AMADOU KONOTIÉ COULIBALY, PAOLO FANTI(\textsuperscript{1})
Istituto di Entomologia «G. Grandi» dell’Università degli Studi di Bologna

\textit{Eucelatoria bryani} Sabr. (Diptera Tachinidae) rearing on the factitious host \textit{Galleria mellonella} L. (Lepidoptera Galleriidae): effect of host age at exposure to the parasitoid females(\textsuperscript{2}).

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INTRODUCTION

\textit{Eucelatoria bryani} Sabrosky is one of the most common parasitoids of the noctuid pest \textit{Helicoverpa} (=\textit{Heliotris}) spp. in the southwestern USA (Bryan \textit{et al.}, 1972). Artificial diets have been tested for this tachinid fly (Nettles \textit{et al.}, 1980; Nettles, 1986) in order to develop an \textit{in vitro} rearing method for low-cost mass production and, hence, to enhance the use of this parasitoid in inundative releases against \textit{Helicoverpa} spp. Such a project implies a sound knowledge of the biological and physiological features of the host-parasitoid relationship, in order to evaluate the performance and feasibility in field or to improve the rearing techniques.

Recently we have established that \textit{E. bryani} can successfully develop on the factitious host \textit{Galleria mellonella} L. and are now trying to improve the rearing method. Given that this tachinid fly is a gregarious parasitoid, our first aim was to evaluate how the host age (and so the size) at exposure to the parasitoid females affects parasitization rates and the number and the size of pupae per host.

The influence of host age on the biology of Tachinid flies has been extensively reviewed by Mellini (1986), and, with reference to \textit{E. bryani}, it has been studied by Martin \textit{et al.} (1989) on \textit{Helicoverpa zea} and by Mani and Nagarkatti (1981) on \textit{Helicoverpa} sp. near armigera.

In the present study \textit{G. mellonella} larvae of varying ages were exposed to \textit{E. bryani}, comparing the results obtained when the parasitoid females had or not the opportunity to choose among host ages.

(\textsuperscript{1}) To whom correspondence should be addressed.
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MATERIAL AND METHODS

_E. bryani_ flies came originally from the colony of Dr. W. C. Nettles, Biological Control of Insect Pests Research Laboratory, USDA-ARS, Weslaco, Texas, where they are reared on the natural host _H. zea_. In our laboratory they have been successfully reared for two years on the factitious host _G. mellonella_ at 27 ± 1°C, 60 ± 5% RH and 16:10 D photoperiod. Adult flies are fed with sugar cubes and water supplemented with chloramphenicol (100 mg/l). At these rearing conditions, females can larviposit from the third-fourth day after emergence. In those trials we always used females with no previous parasitization experience.

_G. mellonella_ larvae were reared and kept after Campadelli (1973). The developmental host ages used in the experiments were: a) sixth instar; b) seventh instar 1st day; c) seventh instar 5th day. The host instars were determined by head size (Sehnal, 1966).

Two kinds of experiments were conducted, according to whether the three different host ages were exposed simultaneously or not to the parasitoid females. In the experiment without choice among the host ages, groups of ten females and ten males (?) were collected soon after emergence and kept in plexiglass boxes (20x20x20 cm) containing diet. After six days groups of ten _G. mellonella_ larvae of the same age were put into different containers, as per the host age tested. In this experiment we also tested the exposure time to the parasitoid females (i.e. 30, 60 and 90 minutes). Thus the combinations of the factorial experiment were nine, each with six replicates, except for the host age «seventh instar 1st day» (five replicates). After exposure to _E. bryani_, the host larvae were removed, kept in boxes with diet and inspected each day in order to isolate the parasitized caterpillars in vials. For each parasitized host, the number and weight of pupae, the number of emerged flies and the percentage of females on the adults were recorded.

