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Further simplifications in the composition of oligidic diets for the parasitoid *Exorista larvarum* (L.).*(1)(2)

I. Introduction

Good results have been obtained for the *in vitro* rearing of *Exorista larvarum* (L.) with oligidic diets based on bovine serum and on the homogenate of a factitious host, *Galleria mellonella* L. The diets are in fact so promising that they may permit the mass production of this tachinid (Mellini *et al.*, 1993a,b; Mellini and Campadelli, 1994,a,b; Mellini et alii, 1994).

Albeit being easy to prepare, these diets, however, are not all that inexpensive. Although bovine blood is in fact cheap and can be easily obtained from slaughterhouses, the preparation of the serum and the subsequent steps for its sterilization are time-consuming operations and require the use of expensive instruments and materials. No less expensive is the other ingredient employed in these diets, namely host larval homogenate, which requires a continuous breeding of the host in order to always have a good supply available.

The experiments reported in the present paper were aimed at replacing these ingredients with others less costly and readily available on the market. It should be immediately pointed out that while bovine serum can be replaced without apparently any problems, replacement of the host homogenate may be risky. In fact, there might be negative repercussions not only on parasitoid yields but also on the viability of the adults as doing away with the host homogenate is, so to speak, tantamount to breaking the biochemical bond which presumably tends to link the parasitoid to the host insect. Undoubtedly, the *in vitro* rearing of a parasitoid in the absence of material deriving from it represents a considerable achievement, at least from a theoretical point of view. In fact, this means not only being able to produce the parasitoid outside its natural environment but, even more importantly, to produce it on a trophic substrate which is completely alien to the natural one. From a practical point of view, however, this artificial set-up may have its drawbacks because, in addition to the above-mentioned problems associated with the possible biochemical bonds between the host and the parasitoid, the pabulum

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may turn out to be scarcely palatable to the parasitoid. In fact, given the absence of the natural phago-stimulating action in artificial diets, the newborn larvae may not be induced to penetrate into a substrate which is so different from the natural one, thus causing considerable losses in production.

Amongst the various materials more frequently employed for preparing diets for parasitoids, yeast extract and hen egg yolk were used in our experiments. The former was adopted as it is particularly rich in aminoacids which, in the course of many studies, proved to be essential for the growth of the parasitoid at least during the early stages of larval growth. The latter was used thanks to its high nutrient contents and because it is particularly rich in lipids; we had already employed it in our previous experiments but at much lower doses (2%). In the present study it was decided to considerably increase this amount in consideration of the fact that *Exorista* host larvae feature a highly developed adipose body.

Always with a view to progressively reducing the cost of artificial diets, in future experiments we shall attempt to employ other widely available and even more inexpensive products such as soya meal and powdered milk which have already proven to be promising with other parasitoids.

II. Materials and Methods

The basic methods adopted in the present study are not different from those illustrated in our previous papers concerning the preparation of artificial diets for *Exorista larvarum*.

The standard pabulum from which we started for developing new formulations is made up of the following ingredients: bovine serum (75%), homogenate of the last instar larvae of *Galleria mellonella* L. (20%), trealose + hen egg yolk + other additives (5%).

The purpose of the present study was to investigate the following three topics: 1. replacement of the homogenate, 2. replacement of the serum, and finally, 3. replacement of both these basic ingredients, notwithstanding the good results obtained in the *in vitro* production of *E. larvarum* with both of them.

The ultimate scope was to obtain an artificial diet with ever more inexpensive ingredients and easier to prepare.

The experiment entailed a series of tests conducted with a number of substitute ingredients. More precisely, it entailed:

1. Replacement of *Galleria mellonella* larvae homogenate with:

a. Powdered *Bombyx mori* L. chrysalis commercial preparations available as pet food.

