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A first overall comparison between the *in vitro* and *in vivo* production of the parasitoid *Exorista larvarum* (L.). (*) (1) (2)

INTRODUCTION

We have so far published six papers on the rearing of the tachinid *Exorista larvarum* (L.) on artificial diets. The results of our previous experiments have been highly encouraging to the point of confirming the possibility of mass rearing this parasitoid *in vitro* for biological control purposes. At this stage, a preliminary brief comparison of the production results obtained on the oligidic diets developed and progressively improved by us from both a nutritional and technical point of view and on the factitious *Galleria mellonella* L. host seems to be appropriate.

The quality of the antagonists obtained in such an unnatural way is of the utmost importance. This issue will therefore be more thoroughly investigated in the near future. It can however already be said at this stage that, on the basis of the results so far obtained, the *Exorista* reared by us *in vitro* even in the absence of host material, have proven capable of attacking the host *Galleria* and of giving rise to a second and fertile generation.

In particular, the present paper is focused on the study of the so-called mean diet utilization index. For *in vitro* rearing, this index is given by the ratio of the puparia biomass to the weight of the pabulum supplied. Similarly, for *in vivo* rearing this rate is given by the ratio of puparia weight to that of the *Galleria mellonella* larvae at the expense of which the puparia are formed. From an experimental point of view these rates permit to compare the nutritional properties of the two media employed for the rearing of the parasitoid, while from a practical point of view they permit to determine the optimum amount of food to be used for *in vitro* rearing.

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MATERIAL AND METHODS

A. *In vitro* rearing.

In setting up these tests, particular attention was given to determining the amount of diet required on a *per capita* basis in order to ensure the normal development of the parasitoid larvae without any wastage or shortage. Instead of the usual 5 cm diameter Petri dishes used in collective experiments, small wells suitable for the study of single specimens, or holding at most 2 or 3 individuals, were employed. In fact, thanks to the small diameter of these containers, it is possible to introduce small quantities of food while avoiding the risk of the pabulum overly thinning out.

1) Tests employing multi-well plates with 24 wells having a diameter of 16.5 mm.

The wells, cylindrical in shape, have a base area of 2.13 cm^2 and a height of 1 cm. Each plate was divided into three sections, each comprising 8 wells in which were introduced 1, 2 or 3 eggs, respectively. Pabulum amounts varied from 800 down to a minimum of 200 mg/well as shown in the following table.

Diet	Ration/well (mg)	Ration/egg (mg)			Pabulum thickness (mm)
		1 egg/well	2 eggs/well	3 eggs/well	
A	800	800	400	266	3.75
B	600	600	300	200	2.81
C	400	400	200	133	1.87
D	200	200	100	66	0.93

It should first of all be noted that the actual amount of food available for each larva in the wells containing more than one egg is far greater than the one shown. In fact, as usual, a small number of the eggs do not hatch and a considerable number of newborn larvae abandon the medium.

The diet employed, considered to be the most appropriate on the basis of previous experiments (Mellini and Campadelli, 1996) was made up as follows:

Skim milk	*Galleria* larval homogenate	Yeast	Egg yolk	Saccharose
75%	5%	6%	12%	2%

Four replications were made using as many multi-well plates.

2) Tests employing multi-well plates with 96 wells having a diameter of 7 mm.

The wells employed in the previous tests were found to be too "large". In fact, on the one hand the greater amount of food was seen to be excessive so that it was only partially exploited, all the more so as 2-3 larvae rarely ever develop in each well. On the other hand, when the ration was reduced, the pabulum tended to quickly dry out as it was too thin. Tests were therefore conducted using plates with much smaller diameter wells having a base area of only 0.384 cm^2 and the usual height of 1 cm. This permitted the medium to be fairly thick so that very small amounts of diet could be tested, from a maximum of 225 mg to a minimum of 50 mg, said amounts being progressively reduced in 25-mg intervals. In each plate, each of the eight trials included a row of 12 wells. The experimental set-up is shown in the table below.

Diet	Ration/well (mg)	Pabulum thickness (mm)
A	225	5.85
B	200	5.20
C	175	4.55
D	150	3.90
E	125	3.25
F	100	2.60
G	75	1.95
H	50	1.30

Given the small amount of pabulum used in this test, only one egg was introduced in each well.

It is worth noting that, as far as shape and size are concerned, these wells are vaguely comparable to the *Galleria* host. Moreover, for amounts of pabulum over 150 mg, the weight of the diet corresponds to that of the host larvae in the intermediate stages of their last instar, that is when they are parasitized.

As the third instar larvae, in particular the mature ones, tend to abandon their tight wells and to look for a more convenient place where to pupate, a parafilm sheet was placed between the plate and its lid after the second moulting. Small holes were then made in the film over each well so as to favour air change as much as possible. Cotton wool wads soaked in sterile water were placed in the large Petri dishes containing the plates in an attempt to slow down pabulum dehydration especially in the wells in which the pabulum layer was rather thin.

The same diet as for the previous tests was employed.

B. *In vivo* rearing.

In routine laboratory tests, *Galleria* larvae are parasitized in the intermediate stages of their last instar. This is done not only to offer the antagonist a host at a more advanced stage of growth but also to avoid the host rejecting the as yet unhatched macrotypical eggs of the antagonist as it pupates. In fact, at temperatures varying from 25 to 27 °C, embryogenesis lasts on average about 3 days. After oviposition, the host larvae are no longer fed so as to simplify the production of the parasitoid by exploiting its idiobiont characteristics. This means that not only can they no longer grow, and this at a crucial stage in which weight increase is very acute (in absolute terms), but they actually start losing weight before the parasite, after having hatched, can take them over. In fact, fasting larvae empty their food channel and practically move about continuously in their rearing containers, letting out considerable quantities of silk threads and finally, in most cases, making up their cocoon. The outcome is that the average weight of host larvae drops from around 150-180 mg at the time of contamination to 128-153 mg at the moment of penetration by the newly hatched antagonist larvae. This means a sharp weight loss of around 15% on average (with a minimum of 9.28% and a maximum of 20.5% as recorded on 20 sample larvae).