In the experiment with choice among the host ages, thirty females and thirty males were collected soon after emergence and kept as described above in a plexiglass cage (40x30x30 cm). After six days, three groups (corresponding to the host ages tested) of ten _G. mellonella_ larvae were placed simultaneously in the cage, thereby keeping the same ratio parasitoid females/host larvae as in the experiment without choice. The exposure time was always 60 minutes. There were six replicates. After exposure to _E. bryani_ females, the host larvae were kept as described above and the same parameters as in the experiment without choice were recorded.

**Statistical analysis.** Data of the experiment w/o choice were subjected to analysis of variance in a factorial design to determine the effect of host age, exposure time and their interaction; the data of the experiment with choice were

(?) The sex of adults can be easily recognized, as males have long claws and pulvilli (Sabrosky, 1981), as in many other Tachinid species.
subjected to one-way ANOVA. In both experiments, analysis of covariance was also used for the variables «pupal weights» and «adult emergence», assuming, respectively, the number of pupae per host and the pupal weights as concomitant variables. Tukey HSD test was used for mean separation where significant (p<0.05) statistical difference occurred. Percentages were angular transformed using the formula proposed by Mosteller and Youtz (1961), which corrects the biases due to different cell sizes for small samples (Snedecor and Cochran, 1980). Because of non-normality of distribution, data related to the parameter «number of pupae per host» were square-root transformed [\(\sqrt{\text{observed value} + 1}\)], according to Snedecor and Cochran (1980). Means and standard errors in all tables and figures are based on untransformed values.

All the analyses were performed using the computer statistical package CSS ver. 2.1 (CSS, 1987).

RESULTS

Experiment w/o choice. The percentage of parasitization(\(^\text{(*)}\)) was influenced in a highly significant way by host age (F=10.7; d.f. 2, 42; p<0.001). Neither exposure time (F=1.0; d.f. 2, 42; n.s.) nor interaction (F=0.9; d.f. 4, 42; n.s.) were significant. The younger the host, the higher the parasitization rate (table 1); this does not mean a lower acceptability or suitability of the elder host, as we can see by considering the number of *E. bryani* pupae developed per host (table 2).

Both the factors tested affected the number of pupae per host («host age» F=43.7; d.f. 2, 367; p<0.001; «exposure time» F=6.67; d.f. 2, 367; p=0.002), while the interaction was not significant (F=0.47; d.f. 4, 367; n.s.). In the caterpillars attacked in the fifth day of last instar the number of puparia almost doubled; an exposure of 30 min showed a significantly lower value compared to 60 and 90 min.

Tab. 1 - Parasitization rates (mean ± s.e.) as related to host age and exposure time (experiment w/o choice). In the same column means followed by the same letter do not differ significantly (Tukey HSD test, \(\alpha=0.05\)).

<table>
<thead>
<tr>
<th>Host age</th>
<th>Par. rate (%)</th>
<th>Exposure time</th>
<th>Par. rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VI</td>
<td>81.6 ± 3.4 a</td>
<td>30 minutes</td>
<td>75.9 ± 5.2 a</td>
</tr>
<tr>
<td>VII 1st</td>
<td>78.3 ± 4.0 a</td>
<td>60 minutes</td>
<td>67.1 ± 5.9 a</td>
</tr>
<tr>
<td>VII 5th</td>
<td>52.7 ± 5.5 b</td>
<td>90 minutes</td>
<td>68.4 ± 5.9 a</td>
</tr>
</tbody>
</table>

\(^\text{(*)}\) The host was defined as «parasitized» when at least one *E. bryani* maggot developed to pupa.
Tab. 2 - Number of pupae/host (mean ± s.c.) as related to host age and exposure time (experiment w/o choice). In the same column means followed by the same letter don’t differ significantly (Tukey HSD test, α=0.05).
VI=sixth instar; VII 1st=seventh instar 1st day; VII 5th=seventh instar 5th day.