Diet formulation:

Diet	Serum	Homogenate *Galleria* larvae	Powdered *Bombyx* chrysalis	Yeast	Additives
A	75%	10%	–	10%	5%
B	75%	5%	5%	10%	5%
C	75%	–	10%	10%	5%

b. Powdered yeast extract.
Diet formulation:

Diet	Serum	Homogenate	Yeast	Additives
A	75%	10%	10%	5%
B	75%	5%	15%	5%
C	75%	–	20%	5%

c. Egg yolk.
Diet formulation:

Diet	Serum	Homogenate	Egg Yolk	Additives
A	75%	10%	10%	5%
B	75%	5%	15%	5%
C	75%	–	20%	5%

d. Egg yolk and yeast in various percentages.
Diet formulation:

Diet	Serum	Homogenate	Egg Yolk	Yeast	Trealose
A	75%	10%	3%	10%	2%
B	75%	5%	8%	10%	2%
C	75%	–	13%	10%	2%
D	75%	–	18%	5%	2%

2. Replacement of bovine serum with increasing percentages of sterile water.
Diet formulation:

Diet	Serum	Water	Homogenate	Yeast	Egg Yolk	Trealose
A	72%	–	10%	8%	8%	2%
B	47%	25%	10%	8%	8%	2%
C	22%	50%	10%	8%	8%	2%
D	–	72%	10%	8%	8%	2%

3. Total elimination of the bovine serum and progressive replacement of the homogenate
Diet formulation:

Diet	Serum	Water	Homogenate	Yeast	Egg Yolk	Trealose
A	–	72%	10%	8%	8%	2%
B	–	77%	5%	8%	8%	2%
C	–	82%	–	8%	8%	2%

4. Total elimination of the bovine serum and of the homogenate.
As in preliminary experiments no puparia were seen to develop with diets con-

taining yeast alone or egg yolk alone, the experiment was conducted with the following diet formulation:

Diet	Serum	Homogenate	Water	Yeast	Egg Yolk	Trealose
A	–	–	78%	15%	5%	2%
B	–	–	78%	10%	10%	2%
C	–	–	78%	5%	15%	2%

Four replications were made for each test, in some cases 5 or even 7. In all tests, about 500 mg of pabulum were available to each larva, an amount far in excess of their actual requirements. From a theoretical point of view at least, therefore, and as long as the pabulum was not polluted, the supply of food was such as to permit the larvae to develop to their full extent on condition, of course, that the diet be suitable. Thirty-five eggs were thus placed in each 5-cm Petri dish. It should however be noted that about 10-15% does not hatch and that a certain percentage of newborn larvae, more or less variable but in any case considerable, leaves the pabulum, creeping up the walls of the container, and eventually dying on the lid.

III. Results

1. Replacement of the homogenate with:

a) Powdered *Bombyx mori* chrysalises.

The following table shows the mean values for each of the parameters considered in the four replications:

Diet	No. of puparia	Mean weight (mg)	Emergence (%)
A	28	45.56	96.42
B	28	47.18	82.14
C	37	43.60	83.78

Despite puparia yields being low owing to the fact that in the first two replications the pabulum was subject to early and massive pollution by moulds, as can be seen yield does not drop as the amount of homogenate is reduced. Indeed, in the trial in which the homogenate was completely replaced with powdered *Bombyx* chrysalises, yield tends to increase. It is worth noting that, at equal weights, this latter product is much richer in nutrients than the homogenate as it is practically anhydrous. As can further be seen, the mean weights of the puparia are essentially the same in all three tests. A certain drop in emergence can however be noted in the diets B and C employing powdered chrysalises.

All in all, it may be concluded that in bovine serum-based diets with fresh *Galleria* larvae homogenate, the latter may be satifactorily replaced with powdered preparations made up of ground silk worm chrysalises. These preparations are fairly inexpensive and readily and widely available on the market as they are employed for animal feed.

b) Powdered yeast extracts.

The rationale behind this experiment is identical to that of the previous one.

The purpose is once more to replace fresh *Galleria* larvae homogenate, a typical laboratory preparation, with another possibly equally valid but more readily available and less expensive commercial ingredient. The following table shows the mean values of the parameters considered in the four replications.

Diet	No. of puparia	Mean weight (mg)	Emergence (%)
A	27	49.61	96.29
B	36	52.17	77.77
C	65	49.20	92.30

As can be seen, the number of puparia tends to increase as the amount of yeast increases with the corresponding reduction in the amount of homogenate. This tendency is particularly marked in diet C where the number of puparia was seen to increase twofold as compared to the starting diet. This is true for all four replications. Such a result may be surprising; however, it should be considered that, at equal weights, powdered yeast extract contains much more aminoacids and proteins than the fresh homogenate. Not surprisingly, in fact, yeast in general has proven to be an important ingredient, giving good results in the *in vitro* rearing of parasitoids. Moreover, in our particular case a further explanation may be that the high yeast contents employed by us reduce the more or less marked tendency of the newborn larvae to abandon the pabulum. This hypothesis seems to be borne out by the fact that the mean weight of the puparia is similar for all three diets, being around 50 mg, which is decidedly much higher than that of puparia obtained from *in vivo* experiments.