The parasite larvae usually attain maturity within the host larvae, quite frequently in their intermediate larval-pupal form and exceptionally in pupae. For this growth pattern to occur, hatching of the parasitoid must coincide with the pupal apolysis of the host, that is penetration of newborn larvae must take place during the eopupal stage of the host or immediately afterwards, during the host's short pharate stage. In about 90% of cases the puparia form outside the cocoon, but at times also within it, and, more rarely, even within the remains of their victims.

It should be noted that the internal organs of the host are never fully consumed, not even in the case of superparasitization by three larvae.

Pabulum utilization index was calculated by weighing the host larvae immediately after egg laying, after which they were isolated one by one. The weights thus recorded were adjusted on the basis of a correction factor determined, as described above, on a sample of larvae which had been set aside for this purpose. Subsequently, within two days of their formation, the weight of the puparia which had developed at the expense of each host was determined.

RESULTS

1. Average pabulum utilization index.

This rate is to be understood as the puparia or puparium weight to pabulum weight ratio.

A. *In vitro* rearing.

a) Tests employing multi-well plates with 24 wells having a diameter of 16.5 mm.

The results obtained from the 4 replications were rather scarce. In fact, only 65 puparia were obtained from the total of 192 eggs employed. The limited surface (2.13 cm^2) of the wells may in part account for this as it tends to facilitate the migratory behaviour of the newborn larvae. The findings are summarized in the table below. In drawing it up, it was not possible to take into account the initial distribution of the eggs. Reference had therefore to be made to the actual number of puparia formed in each well. The greatest amount of data for each treatment, therefore, applies to the wells with only one egg. Some of this data refers to the wells with two or three eggs, in which only one, however, developed into a puparium. Obviously, the least amount of data was recorded for the wells containing three eggs.

Diet	1 puparium/well			2 puparia/well			3 puparia/well		
	No. of cases	Mean weight (mg)	Utilization index	No. of cases	Mean weight (mg)	Utilization index	No. of cases	Mean weight (mg)	Utilization index
A	10	45.37	5.67	3	46.80	11.70	2	46.65	17.49
B	10	55.26	9.21	5	55.28	18.42	-	-	-
C	11	49.90	12.47	2	34.35	17.17	-	-	-
D	8	21.47	10.73	-	-	-	-	-	-

Notwithstanding the scarcity of the data obtained, some conclusions may nevertheless be drawn. As the initial amount of food available was extremely high, the utilization index progressively increased as said amount decreased and the number of puparia formed per well increased.

As far as single puparia are concerned, diet D, that is the one with the lowest amount of food, seems to be an exception to the rule, the utilization index being less than that of diet C. This finding is due to the marked loss of weight of the puparia ascribable to the drying up of the diet distributed in layers which were too thin and which thus constituted an obstacle to the normal feeding activity of the parasite larvae.

As far as the wells with two puparia are concerned, the exception to the rule appears to be diet C (no data being available for diet D) in which the utilization index was slightly lower due to a marked drop in puparia weight.

The development of all three co-tenant puparia was recorded in only two wells of diet A, that is the one with the greatest amount of food. Notwithstanding, the utilization index was found to be amongst the highest recorded as the weight of these puparia was similar to that of single and double puparia for the same treatment.

In conclusion, the average diet utilization index increased from a meager 5.67 in the wells containing 800 mg of pabulum and only one puparium to as high as 18.42 in the wells containing 600 mg of pabulum with two puparia.

b) Tests employing multi-well plates with 96 wells having a diameter of 7 mm.

The findings obtained from this test are also scarce, 135 puparia having developed from the 384 eggs introduced. Nevertheless, unlike in the previous test, the data are fairly uniformly distributed across all the diets except for the last one where a sharp decline was recorded for all biological parameters.

The findings of the four replications are summarized in the table below.

Diet	Diet weight (mg)	No. of puparia	Mean puparia weight (mg)	Mean utilization index	Emergence (%)
A	225	20	40.82	18.14	85.00
B	200	17	39.67	19.83	94.11
C	175	21	35.68	20.38	90.47
D	150	20	33.79	22.52	80.00
E	125	18	26.50	21.20	77.77
F	100	17	22.95	22.95	88.23
G	75	18	17.83	23.77	83.33
H	50	4	10.45	20.90	25.00

The mean utilization index starts from a minimum of 18.14 in diet A (the one with the greatest amount of food), that is to say from the highest values recorded in the previous test in which three puparia were formed simultaneously per well. This value tends to gradually increase as the amount of food available decreases, reaching a maximum of 23.77 in the treatment with only 75 mg of diet. In these wells the pabulum was almost completely consumed, being replaced by abundant excrements in a thin irregular pasty layer of a light brown colour paler than that of the diet. In the wells with a greater amount of diet, instead, a certain quantity of pabulum was always left over which, however, owing to the restricted space of the well, was mixed with the droppings by the continuous movement of the mature larva in search for a suitable place where to pupate.

In treatment H, the one with the least amount of food, instead of continuing to increase, the utilization index slightly drops as the pabulum, being scarce, tends to dry out quite rapidly, thus becoming unsuitable for consumption.