<table>
<thead>
<tr>
<th>Host age</th>
<th>pupae/host</th>
<th>Exposure time</th>
<th>pupae/host</th>
</tr>
</thead>
<tbody>
<tr>
<td>VI</td>
<td>2.6 ± 0.1 a</td>
<td>30 minutes</td>
<td>2.7 ± 0.2 a</td>
</tr>
<tr>
<td>VII 1st</td>
<td>2.8 ± 0.1 a</td>
<td>60 minutes</td>
<td>3.7 ± 0.2 b</td>
</tr>
<tr>
<td>VII 5th</td>
<td>5.0 ± 0.3 b</td>
<td>90 minutes</td>
<td>3.6 ± 0.2 b</td>
</tr>
</tbody>
</table>

The analysis of *E. bryani* pupal weights showed high significance due to host age (F=353.8; d.f. 2, 366; p<0.001) and to the interaction between the two factors (F=7.41; d.f. 4, 366; p<0.001); the values presented in table 3 are thus based on the combinations of the two treatment levels. The pupae that developed in hosts attacked on the fifth day of seventh instar are heavier than the others, and for an exposure time of 90 min all the three means differed from each other. At the same level of the treatment «host age» pupal weights were found to be lower for longer exposure times, an effect probably due to a greater superparasitization.

Indeed, the analysis of the concomitant variable «number of pupae per host» showed high significance (F=245.9; d.f. 1, 366; p<0.001). We thus examined the relationship between this variable and mean pupal weight, fitting a curve for each one of the three host ages tested.

Figure 1 shows the observed mean values and the fitted curves. As the number of pupae per host increased, their mean weight dropped in an exponential decay way, as \( Y = e^{(k - bx)} \). At parity of *E. bryani* pupae per host, the weights were higher the older the *G. mellonella* larvae, this is probably due to the greater size of the hosts and so to greater available resources and less overcrowding.

The adult emergence of *E. bryani*, calculated as the percentage ratio of adults and puparia, was influenced both by host age (F=8.11; d.f. 2, 366; p=0.001) and by the concomitant variable «pupal weight» (F=125.0; d.f. 1, 366; p<0.001). Neither the exposure time (F=0.1; d.f. 2, 366; n.s.) nor the interaction (F=1.1; d.f. 4, 366; n.s.) was significant. The emergence rate was

Tab. 3 - Pupal weight in mg (mean ± s.c.) as related to the combination of the factors «host age» and «exposure time» (experiment w/o choice). Means followed by the same letter (lowercase in the same column, uppercase in the same row) do not differ significantly (Tukey HSD test, α=0.05).
VI=sixth instar; VII 1st=seventh instar 1st day; VII 5th=seventh instar 5th day.

<table>
<thead>
<tr>
<th></th>
<th>30 minutes</th>
<th>60 minutes</th>
<th>90 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>VI</td>
<td>11.3 ± 1.0 aA</td>
<td>7.8 ± 0.6 aB</td>
<td>8.6 ± 0.8 aB</td>
</tr>
<tr>
<td>VII 1st</td>
<td>10.8 ± 0.8 aA</td>
<td>9.0 ± 0.7 aA</td>
<td>13.6 ± 1.3 bB</td>
</tr>
<tr>
<td>VII 5th</td>
<td>24.5 ± 1.2 bA</td>
<td>20.8 ± 1.0 bB</td>
<td>19.2 ± 1.0 cB</td>
</tr>
</tbody>
</table>
lower (45%) in hosts attacked in sixth instar and raised to about 85% in the older hosts (tab. 4).

The percentage of females on the adults of emerged *E. bryani* was also analyzed. This variable was influenced neither by host age (F=1.2; d.f. 2, 42; n.s.) nor by exposure time (F=0.1; d.f. 2, 42; n.s.) nor by the interaction of the two factors (F=0.7; d.f. 4, 42; n.s.), even if there is a trend of lower values in the treatment levels corresponding to high superparasitization (tab. 5).

Tab. 4 - Emergence rate (mean ± s.e.) as related to host age and exposure time (experiment w/o choice). In the same column means followed by the same letter do not differ significantly (Tukey HSD test, α=0.05).

