ± 3.7	55.4 ± 5.4	15.5 ± 3.7	81.1 ± 1.9	31.9 ± 8.9	11.0 ± 0.3	14.2 ± 0.5
HZPH	69.6 ± 2.8	93.9 ± 3.6	53.8 ± 3.6	89.5 ± 4.9	40.4 ± 3.8	80.1 ± 5.7	74.8 ± 4.0	89.4 ± 3.6	14.3 ± 0.6	24.1 ± 0.5

The puparial weights of both *E. bryani* and *P.laxa* were higher on the HZPH than on the meridic diet and the weights of *P. laxa* were higher than those of *E. bryani* on both diets. The results of ANOVA were similar for percentage yields of: third instars, pupariae, adults and adult emergence. While the species effects were not significant, the diet effects and the interactions between diet and species were significant. This means that although the yields of *E.bryani* were the same on meridic and HZPH diets, yields of *P. laxa* were significantly higher on the HZPH than they were on the meridic diet. The species effects (Table 3) were

Table 3. Results of statistical analyses of data in Table 2

Source of variation	df	F	P
Puparia weight			
Diet	1	176.98	< 0.01
Species	1	166.91	< 0.01
Diet x species	1	34.77	< 0.01
Error	268		
Percent developing to third instar			
Diet	1	19.56	< 0.01
Species	1	3.01	0.11
Diet x species	1	31.94	< 0.01
Error	12		
Percent developing to puparia			
Diet	1	6.51	0.02
Species	1	4.72	0.05
Diet x species	1	25.71	< 0.01
Error	12		
Percent developing to adults			
Diet	1	25.11	< 0.01
Species	1	0.031	0.86
Diet x species	1	60.52	< 0.01
Error	12		
Percent adult emergence			
Diet	1	13.72	< 0.01
Species	1	3.61	0.082
Diet x species	1	27.09	< 0.01
Error	12		

much closer to being significant for puparia yields ($P = 0.051$) than for adult yields ($P = 0.86$).

Comparison of tachinid puparia weights: *in vitro* versus *in vivo*. Compared to the meridic diet in table 2, HZPH increased puparia weights of *E. bryani* and *P. laxa* by 29% and 69%, respectively. As shown in Table 4 these improvements are not quite as impressive when the highest weights obtained *in vitro* (Tables 1 and 2) are compared to weights obtained from tachinids reared *in vivo* on *H. zea* larvae. The percentages (weight *in vitro*/weight *in vivo*) for each diet were almost identical for each species: 39-43% for the meridic and 51-52% for the HZPH diet. Based on maximal *in vitro* puparia weights despite the differences in size, each diet had about the same effect on the percentage increase for each species.

Table 4. - Comparison (as percentages) of maximal *in vitro* puparial weights from tables 1 and 2 with *in vivo* puparial weights*.

Data from :	Diet	Percent (<i>in vitro/in vivo</i>)	
		<i>E.bryani</i>	<i>P.laxa</i>
Table 1	Meridic-without insect components	43.3	39.2
Table 2	HZPH -pupal homogenate	52.0	51.1

a - Based on puparial weights obtained *in vitro* (Tables 1 and 2) and unpublished *in vivo* puparial weight furnished by F.D. Brewer of *E. bryani* (27.5 mg/insect) and *P.laxa* (47.2 mg/insect) reared *in vivo* on *H.zea*. Percent = (*in vitro* puparial weight x100)/ *in vivo* puparial weight.

DISCUSSION

Host factor effects. The presence of host materials in artificial diets strongly affects the development and quality of many parasitoids. Nettles (1990) discussed the role of host factors in nutrition of parasitoids reared *in vitro*. Several host chemicals were proved to be essential for growth and development of parasitoids (Irie *et al.*, 1987; Nettles, 1986a ;1987a; Greany, 1986; Lawrence, 1990) including host hormones (Nenon, 1972; Grenier, 1987; Fanti, 1990; Fanti and Bratti, 1991; Lawrence, 1986).

Additional support for the role of host factors comes from the beneficial effects of rearing parasitoids in hosts before transferring them to artificial diets devoid of insect components. This phenomenon, discovered by Nettles *et al.* (1980), has

been confirmed by Bratti (1989) and Volkoff *et al.* (1992) in several other species.

Further evidence for the role of host factors in tachinid nutrition comes from the striking differences between species when host-reared tachinids of three different ages were transferred to the meridic diet. For *E. bryani* the age effects for yields of pupariae and adults were mostly evident between the first and second instars and there was no difference between second and third instars. However, puparia and adult yields of *P. laxa* increased with each of the three instars and the effects of previous host feeding on *P. laxa* were remarkable. Mean yields of adults were as low as 3.7% from first instars and as high as 76.1% from third instars. The results indicate that *P. laxa* is dependent on a greater number of host factor(s) than is *E. bryani*.