B. *In vivo* rearing.

The table below shows data relating to 60 host-parasite systems.

No. of systems	*G.mellonella* larvae		*E. larvarum* puparia	
	Mean weight upon oviposition (mg)	Mean weight upon parasite egg hatching (mg)	Mean weight	Mean utilization index
30 with 1 puparium	164.76	140	39.38	28.12
20 with 2 puparia	176.49	150	27.37x2	36.49
10 with 3 puparia	225.35	192	26.61x3	41.57

A rather exceptional finding is that of the formation of four puparia at the expense of the same victim. This phenomenon may occur when the weight of *Galleria* larvae is greater than 200 mg. Even in this case, however, one or two puparia are always dwarfs, weighing about 10 mg and rarely resulting in adult emergence.

The mean utilization index, understood as the puparium or puparia to victim weight ratio, represents the pabulum exploitation level. For the reasons already given, this index is not calculated on the basis of the mean weight of the host at the time of oviposition by the parasite but at that of egg hatching, which occurs about three days later. As can be seen, this index is not very high for victims in which only one puparium has developed as they are only partially exploited. It should however be noted that the utilization index varies considerably in relation to the weight of the host in each system and to the eventual presence of parasite larvae which have died at the second and/or third instar, ranging from a minimum of 20.83 to a maximum of 32.79. Even in the case of monoparasitization, the utilization index is always far greater than the maximum values recorded under the best *in vitro* rearing conditions, which is to say in small wells with a small amount of pabulum, almost doubling in the case of superparasitization with three puparia. In fact, the utilization index progressively increases as superparasitization level increases, reaching a very high maximum of 41.57.

2. Mean puparia weight.

As usual, the puparia were weighed one-two days after their formation as, during adult formation, they undergo a progressive loss of weight which Campadelli (1980) has calculated to be around 11-16% for *Pseudogonia rufifrons* Wied.

A. *In vitro* rearing.

In 16.5 mm diameter wells, the weight was seen to be practically the same for treatments employing 800 and 600 mg of diet both in the case of one or two puparia being formed (see table on page 186). A considerable drop in weight was observed for the treatment with 400 mg of diet in which two puparia per well were formed. In the 200-mg treatment, in which only one puparium was formed per well regardless of the number of eggs introduced, the mean weight was found to drastically drop by more than half. Such a marked drop is however not ascribable to the low amount of diet but rather to its thinness (0.93mm), which favours its dehydration and thus constitutes an obstacle to the feeding activity of the larvae. In fact, in wells with 400 mg of diet and in which two puparia were formed, their mean weight was over 50% greater. This means that while the same amount of food, i.e. 200 mg *per capita*, is insufficient in the large wells for a single larva, it is quite sufficient for more than one larva in the same well.

In the 7mm diameter wells, the mean weight of the puparia, which is quite high for treatments A and B with the greater amount of diet (see table on page 187), tends to progressively decrease, markedly dropping beginning from the treatment with 125 mg of pabulum and reaching a minimum of 10.45 mg in the last treatment with only 50 mg of diet, the latter tending to dry out quite rapidly as it is only 1.3 mm thick.

More in general, in the 5cm diameter Petri dishes used for our previous experiments with diets which were suitable both from a nutritional and physical point of view, the mean weights were overall greater than 45 mg. Indeed, they were very often above 50 mg even reaching, in certain tests, values of around 60 mg (Mellini and Campadelli, 1996a, 1996b). Some maximum individual weights, albeit in isolated cases, were found to be even greater than 100 mg, that is three times the mean weight of *in vivo* reared puparia.

Unlike in the *Galleria* host, dwarf puparia (that is, with weights of less than 20 mg down to as low as 7-8 mg) were rarely ever formed on the artificial diet. The formation of dwarf puparia on artificial diets may occur in the case of the pabulum having been contaminated at an early stage or when it is too hard, almost never because of pabulum shortage or competition as, vice versa, is the case in highly superparasitized hosts. In fact, the amount of artificial diet so far made available in our experiments is far greater than that actually required.

It should be pointed out that unlike in *in vivo* rearing, in *in vitro* conditions abnormal puparia of mean or high weight are not infrequently formed. Albeit maintaining their larval characteristics, such as deep intersegmental grooves, the majority of these puparia are perfectly viable and lead to adult emergence.

B. *In vivo* rearing.

In order to obtain some general preliminary data, two groups of fifty puparia were collected no more than two days after their formation from the bottom of two containers with parasitized larvae of the kind generally employed for rearing *Exorista*. In this case host weight and level of parasitization could obviously not be taken into account.

The data thus obtained is summarized in the table below.

As can be seen, the mean weight of the hundred puparia was 33.28 mg. Actually, a number of puparia were found to weigh from between 15 to 20 mg,

	No. of puparia	Mean weight (mg)	Min weight (mg)	Max weight (mg)
1st sample	50	31.75	9.5	52.6
2nd sample	50	34.82	8.2	58.1

exhibiting thin and fragile walls and tending to a yellowish colour. The great majority of these puparia derive from hosts which have been subject to high levels of superparasitization; only a dozen, exhibiting thick and rigid dark brown walls, weighed more than 50mg; it is very likely that the latter puparia mainly formed in monoparasitized hosts.

A more detailed analysis of puparia weights in relation to that of their victims and to superparasitization levels appears on page 188, where the utilization index is also discussed. As can be seen, the mean weight of the thirty solitary puparia is 39.38 mg, dropping to 27.37 mg for the puparia formed in pairs on the same victim and to 26.61 in the case of three co-tenant puparia per host. The overall mean weight for the entire batch of a hundred puparia is 30.74 mg, a value which is fairly close to that recorded for random samples of the overall population.