<table>
<thead>
<tr>
<th>Host age</th>
<th>Emerg. rate (%)</th>
<th>Exposure time</th>
<th>Emerg. rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VI</td>
<td>45.0 ± 3.4 a</td>
<td>30 minutes</td>
<td>66.5 ± 3.6 a</td>
</tr>
<tr>
<td>VII 1st</td>
<td>63.6 ± 3.3 b</td>
<td>60 minutes</td>
<td>58.8 ± 3.2 a</td>
</tr>
<tr>
<td>VII 5th</td>
<td>84.4 ± 2.3 c</td>
<td>90 minutes</td>
<td>61.8 ± 3.6 a</td>
</tr>
</tbody>
</table>
Tab. 5 - Percentage of females on emerged adults (mean ± s.e.) as related to host age and exposure time (experiment w/o choice). In the same column means followed by the same letter don’t differ significantly (Tukey HSD test, α=0.05). VI=sixth instar; VII 1st=seventh instar 1st day; VII 5th=seventh instar 5th day.

<table>
<thead>
<tr>
<th>Host age</th>
<th>females (%)</th>
<th>Exposure time</th>
<th>females (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VI</td>
<td>50.4 ± 5.1 a</td>
<td>30 minutes</td>
<td>48.4 ± 5.5 a</td>
</tr>
<tr>
<td>VII 1st</td>
<td>42.1 ± 4.3 a</td>
<td>60 minutes</td>
<td>43.1 ± 3.9 a</td>
</tr>
<tr>
<td>VII 5th</td>
<td>40.9 ± 5.6 a</td>
<td>90 minutes</td>
<td>42.3 ± 5.9 a</td>
</tr>
</tbody>
</table>

**Experiment with choice.** As stated *supra*, data were analyzed only in relation to host age upon attack by parasitoid females. The percentage of parasitization was influenced in a highly significant way by this factor (F=22.7; d.f. 2, 15; p=0.001), although in this experiment the older the host, the higher the parasitization rate (tab. 6). Also the number of pupae per host is higher when the attack occurred on the fifth day of seventh instar (F=82.1; d.f. 2, 122; p<0.001), and the difference among host ages is much greater than those observed in the experiment w/o choice. The pupal weights were influenced by the concomitant variable «number of pupae per host» (F=164.9; d.f. 1, 122; p<0.001), while the Tukey test detected no difference among the means related to host ages. Given the corresponding mean number of pupae per caterpillar, the weight values correspond to those predicted by the fitted curves of the experiment w/o choice.

Adult emergence rate was influenced by the covariate pupal weight (F=24.33; d.f. 1, 121; p<0.001) and by host age (F=5.0; d.f. 2, 121; p=0.001), probably as an effect of superparasitization (tab. 7).

Also in this experiment adult sex-ratio was not influenced by host age (F=1.1; d.f. 2, 15; n.s.).

**DISCUSSION**

This study shows that *G. mellonella* larvae are a suitable host for *E. bryani* in both the instars tested, although the age of the host when attacked and the

Tab. 6 - Parasitization rate, number of pupae per host and pupal weights (mean ± s.e.) as related to host age (experiment with choice). In the same column means followed by the same letter don’t differ significantly (Tukey HSD test, α=0.05). VI=sixth instar; VII 1st=seventh instar 1st day; VII 5th=seventh instar 5th day.

<table>
<thead>
<tr>
<th>Host age</th>
<th>Paras. rate (%)</th>
<th>pupae/host</th>
<th>pupal weights</th>
</tr>
</thead>
<tbody>
<tr>
<td>VI</td>
<td>61.7 ± 7.0 a</td>
<td>1.4 ± 0.1 a</td>
<td>15.5 ± 1.2 a</td>
</tr>
<tr>
<td>VII 1st</td>
<td>51.7 ± 10.1 a</td>
<td>1.4 ± 0.1 a</td>
<td>17.4 ± 1.2 a</td>
</tr>
<tr>
<td>VII 5th</td>
<td>95.0 ± 3.4 b</td>
<td>7.2 ± 0.5 b</td>
<td>17.9 ± 1.2 a</td>
</tr>
</tbody>
</table>
Tab. 7 - Emergence rate and percentage of females on emerged adults (mean ± s.e.) as related to host age (experiment with choice). In the same column means followed by the same letter don’t differ significantly (Tukey HSD test, α=0.05). VI=sixth instar; VII 1st=seventh instar 1st day; VII 5th=seventh instar 5th day.