The percentage of emerged adults is similar in the diet with the highest amount of homogenate and in that where it is totally lacking, having been totally replaced with the highest amount of yeast. Albeit being lower in the intermediate diet, B, the percentage of emerged adults is nevertheless within acceptable limits.

It can therefore be concluded that the host-derived ingredient employed in bovine serum-based diets may be totally and profitably replaced, at least with reference to the three parameters considered, with increasing amounts of yeast.

c) Fresh hen egg yolk.

As in the previous experiment with yeast, the present tests were aimed at establishing whether egg yolk can be used as a substitute for *Galleria* larvae homogenate. The results of the four replications are shown in the table below.

Diet	No. of puparia	Mean weight (mg)	Emergence (%)
A	77	47.91	62.33
B	46	43.68	52.17
C	–	–	–

As can be seen, homogenate reduction from 10 to 5%, accompanied by a corresponding increase in the amount of egg yolk, caused a drastic drop in puparia

production and a certain reduction in their mean weight as well as a slight delay in their development. The diet devoid of homogenate, which had been completely replaced with egg yolk, gave even poorer results, larval development being slow and only partial and never going beyond the 2nd instar. These larvae were seen to survive for about a month, feeding themselves in their subvertical wells opening to the surface, which had been invariably dug into the trophic substrate.

The percentage of emerged adults, calculated as usual on the basis of the number of formed puparia, was unusually low and tended to diminish in the diet in which the egg yolk prevailed over the homogenate.

It can therefore be concluded that, despite the good results obtained by us with other diets at least as far as bovine serum-based diets are concerned, unlike yeast, egg yolk cannot be considered a satisfactory substitute for host larvae homogenate.

d) Yeast and egg yolk in varying proportions.

The following table shows the mean values obtained for the four replications.

Diet	No. of puparia	Mean weight (mg)	Emergence (%)
A	58	50.37	87.93
B	49	55.56	91.83
C	69	59.31	82.60
D	86	63.70	62.79

On the basis of the above results the following considerations may be made:

- The absence of host larvae homogenate in bovine serum-based diets, when appropriately compensated for by powdered yeast extracts eventually added with fresh egg yolk, does not compromise in any way parasitoid yield (see diets C and D).
- The number and mean weight of puparia are clearly higher in the two diets devoid of homogenate but containing a greater amount of egg yolk. The latter ingredient, therefore, appears to be particularly suitable for the preparation of artificial diets for *Exorista* even if it cannot be considered as a substitute of the homogenate as the results of the previous experiments show. Growth rate was also seen to markedly increase.
- The remarkable increase in puparia weight obtained by considerably increasing the amount of egg yolk is accompanied by a marked decline in the percentage of emerged adults. This phenomenon is not necessarily ascribable to the diet composition itself but rather to the excessive weight attained by the puparia, which not infrequently is close to or greater than 100 mg. In fact, the mean weight recorded in the tests with the diet containing the highest amount of egg yolk (18%) was nearly twice that recorded for puparia grown *in vivo* (63.70 vs 35 mg). It should however be added that the puparia of this experiment were weighed later than usual, that is one or two days prior to the adults' emerging. If they had been weighed on the same day in which they formed, their weight would have been 11 to 16% greater, as can be inferred on the basis of the estimates made by Campadel-

li (1980) for another tachinid, *Pseudogonia rufifrons* Wied.
- Even if egg yolk is a very good ingredient, its amount in the diet should never be more than about 10%.
- It should always be borne in mind that the above considerations refer exclusively to diets the main constituent of which is bovine serum (75%).

2. Replacement of bovine serum with sterile water.

The results of the four replications are shown as general mean values in the table below.

Diet	No. of puparia	Mean weight (mg)	Emergence (%)
A	61	59.68	88.52
B	67	58.40	92.53
C	50	58.26	76.00
D	48	55.35	64.58

This series of diets was particularly rich in nutrients as, in addition to the homogenate, they also contained egg yolk and yeast in equal amounts (8%). Only the amount of bovine serum was progressively reduced, reaching zero in the last diet (D), where it was completely replaced with sterile water.