In conclusion, therefore, under *in vivo* conditions, and more precisely in the *Galleria mellonella* factitious host, mean puparia weight was found to be lower than that recorded *in vitro* on condition that the artificial diets be appropriate and abundant. This finding is perfectly comprehensible in the case of superparasitized hosts, given their limited size. It is not, however, at all clear why this should be so in the case of monoparasitized hosts as under these conditions food supply is abundant, so much so that quite a lot is still left over after the parasite has abandoned its victim.

Perhaps the marked increase in weight under *in vitro* conditions does not so much depend on the abundance of the pabulum or on the considerable "room" available as compared to *in vivo* conditions, but rather on certain qualitative characteristics of the artificial diets probably linked to the considerable amount of egg yolk employed.

3. Puparia yield.

Puparia yield is determined on the basis of the number of puparia formed in relation to the number of eggs introduced. It should first of all be noted that a certain number of the latter, around 10%, fail to hatch as in some cases embryogenesis has either not begun or it has been aborted, or in other cases because the larvae, despite appearing to have been fully formed, fail to emerge.

A. *In vitro* rearing.

In the case of a single specimen per well, puparia yield in the 16.5mm diameter wells is more or less the same for all treatments. With two puparia per well there is a marked drop in yield which becomes drastic in the case of three puparia as the amount of food available in the well is progressively reduced. In fact, three puparia developed in only two of the wells, and precisely those which had been supplied with the maximum amount of diet (800 mg). In order to correctly interpret the table on page 186, it should be borne in mind that it refers to the actual number of puparia formed and not to the number of eggs initially introduced according to the basic design adopted for the experiment (see page 184). This means that the single puparium per well column also contains the data referred to wells of the same treatment in which two or three eggs had been introduced but which only gave one puparium. Puparia yield on the overall test was 33.85%. This poor result is also in part accountable to the experimental set-up in which the wells with little diet and more than one egg represent an unfavourable condition.

Puparia yield was practically the same thoughout all treatments in the small 7mm diameter wells except in the last treatment employing only 50 mg of diet, in which a sharp drop in yield was observed.

The overall yield of this test was slightly greater than in the previous one, being 38.98% except in the last treatment, which was a complete failure.

In other tests using the same diet but in 5 cm diameter Petri dishes and with 30 eggs, yields of even 50% and slightly over were attained. This better result also depends on the fact that in these larger containers the diet, which was present in greater quantities, was able to preserve its original characteristics for a longer time, while, vice versa, in the small wells supplied with only small amounts of diet, the pabulum tended to quickly dry up. Furthermore, the migration of the newborn larvae in the larger containers was more limited owing to the greater distances they had to travel in order to reach the walls of the container. It should in fact be recalled that *in vitro* the newly hatched larvae take some time to sink into the pabulum, wandering over it for a considerable length of time. Vice versa, *in vivo* the larvae already begin sinking into host by piercing the integument in the area immediately below its cephalic pole even before having fully emerged from the corion. In some cases, however, as observed by Hafez (1953), they move about on the host body for some minutes.

B. *In vivo* rearing.

Puparia yield was rather low even on the *Galleria mellonella* factitious host, which is a perfectly suitable medium. This can be seen in the following table in which all three groups of larvae were variously parasitized by the same *Exorista* females employed for the *in vitro* tests.

Samples	No. of puparia	Yield (%)
50 larvae with 1 egg each	15	30
50 larvae with 2 eggs each	32	32
50 larvae with 3 eggs each	39	26

The low puparia yield obtained in relation to the number of eggs attached to the host depends only to a small degree on the unfavourable characteristics of the egg (i.e., partial or total failure of embryogenesis, failure of the newborn larvae to emerge). In fact, it is mainly due to the fact that the eggs become detached from the host integument, a phenomenon which occurs quite frequently already within the first minutes after oviposition. In a batch of 46 host larvae employed in another study, out of the 169 eggs with which they were parasitized 36, corresponding to 21.30%, were found to be lying on the bottom of the container about an hour after oviposition. The detachment continued, albeit less intensely, favoured by the fact that the larvae became entangled in the intricate bundle of silky threads issuing from them during their practically continuous wandering about in the foodless containers and by their constant rubbing together. This phenomenon appears to be particularly marked in the case of oviposition by young females of less than 5 to 6 days old. This is presumably due to the fact that during ovipositioning these females do not exert the necessary pressure with the tip of their factitious ovipositor against the body of the host so as to make the ventral surface of the corion, which is lightly concave and sticky, adhere properly to it.

Ovipositioning on larvae which are not sufficiently far from forming a cocoon is another cause conducive to the loss of eggs. In fact, embryogenesis takes about three days so that all the larvae parasitized in the three days before moulting reject, together with their exuvia, the as yet unhatched parasite eggs.

Finally, it should be noted that in the case of superparasitized hosts, the parasite frequently exhibits a competitive behaviour which leads to the elimination of one or more of the competitors.

Bearing all these considerations in mind, it is not therefore at all surprising that, as shown in the table above, out of the overall 300 eggs only 86 puparia developed, corresponding to a mean yield of 28.66%.

4. Emergence rates.

As usual, emergence rates were determined on the basis of the number of puparia formed.

A. *In vitro* rearing.

Fifty-seven adults emerged from the 65 puparia formed in the 16.5 mm diameter wells (see table on page 186), corresponding to an overall mean of 87.7%, with a minimum emergence rate of 72% in the case of the puparia formed in the wells with only 200 mg of diet.