<table>
<thead>
<tr>
<th>Host age</th>
<th>Emergence rate (%)</th>
<th>Females (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VI</td>
<td>85.1 ± 5.1 a</td>
<td>44.3 ± 4.4 a</td>
</tr>
<tr>
<td>VII 1st</td>
<td>98.9 ± 1.1 b</td>
<td>59.6 ± 10.7 a</td>
</tr>
<tr>
<td>VII 5th</td>
<td>76.6 ± 3.2 a</td>
<td>49.3 ± 2.4 a</td>
</tr>
</tbody>
</table>

The possibility of choice for the parasitoid females among the host instars can greatly influence the behavior of the parasitizing females and/or the development of the maggots.

Comparing the parasitization rates in the two experiments shows that, when a choice is possible, older hosts are much more preferred than smaller ones and this involves a higher superparasitization. When the parasitoid females have no choice, there is apparently a kind of regulations in the larviposition, that is, the parasitization rate in smaller hosts is higher but the number of pupae per host is lower. It is well known that tachinid flies have generally not developed the Hymenopteran strategies to avoid superparasitization (Askew, 1971). Yet E. bryani has an interesting feature in this regard: Burks and Nettles (1978) observed that the presence of host hemolymph on the cuticle of Helicoverpa (=Heliothis virescens) acts as a deterrent of E. bryani larviposition and suggested that hemolymph may contain a chemical cue that deters multiple parasitism in nature.

In the present work the number of parasitoid attacks and the number of maggots deposited inside each host were not detected, so it was not possible to evaluate exactly why more pupae developed in older hosts. This can be due either to a greater larviposition (more attacks or more maggots per attack, or both) or to the greater trophic resources available for maggot development, as argued by Martin et al. (1989) with regard to E. bryani on H. zea.

The relationship between host age and number of developed E. bryani pupae per host was observed by Martin et al. (1989) on H. zea, by Mani and Nagarkatti (1981) on H. sp. near armigera, and by Brewer and King (1980) on H. virescens. Other tachinid species that exploit the same behavior are Lexophaga diatraeae on Diatraea saccharalis (King et al., 1976, 1981) and on G. mellonella (Grenier, 1981), Athrycia cinerea on Mamestra configurata (Wylie, 1977), Carcelia bombylans on Dendrolimus spectabilis (Kawanishi, 1978) and Drino munda on many noctuids (Chautani and Hamm, 1967).

The pupal weights were a function of their number per host and of host size; they also influenced the emergence of E. bryani adults, as it was observed in other hosts, i.e. H. virescens (Ziser et al., 1977), H. zea (Martin et al., 1989) and H. armigera (Shekharappa et al., 1988). In the experiment w/o choice and
with a 1:1 ratio parasitoid females/host larvae, this fact implied heavier pupae in the older hosts, while in the experiment with choice, because of the greater difference in superparasitization, there was no difference among means as related to host age.

In regard to *E. bryani* adult sex-ratio, we detected no significant differences among host ages but did find a trend to male bias when superparasitization or maggot overcrowding was greater. Mani and Nagarkatti observed a male biased sex-ratio when *E. bryani* larviposited in younger instars, and Ziser *et al.* reported that maggot overcrowding in *H. virescens* is linked to an increase in the proportion of males, as quoted in many hymenopteran species (Waage, 1980). Mellini (1986) pointed out that the ability to regulate sex allocation in Dipterous parasitoids is unknown and the explanation of these observations may be a greater mortality in the female maggots as they need more trophic resources.

The host exposure times tested did not affect maggot development as markedly as host age and size, even if longer exposure times involved lower pupal weights and so a reduced adult emergence as an indirect effect of a greater superparasitization.