As can be seen in the last two diets, the production of puparia tends to drop as the amount of serum is reduced. Their mean weight, on the other hand, remains practically constant and in any case above that recorded for *in vivo* experiments, where it rarely exceeds the mean value of 40 mg. A considerable uniformity of weight, moreover, was observed within each diet together with a marked synchronization in chrysalis formation. The percentage of emerged adults, however, appears to be lower in the last two diets, and is especially low in the last test with the diet devoid of bovine serum.

In conclusion, the removal of serum, albeit compensated for by considerable amounts of yeast and egg yolk, would seem to have, below a certain level, negative effects on adult emergence alone. Notwithstanding this fairly clear indication, it was nevertheless decided to conduct further experiments in which the serum was totally eliminated in order to confirm the results reported above.

3.Total elimination of bovine serum and progressive elimination of the homogenate.

The following table shows the mean values obtained for the four replications of this experiment.

Diet	No. of puparia	Mean weight (mg)	Emergence (%)
A	51	48.46	90.21
B	49	42.10	81.52
C	54	40.11	77.81

It should first of all be noted that puparia yield does not vary, being fairly satisfactory for all three diets, while the mean weight of the puparia tends to

decrease in the diet with only 5% homogenate and in the one completely devoid of this ingredient, which was compensated for by increasing the amount of water to 77 and 82%, respectively. In any case, mean weights never drop below 40 mg, which is greater than those normally attained in the host, *Galleria*. Larval growth rates tend to increase (on average by less than one day over about a week at 25-26 °C) as the amount of homogenate in the diet is reduced.

It may also be observed that the rate of emergence tends to decrease although not to alarming levels. This finding would seem to be in contradiction with the results obtained in the previous experiment where emergence values were lower even when the homogenate was present in the diet.

In conclusion, yeast and egg yolk in equal quantities of 8% may be considered to satisfactorily replace bovine serum and host larva homogenate at least as far as simple parasite production is concerned, that is to say in quantitative rather than in qualitative terms.

Given the discrepancies between the results of this experiment and those of the previous one, however, a number of additional tests were conducted which are described in the following section.

4. Total elimination of serum and homogenate and their replacement with various mixtures of yeast extract and egg yolk diluted in sterile water.

Two preliminary experiments were conducted, the purpose of which was to test, in addition to several mixtures of yeast and egg yolk, also diets made up of either of these two ingredients alone previously diluted in water. It was seen that puparia never developed with diets containing yeast or egg yolk alone. Indeed, the first larval instars, albeit living at length (even up to about a month) and growing considerably within their "respiratory funnel", were never able to moult. These diets were therefore abandoned in subsequent experiments.

The following table shows the mean values for the 7 replications.

Diet	No. of puparia	Mean weight (mg)	Emergence (%)
A	85	37.66	76.47
B	91	37.39	81.13
C	132	35.43	70.45

Puparia yield is rather good for all three diets, but it is undisputably much higher in the diet containing the highest amount of egg yolk. This result may not necessarily be due to the higher nutritional value of this diet but rather to the fact that its characteristics are such as to induce the newborn larvae not to abandon the pabulum. This assumption is borne out by the fact that, as can be seen, the mean weight of the puparia is practically identical in all three diets and, moreover, similar to that of the puparia reared on the host, *Galleria*. The advantage afforded by the higher puparia yield in the diet containing the highest amount of egg yolk was however partly offset by the lower emergence rate.

As far as adult yield is concerned, that is in quantitative terms at least, it may be concluded that the three diets examined here give similar results (data not yet

available for a qualitative evaluation). Given that these diets are extremely easy to prepare, further research shall be carried out in an attempt to identify the optimum yeast/egg yolk ratio, which in any case can be expected to range from 1:1 to 1:3.

The results of these experiments are worthy of attention. In fact, a good production of *E. larvarum* is achieved by eliminating not only the bovine serum, which has been the basic ingredient of a large number of the diets tested, but also, at the same time, the host-derived material. The final pabulum thus prepared has an extremely simple composition as it contains only four ingredients, namely water, yeast, egg yolk, and trealose, plus gentamicine and agar as the gelling agent. As further confirmation of its validity, it is worth noting that in all three tests and in their various replications, adults often emerged even from defective (with wrinkled cuticle) or "dwarf" (with thin and yellowish cuticle) puparia.

