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Formulas for "inexpensive" artificial diets for the parasitoid *Exorista larvarum* (L.). * (1)

(Ricerche eseguite con il contributo del M.U.R.S.T. 40%)

INTRODUCTION

The present paper, the fifth in the series, reports a study conducted for the purpose of further improving artificial diets for *Exorista larvarum* (L.) in terms of preparation techniques and composition with a view to the mass production of this parasitoid for the biological control of defoliating Lepidoptera larvae.

In particular, given the success of previous experiments (Mellini et al., 1993a; Mellini et al., 1993b; Mellini and Campadelli, 1994a; Mellini and Campadelli, 1994b; Mellini et al., 1994; Mellini and Campadelli, 1995), the present study was conducted in order to improve the cost efficiency of the diets by employing widely available and inexpensive ingredients. Starting from the basic pabulum, which, in addition to water, comprises only three nutrients (egg yolk, yeast and trealose), attempts have been made to replace trealose with saccharose. Other yeast-based products were also tested in order to find a suitable and inexpensive substitute. The possibility of replacing at least in part the expensive yeast with soy flour or with powdered milk was also investigated and an attempt was made to partially replace egg yolk with soy flour, which is one of the cheapest ingredients available and already widely employed in animal rearing. A further objective of the study was to identify the ideal ratio of yeast to egg yolk, that is of the two main ingredients of the diets recently developed by us.

Sterile water has been generally used as a diluent. After our success, however, with powdered milk, wholly skimmed milk was adopted as the diluent, it being noteworthy also from a nutritional point of view as it contains various nutrient ingredients such as protein (3.2%), lactose (5%) and lipids (0.1%).

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(1) Studies on Diptera Tachinidae. LXVIII contribution.

Our experiments also entailed close control of the viability of the eggs employed especially after having noted a considerable difference between the number of cultured eggs and that of the puparia obtained (usually more or less below 50%), which was not accompanied by any evident mortality of the larvae in the pabulum. For this purpose, a number of *Galleria* larvae supporting tachinid's eggs were set aside and not used for obtaining the *Exorista* eggs employed in the experiment but grown in order to verify the formation of parasitoid puparia. A very high level of superparasitization was found in all samples so that the viability of the eggs and the full efficiency of *E. larvarum* after three years of continuous rearing on the factitious host *G. mellonella* were confirmed.

MATERIALS AND METHODS

The procedure was the same as that of the previous experiments. It is nevertheless worth remembering that as in past experiments the diets were added with ascorbic acid (0.01%) and gentamicin sulphate (0.03%).

Unless otherwise specified, four replications were made for each of the underlisted tests, each Petri dish being supplied with 30 parasitoid eggs. In case of considerable contamination being detected in the diet, the material was discarded and the trial repeated.

1. Replacement of trealose with saccharose.

The possibility was investigated of employing the widely used and inexpensive saccharose in place of the very expensive trealose so far employed both by us and by many other researchers in the preparation of artificial diets for the parasitoid. The possible advantage to puparia production of doubling the dose commonly employed of this ingredient was also examined given that there was no danger of the osmotic pressure increasing as the pabulum was "solidified" by agarization.

Diet formulation:

Diet	Water	Yeast	Egg yolk	Trealose	Saccharose
A	74%	10%	14%	2%	-
B	74%	10%	14%	4%	-
C	74%	10%	14%	-	2%
D	74%	10%	14%	-	4%

2. Yeast selection.

Given the high cost of the yeast extract (Sigma code Y-0550) so far employed (I), the possibility of replacing it with a less refined and much cheaper commercial product such as debittered yeast (II) or the even cheaper "standard" natural yeast (III) was examined.

Diet formulation:

Diet	Water	Type of yeast			Egg yolk	Saccharose
		I	II	III		
A	72%	12%	-	-	12%	4%
B	72%	-	12%	-	12%	4%
C	72%	-	-	12%	12%	4%

3. Replacement of yeast with soy flour.

Soy flour is a low-cost product with a high protein content quite often used in the preparation of artificial diets for insects in general, amongst which parasitoids. Part of the present study was aimed at investigating the possibility of replacing the undoubtedly effective but expensive Sigma yeast with this ingredient.

Diet formulation:

Diet	Water	Yeast	Soy flour	Egg yolk	Trehalose
A	76%	4%	-	16%	4%
B	72%	2%	6%	16%	4%
C	76%	-	4%	16%	4%

4. Replacement of yeast with powdered milk.

As replacement of yeast with soy flour proved unsuccessful, an attempt was made to replace the former with another low-cost product, powdered milk. Unlike for replacement with soy flour, in this case the yeast was not fully replaced with the substitute ingredient as yeast was found to be an essential component of the diet.