Similarly, in the small 7mm diameter wells (see table on page 187), the overall mean emergence rate was 85.49%, with only slight variations, the lowest emergence rate being as low as 25% in the treatment employing 50 mg of diet. In fact, no adults emerged from puparia weighing 11 to 12 mg or less. Out of the 4 puparia obtained with this treatment, only one imago emerged from the puparium which weighed 13.4 mg.

It should be noted that these rates are amongst the highest encountered throughout our *Exorista in vitro* rearing experiments and this despite the fact that even though a number of the puparia formed in the small 7mm diameter wells were defective from a morphological and chromatic point of view, they nevertheless gave rise in many cases to fully formed adults. On suitable diets placed in 5cm diameter Petri dishes, medium emergence rates were around 80%. Under these conditions, dwarf puparia were not formed. Indeed, the opposite was seen to occur, giant puparia of over 70-80 mg in weight shows a considerable drop in emergence rates.

Unlike *in vivo*, in *in vitro* conditions atypical puparia were not infrequently formed which were seen to feature the morphological characteristics typical of the larvae and to usually lead to adult emergence.

It should also be borne in mind that as the puparia reared on artificial diets undergo a series of manipulations the viability of newly formed puparia in particular may to some extent be adversely affected. Manipulations include removal of the puparia from the pabulum, cleaning them from residues of food and excrements, weighing them and finally transferring them to other containers.

B. *In vivo* rearing.

The emergence rates of the puparia formed at the expense of the *Galleria* host were little higher than those recorded in the *in vitro* tests reported in the present paper. Ninety-eight adults, corresponding to an emergence rate of 86.72%, were obtained out of a sample of 113 puparia collected on the bottom of a general *Exorista* rearing container. Out of a second sample of 58 puparia obtained from *Galleria* larvae remaining from the partial removal of the eggs of the tachinid employed in the experimental section of the present study, 51 adults developed, corresponding to an emergence rate of 87.93%. Actually, the emergence rate may be considered to be 94.87% as four of the puparia which failed to emerge were of very small dimensions, far below those normally conducive to emergence. This observation permits us to conclude that emergence failure under *in vivo* conditions depends not so much on the general unfavourable physiological characteristics of the pupae but rather, and to a large extent, on their small dimensions resulting from over-superparasitization.

Vice versa, excluding sporadic cases of dwarfism or, alternatively and more frequently of gigantism and of damaged puparia due to manipulation, under *in vitro* conditions emergence failure was seen to involve puparia which, despite being apparently quite normal in terms of size, shape and pigmentation, were obviously scarcely viable.

Dissection of both the *in vitro* and *in vivo* reared puparia from which imago failed to emerge revealed that in most cases the tachinid had died at the eopupal stage or, less frequently, at more or less later stages of development of the pupa, and, occasionally, when the adult was fully formed and completely pigmented. Under both experimental conditions some cases were even observed of the adult having broken through the cephalic cap but failing to fully emerge from the puparium. This latter situation is presumably favoured by the fact that puparia are free on the smooth bottom of the empty glass container so that in its attempt to emerge the adult has nothing to get a hold of.

Conclusions

In this first overall comparison of the results obtained from the *in vitro* and *in vivo* rearing of *Exorista larvarum* only a number of biological parameters have been considered. Given the importance of the issue, a more detailed and thorough analysis is envisaged in the future. As of now, however, it can already be safely said, at least on the basis of repeated laboratory results, that adults reared on our artificial diet are perfectly capable of successfully attacking the larvae of the factitious host *Galleria mellonella* and of giving rise to a new generation equally capable of doing the same. This does not mean, of course, that, in view of the possibility of field applications of this parasitoid, further studies are not required.

In this paper, attention has been first and foremost given to the diet utilization index with a view to defining the optimal amount of diet *per capita* so as to ensure maximum pabulum exploitation and thus avoid wastage. The ultimate goal is of course that of reducing the production costs of the parasitoid. The topic of food rations was dealt with in the second paper of the series (Mellini *et al.*, 1993). It has been discussed here again not only because of its considerable practical importance but also because in the meantime the composition of the diet has been radically altered so that it was felt that a comparison with *in vivo* conditions was called for, given also that essential changes have been made to the rearing techniques.

In the present study the parasitoid was reared individually in small 7mm diameter wells which not only permitted to reduce the amount of food to extremely low levels but also afforded "environmental" conditions fairly similar to those of the live host. The mean utilization index in these wells tends to slightly increase as the amount of food decreases, going from 18.14% with 225 mg of diet to 23.77% with only 75 mg of diet. This means that even with minimum doses weighing much less than the *Galleria* larvae, the pabulum is not fully exploited but rather that the weight of the puparia tends to drop. This would seem to indicate that the diets possess qualitative characteristics which, in some respects, are inferior to those of the host, at least as far as the specific requirements of *Exorista* are concerned. It should nevertheless be borne in mind that too small amounts of food lead to alterations in the physical characteristics of the pabulum such as its more or less rapid dehydration, thus making it become unsuitable. In larger wells with a diameter of 16.5 mm, in which 1 to 3 eggs were placed, it was seen that the utilization index increased as the number of puparia formed per well increased. All these tests have shown that, considering the various biological parameters, the optimum *per capita* dose of diet is around 175 to 200 mg. It is important however that the pabulum in the containers be of a certain thickness, that is around 3 to 4 mm. This does not only prevent it from rapidly drying out but, in case of massive pollution by bacteria and moulds when the larvae are at the second instar, it also permits them to continue growing in the deeper unpolluted layers of the pabulum even if this means that their final weight is markedly less. Obviously, the index reported here refers to the type of diet employed which, it should be noted, was amongst the best so far prepared by us. On the basis of our experience it may however be said that this index is not much different for other diets of even quite different composition prepared and employed by us. All in all, it may be said that, besides its nutritional properties, the important thing is that the diet also be suitable from a physical point of view.