In a subsequent experiment, repeated twice, another three diets were tested, which differed only in that 5% water was replaced with an equal amount of larval homogenate. This small amount of host material proved to be sufficient for obtaining a considerable increase in the mean weight of the puparia, which rose from about 35-37 mg to more than 50 mg, although puparia yield and emergence rate remained unchanged.

IV. Conclusions

As the bovine serum- and *G. mellonella* larva homogenate-based diets previously formulated by us (Mellini *et al.*, 1993a,b; Mellini and Campadelli, 1994a,b) were found to give good results, experiments were pursued in an attempt to identify alternative commercial ingredients which, in addition to being suitable, are also easily available and inexpensive. A series of subsequent experiments gradually led us to the formulation of very simple diets which were completely different from the starting one and devoid of host-derived material.

1. Replacement of *G. mellonella* larva homogenate.

This replacement may be made in various ways, primarily by using powdered *Bombyx mori* chrysalis preparations available on the market as animal feed or additives. Good results, however, were also obtained with ingredients not derived from insects; powdered yeast extract, which had already proven to be a very good additive in previous experiments, was found to be a satisfactory substitute for the homogenate as this was progressively eliminated. Egg yolk proved to be a less suitable substitute; in fact, albeit mixed in with the bovine serum, it was not able to prevent, when used in increasing doses, a progressive decline in puparia production and weight. It was seen that the development of the parasitoid larvae was unable to reach even the first moulting in the diet in which the homogenate had been completely replaced with egg yolk, even if the larvae managed to survive for over a month and to grow considerably in size. Finally, in diets containing a mixture of yeast and egg yolk in varying proportions, although an increase in puparia production and weight was observed when the amount of egg yolk prevailed, this was nevertheless accompanied by a decline in the emergence rate. To some extent, this finding may be accounted for not so much by the nutritional properties of the

substrate as such but rather by the excessive weights attained by the puparia, sometimes over 100 mg as compared to the mean weight of 35-40 mg for *in vivo* puparia.

It can be concluded, therefore, that the homogenate may be safely substituted by yeast and egg yolk, which are not derived from insects. In particular, mixtures of both these ingredients in equal parts proved to be especially good substitutes. Bratti and Mariani (1995) have also been able to eliminate host-derived ingredients from the pabulum for *Exorista*; by using liquid media for *in vitro* tissue cultures added with egg yolk, they were able to breed the parasitoid for 5 consecutive generations.

The elimination of host-derived material from artificial diets prepared for parasitoids is generally considered today an important step forward in this field of research, but, even if interesting and important from a theoretical point of view, it need not be from a practical one. In fact, the sharp interruption of the biochemical bond linking the parasitoid and the host, may have negative repercussions. For instance, the behaviour of the parasitoid in finding out the host may be adversely affected. It has in fact been demonstrated that the females of certain polyphagous Tachinids prefer attacking hosts belonging to the same species on which they have developed. It should be added that, in the case of *Exorista*, as with all other Tachinids which directly contaminate their host, the host must be reared in parallel so as to have a supply of larvae for the parasite to deposit its eggs. The females, in fact, usually attach their eggs to moving larvae, depositing them on inanimate objects, particularly those sticking up from the bottom of their cages, only after having been deprived of living larvae for long periods of time. We have as yet been unable to induce them to electively oviposit on inert substrates so that the eggs are spread out all over the bottom of the cages. Furthermore, the lack of the host may also lead to a decline in female fecundity. In view of all these drawbacks, then, and seeing that the host has to be reared in any case, the addition to the diet of small amounts of host-derived homogenate need not be considered as all that uneconomical.

2. Progressive replacement of bovine serum.

Puparia production and emergence rate were seen to tend to decrease as bovine serum was progressively eliminated in the presence of host homogenate as well as of egg yolk and yeast in equal quantities. These phenomena were particularly marked in the diets where the serum was completely eliminated. Mean puparia weight remained high in all the trials, ranging from 55 to 60 mg, values which are much higher than those recorded for *in vivo* rearing. On the basis of these experiments at least, it may be concluded that it is best not to replace the serum completely with water but rather to use it in quantities of around 20%. Notwithstanding these results, however, it should be borne in mind that the pollution occasionally observed at a more or less early stage and to greater or lesser extents in some of the trials, may have partially altered the final data despite the fact that four replications were made for each trial.