Even stronger support for the host factor effect is provided by the results of feeding the insect medium (HZPH) and meridic diet devoid of insect components. For most criteria (percentages developing to the third instar, pupariae, adults and adult emergence) yields of *E. bryani* were not affected by diet. However, all of these yield criteria were increased for *P. laxa* when the diet was HZPH. The absence or deficiency of host factor(s) had its greatest effect during the puparial stage of *P. laxa* because, compared to the meridic diet, HZPH increased adult yields 5.2-fold from 15.5 to 80.1% and adult emergence 2.8-fold from 31.9 to 89.4%. The smallest effects (1.7-fold increases from meridic to HZPH diet) were for third instars and puparia. Host factor(s) seem to be present in the HZPH diet and absent (or present in low concentrations) from the meridic diet which is devoid of host components. Again *P. laxa* appears to be dependent on a greater number of host factor(s) than does *E. bryani*.

Effects other than host factors. Not all the differences in adult yields between *E. bryani* and *P. laxa* when first, second, and third instars from hosts were reared on the meridic diet must necessarily be attributed to host factors effects. When first instars from hosts were transferred to artificial diets, adult yields were 3.7% for *P. laxa* and 24.7% for *E. bryani*. *E. bryani* females feed on host hemolymph to maintain fecundity (Nettles, 1987b) and in the uterus *E. bryani* grows seven to eight fold from egg located at the beginning of the common oviduct to the egg or larva just before larviposition (Gardenghi and Mellini, 1992). *P. laxa*, unlike *E. bryani*, is handicapped by delays caused by hatching in a vulnerable environment, burrowing through the cuticle, and slower development caused by its larger adult size. In the second instar when the disadvantages of delayed development should not have been a factor, nutritional deficiencies caused by host effects still apparently were evident because yields of adults from second instars transferred to the meridic diet were 63.4% for *E. bryani* and 30.4% for *P. laxa*. By the third instar *P. laxa* caught up to *E. bryani* and host effects no longer were evident; there was no significant difference between species for adult yields (mean yields of 69.2% for *E. bryani* and 76.1% for *P. laxa*).

Pupal homogenate. Martin *et al.* (1989, 1992) reported the presence of dead maggots of *E. bryani* and *P. laxa* inside prepupae of *H. zea* and rarely observed tachinids emerging from host pupae. Nettles (unpublished) observed that *E.*

bryani occasionally emerged from pupae of *Galleria mellonella* L.. While the foregoing suggest that HZPH might be a poor medium for rearing tachinids, this was not the case because for *P. laxa*, HZPH medium produced yields of 90% pupae and 80% adults and was superior to the meridic diet for all parameter measured. Also, the puparial weight of *E. bryani* was higher on the pupal homogenate. For all the remaining *E. bryani* yield criteria there was no significant difference between the meridic and the insect based artificial diets. HZPH is the best artificial diet ever tested for tachinids and the unknown mechanism used by *H. zea* prepupae to kill *E. bryani* and *P. laxa in vivo* was weak or inoperative *in vitro* in the pupal homogenate coddled at 60 °C.

Improvement of artificial medium. Because insect fecundity usually increases with size, increasing weights of *in vitro* reared puparia to levels greater than 39-52% of the weights obtained *in vivo* is a reasonable and desirable goal for mass production. An examination of the potential reasons for the lower puparial weights of the parasitoids reared *in vitro* may lead to improvements in the artificial diet. Inadequate larval feeding on the artificial diet could be a problem. We have observed that tachinid larvae frequently probe and attempt to feed on the air space above the medium and these unproductive actions *in vitro* undoubtedly decrease feeding. The behavior of maggots placed on the surface of the agar-based medium varies considerably and some individuals do and some do not feed deeply. Those feeding heavily and deeply into the diet are larger than those feeding only on the surface. However, at most the quantity of food ingested *in vitro* does not match that which occurs *in vivo* because maggots always surrounded by host tissues are able to feed constantly and this probably helps to account for the larger size of the host reared maggots.

Although agar prevents *E. bryani* and other species from drowning, ingestion of agar-containing artificial media may be harmful to tachinid larvae because at least four species, including *E. bryani* and *P. laxa*, have blocked alimentary tracts (Gardenghi and Mellini, 1990; Gardenghi *et al.*, 1991; 1992). This barrier may cause indigestible and poorly digested materials such as agar and gums and cellulose in soy flour to have harmful effects which partially overcome the beneficial effects of agar (Nettles *et al.*, 1980) and soy flour (Nettles, 1986b). While agar probably is more beneficial than harmful and its presence in the medium clearly is not a disaster, replacement of agar with inexpensive digestible gelling agent may be beneficial. Also, removal of harmful substances which may be present both in the pupal homogenate and in meridic diets probably will be necessary. Finally essential or beneficial host factors must be identified and added to artificial diets in order to obtain optimal growth and development of tachinids reared *in vitro*.