In the rearing techniques of *E. bryani* on the factitious host *G. mellonella*, the more suitable host age for larviposition is the seventh instar fifth day, so long as no younger hosts are exposed simultaneously and too great a superparasitization is prevented. Moreover, parasitizing hosts at the end of their feeding activity would simplify the rearing operations, because there is no subsequent need to feed *G. mellonella* larvae after the exposure to parasitoid females.

ACKNOWLEDGEMENTS

We thank Dr. W.C. Nettles, Biological Control of Pests Research Unit, Weslaco, Texas, for supplying us with the insects used to establish the *E. bryani* colony.

**Key words:** *Galleria mellonella*, Lepidoptera, *Eucleatoria bryani*, Tachinidae, host-parasitoid relationship, host suitability.

SUMMARY

In the present study the possibility of rearing the gregarious parasitoid *Eucleatoria bryani* on the factitious host *Galleria mellonella* is reported. The percentages of parasitization and the pupal yields of *E. bryani*, as related to host age and exposure time to the parasitoid females, were examined, comparing the results recorded when the parasitoid females had or not the opportunity to choose among host ages.

The highest pupal yields of *E. bryani* were found in older hosts and with longer exposure times. The pupal weights and the emergence of *E. bryani* adults were a function of their number per host and of host size.

This study shows that *G. mellonella* larvae are a suitable host for *E. bryani* in both the instars tested, although the age of the host when attacked and the possibility of choice for the parasitoid females among the host instars can greatly influence the behavior of the parasitizing females and/or the development of the maggots.
Allevamento di *Eucelatoria bryani* Sabr. (Diptera Tachinidae) sull’ospite di sostituzione *Galleria mellonella* L. (Lepidoptera Galleriidae): influenza dell’età dell’ospite al momento dell’esposizione alle femmine del parassitoidi.

**RIASSUNTO**

Nel presente lavoro viene presentata la possibilità di allevare il parassitoido gregario *Eucelatoria bryani*, abitualmente moltiplicato su *Heliothis* spp., sull’ospite di sostituzione *Galleria mellonella* L.

Sono state esaminate le percentuali di parassitizzazione e la resa in pupari del tachinide in base al tempo di esposizione alle femmine ovidenenti e all’età delle larve del lepidottero. Sono state effettuate prove in cui larve di diversa età erano ospitate alla contaminazione contemporaneamente (per valutare la capacità di discriminazione da parte delle femmine) ovvero separatamente.

Le osservazioni hanno evidenziato una maggiore resa in pupari in ospiti di taglia maggiore e per esposizioni prolungate ed una flessione dei pesi dei parassitoidi e delle loro percentuali di sfruttamento in relazione al numero di pupae formate per ospite.

Le larve di *G. mellonella* sono ospiti idonei per lo sviluppo di *E. bryani* in entrambi gli stadi saggiati, ma l’età dell’ospite al momento della parassitizzazione e la possibilità di scegliere fra ospiti di diversa età possono influenzare in misura notevole il comportamento delle femmine larvedenenti e lo sviluppo delle larvetti parasite.


**RÉSUMÉ**

Cette étude se rapporte à la possibilité d’élever le parassitoïde *Eucelatoria bryani* sur l’hôte de substitution *Galleria mellonella*.

Les pourcentages de parasitisme et le rendement en pupaires du parassitoïde, en fonction de l’âge des larves hôtes et de la durée de leur exposition aux femelles du parassitoïde, ont été examinés, tout en comparant les résultats obtenus dans les cas où les femelles avaient ou non le choix entre les âges de l’hôte.

Les plus grands nombres de pupes sont obtenus en parasitizando les larves au 5° jour du dernier stade et pour des durées d’exposition plus longues. Les poids des pupaires et l’émergence des adultes de *E. bryani* sont fonction de leur nombre par hôte et de la dimension de ce dernier.

Cette étude montre que toutes les larves de *G. mellonella* de l’expérience sont aptes pour le parasitisme de *E. bryani*, cependant l’âge de l’hôte et la possibilité pour les femelles de choisir entre les larves peuvent largement influencer le comportement des femelles au moment du parasitisme et le développement des larvetti endophages.

**REFERENCES**