3. Total elimination of the bovine serum and progressive elimination of larval homogenate.

Puparia production was seen not to vary appreciably in diets devoid of serum, which had been replaced with sterile water, and containing yeast and egg yolk in equal quantities. Nevertheless, mean weight tended to diminish although remaining similar to that of *in vivo* reared puparia; a slight reduction in emergence rate was also observed.

4. Total elimination of bovine serum and larval homogenate.

In the final experiments of this study, both the basic components of the diet initially formulated by us for *E. larvarum* were completely eliminated. The new ingredients with which they were replaced were yeast and egg yolk which, in the first experiments of this study, had given good results both when used alone and together. Sterile water making up 78-80% of the whole diet was used as a diluent. In the presence of yeast or egg yolk alone (in addition to 2% trealose which was always present in the diets formulated by us), it was observed that, albeit surviving at length and even growing to considerable size and weight, the larvae were unable to develop to the 2nd instar.

Rather unexpectedly, the three different mixtures tested by us gave fairly similar results. Puparia production, however, tended to be higher in diet C, in which egg yolk prevailed, while the emergence rate was the lowest. Mean puparia weights, moreover, were practically the same for all three diets, ranging from 35 to 37 mg, which is rather low and similar to that recorded for rearing on *Galleria*. The addition of larval homogenate, even in the small amount of 5%, was however sufficient to determine an increase in mean weight by about 15 mg (46.35-52.49).

In conclusion, the oligidic diet originally formulated by us for *E. larvarum* may be profitably and variously integrated with powdered yeast extracts and/or fresh hen egg yolk which are capable at least of determining a considerable increase in puparia weight. Moreover, it is worth noting that these two ingredients together may be used to completely replace the serum and homogenate, representing by themselves (with 2% trealose) and in a mixture with sterile water (78%) a sufficiently satisfactory new diet. In fact, the egg yolk is a trophic source of the first order, being particularly rich in lipids like the larvae of host lepidoptera with a highly developed adipose body, while yeast is a fairly good substitute for the homogenate, being very rich in aminoacids and proteins.

It must however be noted that with this simplified trophic substrate puparia weights diminished, reaching the levels typical of *in vivo* rearing. Moreover, emergence rates also tended to decline more or less appreciably. This trend may be offset by simply adding small amounts of larval homogenate (5%). Recourse to the homogenate should not be considered a drawback since, as has already been pointed out, the host, *Galleria mellonella* has to be in any case reared in order to give a supply of larvae which is indispensable to induce the females of *Exorista* to oviposit. Moreover, the introduction of host material ensures maintenance of the biochemical link between the two symbionts. It may be observed that parasitoid production also tends to improve with the addition of small quantities of bovine serum (about 20%) but, for the reasons already given above, and even if the two ingredients cannot be considered wholly substitutes one for the other, the homogenate is to be preferred.

Finally, it is worth noting that despite the fact that many of the diets examined by us proved to be suitable and satisfactory, there is a substantial difference between the number of the eggs introduced (generally between 30 to 35 per dish depending on the trial) and the puparia production rate, which was usually around 50%. Not considering cases of mould or bacteria pollution, this loss is only partially due to hatching failure (only about 10-20% of the eggs do not hatch) and to larval mortality during growth, which is usually low. In fact, it is mainly due to the reluctance of the newborn larvae to penetrate into the trophic substrate as, unlike in the live host, they very seldom sink in in proximity to the corion. Under these artificial conditions, they often move around on the trophic substrate for shorter or longer distances, reaching the edge of the dish, where a good number crawl up the walls, especially if favoured by the formation of condensate, eventually reaching the lid where they die for lack of food after some days. Losses in production may be further aggravated by pabulum pollution, particularly if this occurs at an early stage, by insufficient pabulum density and, above all, by the presence of a thin liquid film on the pabulum surface which hinders the penetration of newborn larvae. In other words, the main reasons for losses in production are due to physical factors and not to nutritional ones associated with diet preparation methods.