Diet formulation:

Diet	Water	Yeast	Powdered milk	Egg yolk	Saccharose
A	76%	8%	-	14%	2%
B	76%	4%	4%	14%	2%
C	76%	2%	6%	14%	2%

5. Replacement of water with wholly skimmed milk.

The replacement was attempted not so much because of cost efficiency reasons but because of the good results obtained by the partial substitution of yeast with powdered milk. Moreover, it appeared only natural to use the milk directly rather than powdered milk diluted in water as in the previous formulation. Skimmed milk was employed given that the lipid fractions are already largely accounted for by the egg yolk.

Diet formulation:

Diet	Skimmed milk	Yeast	Egg yolk	Saccharose
A	76%	8%	14%	2%
B	76%	6%	16%	2%
C	76%	4%	18%	2%

6. Replacement of egg yolk with soy flour.

This formulation was developed largely on account of its cost effectiveness. Wholly skimmed milk instead of water was employed as a diluent given the good results obtained with the previous formulation.

Diet formulation:

Diet	Skimmed milk	Yeast	Egg yolk	Soy flour	Saccharose
A	76%	6%	10%	6%	2%
B	76%	6%	8%	8%	2%
C	76%	6%	6%	10%	2%
D	76%	6%	4%	12%	2%

7. Determination of the yeast to egg yolk ratio.

The ratio of these two ingredients had already been varied in previous diets, which however employed other ingredients (as in diet No.5) or in which much larger intervals were adopted (Mellini and Campadelli, 1995). In the present formulation the two components were diluted in water and amounted to 28% of the total diet, the ratio between the two ingredients being varied in a complementary fashion and with small intervals.

Diet formulation:

Diet	Water	Egg yolk	Yeast	Saccharose
A	70%	14%	14%	2%
B	70%	16%	12%	2%
C	70%	18%	10%	2%
D	70%	20%	8%	2%

RESULTS

It should first of all be noted that considerable differences in the results of various replications were often recorded for the same diets. These differences are in part due to the inevitable small variations in the physical factors characterizing the pabulum despite the attention given to its preparation.

1. Replacement of trealose with saccharose.

The essential findings of the experiment are summarized in the following table:

Diet	Number of puparia	Mean weight (mg)	Emergence (%)
A	58	48.44	68.96
B	45	44.22	62.22
C	67	47.81	70.14
D	55	43.68	80.00

As can be seen, the number of puparia obtained in trials C and D, in which saccharose was employed, tends to be higher. As far as the amount of sugar is concerned, puparia yield tends to diminish in trials B and D for which the greatest dose (4%) of this ingredient was employed, either in the form of trealose or of saccharose. As regards the mean weight of the puparia, this can be seen not to have practically varied as a function of sugar quality and quantity. Finally, the

percentage of emergence calculated on the number of fully formed puparia, appears to be a little higher in the trials for which a higher dose of saccharose was employed.

It may therefore be concluded that ordinary saccharose, an easily available and cheap ingredient, can safely and satisfactorily be employed as a substitute for the more expensive trealose. Quantitywise, a dose of 2% would appear to be preferable at least in relation to the experimental diets tested by us in which puparia yield tended to be higher when this dose was adopted. In fact, an increase in the amount of the sugars employed was not found to have, at least under the conditions tested by us, the typical phagostimulating effect which this ingredient is generally considered to have on insects, a considerable number of newborn larvae coming to be dispersed on the walls and lid of the container.

2. Yeast selection.

Test results are shown in the following table.

Diet	Number of puparia	Mean weight (mg)	Emergence (%)
A	63	46.05	74.60
B	39	35.92	66.66
C	41	35.24	68.29

Puparia yield was higher in the pabulum containing Sigma yeast extract and lower, and practically the same, in the other two diets. Puparia mean weight was also greater for the Sigma yeast containing diet while lower, and virtually the same, in the other two. Emergence percentage, on the other hand, did not appear to differ significantly amongst the three diets. A finding which is not shown in the table but which nevertheless deserves attention is growth rate, which was seen to be markedly higher, especially during the first instar stage, in the Sigma yeast containing diet. In fact, the larvae on this diet had already reached their third instar stage while those on the other diets were still in their first. This pattern was found to hold in all replications. The greater effectiveness of Sigma yeast for all the vital parameters considered can be reasonably ascribed to its higher aminoacid content.

In conclusion, the use of the two inexpensive yeasts tested here is not convenient. Indeed, the use of the more expensive one is preferable and cost reduction may be attempted by employing it in as smaller doses as possible or by testing other equivalent but less expensive products.

3. Replacement of Sigma yeast with soy flour.

Test results are summed up in the following table.

Diet	Number of puparia	Mean weight (mg)	Emergence (%)
A	59	44.26	76.27
B	38	36.55	50.00
C	22	28.16	36.36

The number of puparia, their mean weight and emergence percentage are

markedly higher in the diet containing the greatest amount of yeast. In fact, in diet C, in which no yeast was present, not only is there a sharp drop in the values of all three of these parameters but the first instar growth rate is also much slower, thus determining a considerable delay in puparia development. The importance of yeast in all *Exorista* diets is therefore once again confirmed, its presence being all the more important in diets which do not contain any host homogenate.