Under *in vivo* conditions, the utilization index was always found to be sensibly or even markedly greater than that reported *in vitro*, the weight factor being essential. In fact, as for the pabulum, the utilization index depends on the weight of the host so that it increases when the latter decreases, further increasing as the level of superparasitization increases. In fact, on a sample of larvae which weighed between 86.4 and 188.9 mg upon hatching of the parasite, an average index of 28.12% was reported in the presence of only one puparium which increased to as much as 41.57% in the case of three co-tenant puparia.

Puparia weight. Provided conditions are optimal, the average weight of *in vitro* formed puparia in the presence of abundant food is generally higher, and considerably so, than that of *in vivo* formed puparia, reaching or even exceeding 60 mg, with isolated individuals reaching or exceeding 100 mg against the average of, at most, 35 to 40 mg of the *in vivo* specimens. A marked drop in weight was seen to occur with food rations of around 200 mg when these were distributed in thin layers and of about 125 mg even when these were distributed in fairly thick layers. Moreover, dimensional variability was considerably less *in vitro* than *in vivo*, as in the former case there were no evident signs of competition for the food. In fact, *in vitro* rearing not only afforded an abundance of pabulum for all the co-tenants but also ample room, at least in the 5cm diameter Petri dishes employed by us, much more than that afforded by the narrow and relatively short funnel of the host. Under *in vitro* conditions, undersized puparia may be formed if the pabulum is initially too compact or becomes so in the course of larval development or if there is not enough of it or, finally, if it becomes contaminated by bacteria or moulds. In no case, however, are these puparia so undersized as those which may be formed in the host.

Under *in vivo* conditions, puparia weight depends to a good extent on the size of the victim but above all on the level of superparasitization. If the latter is high, it often leads to the formation of dwarf puparia and, consequently, to a very marked dimensional variability. *In vivo* reared puparia can individually attain the average weights of *in vitro* reared ones only if solitary and in hosts of considerable weight, i.e. around or above 200 mg. But even in this case the maximum weight attained by *in vivo* puparia is between 55 and 60 mg and this despite the fact that, in theory at least, the amount of food available is such as to permit them to become even larger.

While *in vitro* puparia weight is, therefore, largely dependent on the quality of the pabulum, which quantitywise is almost always more than enough, *in vivo* it depends on the amount of food, which is usually limited. This is all the more so in a host such as *Galleria* which is generally superparasitized and small as compared to natural hosts such as the corpulent larvae of Noctuids.

Puparia yield. Puparia yield is calculated on the basis of the number of cultured eggs. *In vitro*, it is always low, rarely reaching 50%, a level which is attained only when rearing is performed in 5cm diameter Petri dishes with 30 eggs and 13 cc of pabulum. In addition to the 10% of unhatched eggs (a phenomenon due to failed or incomplete embryogenesis or failure of the larva to emerge from the egg-shell) yield is further and markedly reduced by the fact that the newly hatched larvae tend to abandon the medium. Larval mortality rate in the course of development from the second instar onwards is instead very limited. In certain tests, it cannot be excluded that the level of mortality may have been affected by improper handling of the eggs, which have to be detached from the host integument in order to transfer them to a disinfecting solution and then in sterile water before being placed on the diet. The overall yield of the tests reported in this paper was unusually low, being around 33.85% in the 16.5mm diameter wells and 35.15% in the smaller wells with a small amount of food. It should however be noted that in the case of the former, the low yield rate may depend on the fact that in some treatments only small amounts of food were employed and that in others more than one egg were placed in each well.

Notwithstanding, the *in vivo* yield rate was also low (28.66%). Apart from the fact that also in this case a number of the eggs were not viable, the most important adverse phenomenon was represented by the considerable loss of eggs, which tended to become easily detached from the larval integuments already a short time after oviposition. This tendency appeared to be even more marked when oviposition was made by young females which apparently failed to perform this behaviour efficiently. Moreover, the egg-carrying host larvae, when placed in foodless containers, move about continuously, issuing large quantities of silky threads in which the eggs become entangled. Albeit not as important, an additional cause adversely affecting puparia yield is the level of superparasitization which, when excessive, leads to the elimination of a part of the co-tenant endophagous larvae.

Egg loss upon host ecdysis was instead low. This is due to the fact that utmost care was taken in our experiments to place the parasite only on host larvae at their last instar, at a stage when they were sufficiently far from pupal moulting.

Generally speaking, then, the main cause for the marked reduction in puparia yields *in vitro* is the migration of the newborn larvae away from the pabulum, while the detachment of the eggs from the host body is the main one in *in vivo* conditions.

Emergence rates. Emergence rates are calculated on the basis of the number of formed puparia. Both *in vitro* and *in vivo*, no adults emerged from puparia weighing less than 11-12 mg.

Under *in vitro* conditions, a reduction in emergence rates was observed both in dwarf puparia (which are rare) and in giant ones (which are rather frequent) weighing more than 70 mg. This latter phenomenon was not observed *in vivo* as the puparia never attained such high weights. Emergence rates, which in the present study were found to be 83,7% on average, are usually around 80% and normally also involve puparia morphologically atypical maintaining larval characteristics, a phenomenon not observed *in vivo*. It should be recalled that *in vitro* reared puparia undergo a series of manipulations which may to some extent adversely affect their viability. These manipulations include their removal, cleaning them from the residues of diet and excrements, weighing them and finally transferring them into empty containers.