SUMMARY

Starting from a standard diet based on bovine serum and larval homogenate prepared by us for *Exorista larvarum* (L.) and which had already proven to be satisfactory, other completely new formulations were prepared and tested employing widely available commercial ingredients for the purpose of obtaining the ideal oligidic diet.

The findings of the first set of experiments showed that *Galleria mellonella* L. host larva homogenate can be completely replaced with greater or lesser success with a number of other ingredients, namely: a) powdered *Bombyx mori* L. chrysalis preparations, b) powdered yeast extract, c) fresh hen egg yolk, and d) a mixture of the latter two ingredients diluted in bovine serum (75%). Mean puparia weight was seen to increase considerably when these ingredients were used, even in diets completely devoid of host material.

The second set of experiments showed that even bovine serum can be replaced, in the presence of the host homogenate (10%), by simply adding sterile water on condition that the diet contain appropriate amounts of yeast and egg yolk.

Both the bovine serum and the homogenate were successfully and completely eliminated in the third and fourth sets of experiments. In addition to the usual amount of trealose (2%) the diet finally obtained only contained water and the two aforementioned ingredients, the most satisfactory ratio of which appeared to be about 1:1. The adults obtained from this diet were fecund and perfectly capable of giving rise to a new generation of the parasitoid in the laboratory at the expense of *G. mellonella*. Moreover, puparia mean weight was equal to that of *in vivo* reared specimens, while the addition to the diet of even a very small amount of larval homogenate (5%) was sufficient to considerably increase puparia weight (by about 40%). Given the present rearing patterns, the addition of this ingredient does not entail any substantial increase in costs as in fact *Galleria* must be continuously reared anyway so as to have the supply of host larvae required to induce *Exorista* to oviposit; as with all oviparous tachinids which directly contaminate their host, *Exorista* deposits its eggs on inanimate substrates only in abnormal conditions and even then in an unselective manner.

The in-field effectiveness of the parasitoids reared in the laboratory on these simplified diets remains to be verified.

Ulteriori semplificazioni nella composizione delle diete per il parassitoide *Exorista larvarum* (L.).

RIASSUNTO

Col presente lavoro, partendo dalla soddisfacente dieta oligidica a base di siero bovino e di omogeneizzato larvale da noi messa a punto per *Exorista larvarum* (L.), si è giunti, per gradi, alla formulazione di diete completamente nuove a base di prodotti largamente presenti in commercio.

Nella I parte della sperimentazione si è constatato che l'omogeneizzato larvale dell'ospite *Galleria mellonella* L. può essere, con vario successo, completamente sostituito da: a) preparati polverulenti di crisalidi di *Bombyx mori* L., b) estratto in polvere di lievito di birra, c) tuorlo d'uovo fresco di gallina, d) miscele di questi due prodotti diluiti in siero bovino (75 %). Essi possono determinare un forte incremento nel peso medio dei pupari, pure in assenza di materiali provenienti dall'ospite.

Nella II parte si è accertato che anche il siero bovino può essere rimpiazzato, in presenza di omogeneizzato dell'ospite (10%), semplicemente con acqua sterile, qualora siano presenti congrue dosi di lievito e di tuorlo.

Nella III e IV parte sono state completamente eliminati con successo sia il siero che l'omogeneizzato, pervenendo così alla formulazione di una dieta costituita da acqua, dai due prodotti sopra menzionati nonchè dalla solita piccola dose (2 %) di trealosio. Il rapporto tra i due ingredienti al momento alquanto più soddisfacente sembra quello paritario. Gli adulti così ottenuti sono risultati fecondi e capaci di dare origine, in laboratorio, ad una nuova generazione a spese di *G. mellonella*. Il peso medio dei pupari è uguale a quello raggiunto *in vivo*; volendolo aumentare considerevolmente (attorno al 40 %) è sufficiente aggiungere alla dieta l'omogeneizzato larvale nella modestissima misura del 5 %. Tale addizione non costituisce al momento un aggravio di spesa, dovendosi comunque mantenere un allevamento continuato di *Galleria*. Le larve del lepidottero infatti sono indispensabili per ottenere una regolare ovideposizione da parte di *Exorista* che, al pari di tutti i Tachinidi ovipari a contaminazione diretta, depone su substrati inanimati solo in situazioni anormali ed in modo non selettivo.

Non è ancora stata verificata la validità in pieno campo dei parassitoidi prodotti con le dudette diete semplificate.

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