In conclusion, two species of tachinids responded very differently to two agar based diets: one, a meridic diet devoid of insect components and the other, a diet containing host material. Survival (68% puparia, 55% adults) of one-day-old maggots of *E. bryani* transferred to the meridic diet was one of the highest ever recorded *in vitro* on insect-free diets and was much better than the survival of *P. laxa* on the meridic diet. While the insect medium (HZPH) and the meridic diets produced about the same yields of *E. bryani*, the insect diet (HZPH) produced high

yields of *P. laxa*: an average of 90% puparia and 80% adults and these are the highest yields of tachinids ever obtained *in vitro*. Although it is not yet clear whether agar is harmful to tachinids, the agar-based rearing technique produces excellent yields of tachinids when used with a highly active diet such as HZPH. The results demonstrate the excellent potential of *in vitro* rearing for the mass-production and augmentation of tachinids. Immediate future research should be devoted to increasing parasitoid weights and to increasing yields from insect-free meridic diets. Eventually it will be necessary to discontinue the use of the live host for oviposition/larviposition and for nutrition the first day of parasitoid development.

KEYWORDS: *Eucelatoria bryani*, *Palexorista laxa*, Tachinidae, *in vitro* rearing, artificial diet, host effects, biological control

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SUMMARY

Yields of adults were lowest for first instars and highest for third instars of both *Eucelatoria bryani* Sabrosky and *Palexorista laxa* (Curran)(Diptera:Tachinidae) collected from the host *Helicoverpa zea* (Boddie) and reared *in vitro* on the meridic diet devoid of insect components. When placed on the artificial diet as first and second instars, yields of adults of *E. bryani* were superior to those of *P.laxa*; however, for adult yields there was no significant difference between *P. laxa* and *E. bryani* when third instars were used. In experiments begun with one-day-old maggots of *E. bryani* and *P. laxa* obtained from hosts, *P. laxa* performed better on the *H. zea* pupal homogenate (HZPH) diet than on the meridic diet. For *E. bryani* there was no significant difference between diets for most criteria, demonstrating that this species grew and developed surprisingly well on the meridic diet. The maximal mean adult yield (80%) for *P.laxa* fed HZPH is the highest ever reported for tachinids reared *in vitro*. The results demonstrate the excellent potential of *in vitro* rearing when tachinids are fed superior artificial media and the likely importance of the identification of host chemicals for the development of improved artificial media.

Crescita e sviluppo *in vitro* di *Eucelatoria bryani* Sab. e *Palexorista laxa* (Curran) (Diptera Tachinidae) su dieta meridica e su dieta a base di omogeneizzato di pupe di *Helicoverpa zea* (Boddie) (Lepidoptera Noctuidae)

RIASSUNTO

Le rese di adulti ottenute dall'allevamento di larve di prima età, su dieta artificiale meridica, sia di *Eucelatoria bryani* che di *Palexorista laxa*, , sono risultate inferiori rispetto a quelle delle larve di terza età di entrambe le specie. Quando le larve sono state trasferite sulla dieta artificiale sia come primo che secondo stadio, le rese di adulti riferite ad *E.bryani* sono state più elevate rispetto a quelle di *P.laxa*. Tuttavia non ci sono state differenze in merito alle percentuali di adulti tra *P.laxa* e *E.bryani* quando si sono poste in allevamento le larve di terza età.

Negli esperimenti dove si sono utilizzate, per *E.bryani* e *P.laxa*, larve di prima età, rimaste per un giorno all'interno delle larve ospiti, *P.laxa* si è sviluppata meglio su la dieta a base di omogeneizzato di pupe di *Helicoverpa zea* che su dieta meridica. Per *E.bryani* la situazione è differente in quanto non si sono riscontrate differenze nei parametri biologici fra le larve allevate sull'omogeneizzato e sulla dieta meridica.

La percentuale di adulti più alta, manifestatasi per le larve di *P.laxa* su omogeneizzato di *H.zea*, rappresenta il miglior risultato mai riportato per un tachinide allevato *in vitro*. I risultati dimostrano l'eccellente potenzialità della tecnica dell'allevamento *in vitro* qualora i tachinidi vengano allevati su substrati nutrizionalmente adeguati oltrechè la probabile importanza dell'identificazione di eventuali sostanze chimiche presenti nell'ospite per migliorare la composizione della dieta.

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