In vivo emergence rates were generally slightly higher, being around 87-88% in the tests reported in this paper. It should be pointed out that the puparia formed at the expense of *Galleria* did not undergo any manipulation. Some of them, however, were too small for the adult to be able to emerge; this does not exclude that there may have been other reasons for failed emergence depending on the intrinsic characteristics of the puparia themselves.

Dissection of the puparia from which imago failed to emerge revealed that in both experimental conditions the parasitoid had died at the eopupal stage or, more frequently, at more or less later stages of development of the pupa, and, occasionally, when the pharate adult was fully formed and completely pigmented.

More in general, it should in any case be noted that certain negative factors at work *in vitro* are not encountered *in vivo*. This especially refers to the routine manipulations which both the eggs and puparia undergo under the former conditions, and which may to some extent adversely affect their viability, thus lowering some biological parameters such as puparia yield and emergence rates.

Duration of larval growth. No specific research has been conducted so as to be able to compare this parameter in the two experimental conditions adopted. Notwithstanding, some data is available which permits this comparison to be made. In fact, in order to ascertain the viability of the *Exorista* eggs transferred on the diet, the corresponding batch of *Galleria* larvae from which the eggs had been taken was always kept back. A comparison showed that larval growth is usually somewhat slower in *in vitro* conditions, even when these are optimal in terms of diet composition and physical characteristics. At 26-27 °C, in fact, 5 to 6 days after hatching were usually sufficient for the larvae to reach full maturity *in vivo*, while *in vitro* the time required was at least 7 to 8 days. Moreover, if the pabulum is too compact, young larvae take time to dig long deep funnels, while if it is too soft a thin liquid film forms on the surface which the first instar larvae find difficult to sink into, thus running the risk of their posterior tracheal spiracles becoming submerged. In any case, the slower development is due to a large extent to the reluctance of the newborn larvae to sink into the pabulum as well as to a slower growth rate than in *in vivo* conditions. It cannot be excluded that this may be due to the lack of particular substances which are instead present in the host and which may be responsible for quickly triggering off the growth process. Larval growth, however, is just as fast *in vitro* as in the *Galleria* host during the second and third instar. It is also worth recalling that as already pointed out by Hafez (1953) growth rate appears to be accelerated *in vivo* especially by high levels of superparasitization, which bring the endophagous larvae into contact with each other. Clearly, such a phenomenon does not occur *in vitro* as the larvae have more than ample room available so that eventual reciprocal interferences take place exclusively in the last stages of the third instar, in particular when the mature larvae are searching for a place where to pupate. In conclusion, therefore, while the growth rate of the parasitoid is quicker under *in vivo* conditions, *in vitro* conditions are still the most favourable for its increase in weight.

SUMMARY

Unlike in previous tests, instead of in 5cm diameter Petri dishes the present tests were conducted in 16.5 and 7-mm diameter multi-well plates. In particular, the smaller wells were supplied with only one *Exorista larvarum* (L.) egg per well. This was done in order to be able to better compare a number of biological parameters for *in vivo* and *in vitro* conditions.

The highest values for the utilization index of the diet developed in a previous study were recorded with doses of about 75 mg of pabulum per well. Notwithstanding, as such a small amount of food (weighing half as much as that of the *Galleria mellonella* L. larvae subjected to parasitization) leads to the formation of viable but extremely low-weight puparia, the ideal diet appears to be from between 175 to 200 mg. In this case, the utilization index was found to be 20%, leading to the formation of

puparia weighing from between 35 to 40 mg, weights which were somewhat greater than the mean ones obtained *in vivo*. *In vivo*, the utilization index is markedly higher, increasing from mean values of 28% in the case of monoparasitized hosts to 41% in victims in which three puparia were formed.

The mean weight of *in vitro* obtained puparia was generally greater, even by 50% and over, as compared to that of *in vivo* ones. This finding depends on the large amount of pabulum available to the larvae in the 5cm diameter Petri dishes. In 16.5mm diameter cylindrical wells with a considerable amount of diet, puparia mean weight stabilized around 45 to 55 mg with 300 mg of pabulum *per capita*. In 7mm diameter wells with a small amount of diet, puparia weight sharply dropped with rations of 125 mg or less. *In vivo*, in addition to host larva weight upon the parasite hatching, mean puparia weight is largely dependent on the level of superparasitization, which determines a weight variability far greater than that encountered *in vitro*. In fact, the present test showed that while the mean weight of puparia from monoparasitized hosts was 39.38 mg, that of two co-tenant puparia was 27.37 mg. Higher levels of superparasitization frequently lead to the formation of dwarf puparia weighing less than 15 to 20 mg.

Puparia yield with respect to the number of eggs initially present, surprisingly appears to be nearly the same under both experimental conditions. *In vitro*, this rate was found to be adversely affected to a large extent by the marked vagrant behaviour of the newborn larvae which move about at length on the pabulum, often tending to abandon it. Moreover, in the present tests, the use in certain treatments of a minimum amount of pabulum also had the effect of reducing puparia yield as, by quickly dehydrating, it became unsuitable for consumption.

Adverse factors affecting yield in *in vivo* rearing are represented by the ease with which the eggs frequently detach themselves from the host integument as well as by high levels of superparasitization which fairly frequently lead to the loss of many larvae even at advanced stages of development. Another adverse factor encountered not so much under laboratory conditions as in nature, is that oviposition is not rarely performed on larvae which are close to moulting so that, together with their exuvia, they also reject the still unhatched eggs glued to their integuments. For all these reasons, the overall yield in the present study was found to be 35% *in vitro* and, unexpectedly, somewhat lower *in vivo*, i.e. about 30%.

Emergence rates, calculated on the basis of the number of puparia, were around 83,7% *in vitro*. The unemerged puparia comprised to a large extent collapsed and/or giant specimens. *In vivo*, emergence rates were around 87 to 88%. In this case, failed emergence mainly concerned dwarf puparia weighing less than 11-12 mg. It should be noted that *in vitro* emergence rates in previous experiments were generally lower, being around 80%. In both experimental conditions, dissection of the unemerged puparia revealed that the tachinid had died as an eopupa or at various stages of its pupal development, or even as an adult which, albeit completely formed, was unable to open the cephalic cap of the puparium.

On the basis of the results of the present study and with reference to the biological parameters examined, it may be concluded that, especially from a quantitative point of view, there are no substantial differences between the *in vitro* and the *in vivo* yield of this tachinid.

Primo confronto tra la produzione *in vitro* e quella *in vivo* del parassitoide *Exorista larvarum* (L.)

RIASSUNTO

Le presenti prove, anziché nelle solite capsule Petri del diametro di 5 cm, sono state condotte in piastre multicellari del diametro di mm 16,5 e di mm 7. Queste ultime in particolare, rifornite di 1 solo uovo di *Exorista larvarum* (L.) per cella, hanno consentito un migliore confronto di alcuni parametri biologici tra la produzione del parassitoide *in vitro* e quella *in vivo*.

L'indice di utilizzazione della dieta, messa a punto nel precedente lavoro, ha raggiunto i valori più elevati con dosi attorno ai 75 mg/cella. Tuttavia, poiché con razioni così modeste, di peso più che dimezzato rispetto a quello delle larve di *Galleria mellonella* L. sottoposte a parassitizzazione, si ottengono pupari vitali sì ma di peso troppo basso, appare consigliabile l'impiego di razioni attorno ai 175-200 mg che danno, con un indice di utilizzazione sul 20 %, pupari di 36-40 mg, quindi addirittura di peso alquanto superiore alle medie realizzate *in vivo*. *In vivo* l'indice di utilizzazione è decisamente più alto salendo, da medie del 28 %, nel caso di ospiti monoparassitizzati, al 41 % in vittime in cui si sono formati 3 pupari.

Il peso medio dei pupari ottenuti *in vitro* è generalmente superiore, anche del 50 % ed oltre, rispetto a quelli formatisi *in vivo*. Ciò dipende dalla grande quantità di pabulum di cui dispongono le larve in capsule Petri del diametro di 5 cm. In celle cilindriche larghe 16,5 mm, ad alto contenuto di dieta, si è visto che con razioni di 300 mg pro capite, ed oltre, il peso medio dei pupari si stabilizza sui 45-55 mg. In celle larghe solo 7 mm, con basso contenuto di dieta, si è notato che un brusco calo di peso si verifica con razioni di 125 mg ed inferiori. *In vivo* il peso medio è largamente condizionato, oltre che da quello della larva ospite al momento della schiusa dell'uovo, dal grado di superparassitizzazione che porta la variabilità megetica dei pupari a livelli ben maggiori di quelli riscontrati *in vitro*. In questa prova si passa infatti da una media di 39,38 mg, per pupari da ospiti monoparassitizzati, a medie di 27,37 mg per pupari formatisi in coppia. Con livelli più alti di superparassitizzazione si hanno frequentemente pupari nani di peso inferiore ai 15-20 mg.

La resa in pupari, rapportata al numero delle uova impiegate, è sorprendentemente apparsa quasi uguale nelle due condizioni sperimentali. *In vitro* essa risulta fortemente penalizzata dalla spiccata tendenza delle larve neonate a vagabondare a lungo sul pabulum fino, sovente, ad abbandonarlo e, nelle presenti prove, anche dalle tesi con quantitativi minimi di pabulum che, disidratandosi, diviene abbastanza rapidamente inidoneo. *In vivo* la resa è decurtata dalla facilità e la frequenza con cui le uova si distaccano dal tegumento dell'ospite, nonché da alti livelli di superparassitizzazione che sfociano, piuttosto frequentemente, nella perdita di molte larve anche ad accrescimento avanzato; infine, non tanto in laboratorio quanto in natura, dall'ovideposizione su larve abbastanza prossime alla muta, le quali, assieme alla esuvia, rigettano le uova, non ancora schiuse, incollate sui loro tegumenti. Così nel corso di questo lavoro, la resa generale si è collocata attorno al 35 % *in vitro* e, inaspettatamente, ad un livello alquanto più basso, circa il 30 %, *in vivo*.

Le percentuali di sfarfallamento, calcolate sul numero dei pupari, si sono aggirate *in vitro* sull'83,7%; l'aliquota restante è, in buona parte, rappresentata da pupari collassati e/o giganti. *In vivo* le percentuali si sono attestate sull'87-88 %; in questo caso il mancato sfarfallamento coinvolge soprattutto i pupari nani di peso inferiore agli 11-12 mg. Per il vero nelle precedenti sperimentazioni i tassi *in vitro* sono generalmente risultati più bassi, attorno all'80 %. In entrambe le condizioni sperimentali, la dissezione dei pupari non schiusi ha mostrato che il tachinide era morto o come eopupa o come pupa in varie fasi di sviluppo o addirittura come adulto completamente formato ma incapace di aprire la calotta cefalica del pupario stesso.

In conclusione, dalle presenti indagini e con riferimento ai parametri biologici esaminati, risulta che non esistono differenze sostanziali, soprattutto dal punto di vista quantitativo, tra la produzione *in vitro* e quella *in vivo* di questo Tachinide.

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