Another important finding here is the fact that only relatively small doses of yeast are required (in the order of 4% as in diet A) for good results to be obtained. Even this small amount is probably sufficient to make up for the lack in the group of aminoacids present in the diet. It may therefore be concluded that notwithstanding soy flour's rich protein content it is insufficient to completely replace yeast.

4. Replacement of Sigma yeast with powdered milk.

The following table sums up the results obtained for the diets adopting such formulation.

Diet	Number of puparia	Mean weight (mg)	Emergence (%)
A	53	47.28	60.37
B	33	42.96	69.96
C	37	45.15	59.45

Halving the initial dose of yeast and making up the difference with powdered milk leads to a sharp drop in the number of puparia (diet B). Reduction to a quarter (diet C) does not result in any further drop in the number of puparia but larval growth is a few days longer especially owing to the delay in reaching full first instar development. As for the previous tests, the same pattern is observed here where yeast is used in too small amounts.

Mean puparia weight was found instead to be fairly much the same in all three diets; no substantial differences in emergence percentage, which tended to be low, being noted either. Several puparia appeared to be collapsed in all three diets, while a number of adults, albeit properly formed, were unable to open the cephalic cap of the puparium.

In conclusion, yeast was once more found to be an ingredient of primary importance, only puparia weight being satisfactory but not yield or growth rate when powdered milk was used as a substitute.

5. Replacement of water with wholly skimmed milk.

As can be seen in the following table, this substitution gave good results.

Diet	Number of puparia	Mean weight (mg)	Emergence (%)
A	56	47.42	78.57
B	64	51.23	67.18
C	58	53.69	70.68

The number of puparia appeared to be fairly good for all three diets, puparia weight and emergence percentage also being equally satisfactory.

Skimmed milk may therefore be considered a good diluent. It cannot however be denied that the good results obtained may also be ascribable to the considerable amounts of yeast used in the diets, which varied from 4% to 8% as well as to that of egg yolk, ranging from 14% to 18%. Nevertheless, skimmed milk may rightly be considered a good ingredient not only from a nutritional point of view but also thanks to its ease of use in the preparation of small amounts of pabulum for laboratory applications.

6. Replacement of egg yolk with soy flour.

The following table sets out the results obtained with these diets.

Diet	Number of puparia	Mean weight (mg)	Emergence (%)
A	51	39.72	80.39
B	39	37.12	92.30
C	26	31.23	84.61
D	23	19.52	65.21

The decrease in the dose of egg yolk, made up for by a corresponding increase in the dose of soy flour, led to a progressive drop in the number of puparia; mean puparia weight sharply diminishing in diet D which contained the lowest dose of egg yolk and the highest dose of soy flour. Similarly, emergence percentage, which was found to be high in the first three diets, showed a sharp decline in the last diet containing the lowest dose of egg yolk.

It may therefore be concluded that soy flour, albeit a very cheap ingredient already employed by other researchers (for instance, Nettles, 1986) for the preparation of parasitoid diets, cannot replace the more expensive fresh egg yolk as, when used beyond a certain limit, it leads to a drop in all *Exorista* parameters. A complementary trial was conducted in which soy flour was employed in small standard doses in all four diets, the results proving that such an ingredient can be used as a good food integrator. It should however be noted that even in this trial the reduction in the percentage of egg yolk tended to lead to a drop in mean puparia weights.

7. Determination of the optimum yeast to egg yolk ratio.

This issue has already been more or less directly dealt with in previous experiments conducted by us. Nevertheless, given its importance in the preparation of simplified diets employing water as a diluent and in which the two ingredients, together with small doses of saccharose (2%), constitute the only nutrient source, it deserves further discussion here.

Test results are shown in the following table.

Diet	Number of puparia	Mean weight (mg)	Emergence (%)
A	43	29.43	67.44
B	48	45.41	58.33
C	60	41.17	65.00
D	67	43.98	61.19

The diet which gave the worst results in terms of puparia number and weight is diet A in which the two ingredients are present in equal amounts, while that which gave the best results was diet D where egg yolk and yeast are mixed together in amounts of 20% and 8%, respectively. As puparia weights and emergence percentages are practically the same in the intermediate diets B and C and equal to those of diet D, it may be concluded that the smaller number of puparia does not so much depend on nutritional imbalances but rather on a lower capacity of the diet to attract first instar larvae which in fact exhibit a more or less marked tendency to abandon the pabulum.

On the basis of our findings, therefore, the ideal yeast to egg yolk ratio appears to be between 1:2 and 1:3. In any case, the emergence percentage for all these simplified diets was rather low due to the collapse of the newly formed puparia or to the inability of the adults to break through the cephalic cap. Integration of these diets with other ingredients, even if only in small amounts, appears therefore to be advisable.

CONCLUSIONS

In the fourth paper of the series, dealing with the preparation of artificial diets for *Exorista larvarum* (Mellini and Campadelli, 1995), a report was given of a successful attempt to replace the more expensive ingredients employed for these diets with cheaper ones. It was seen that a mixture of powdered yeast and fresh egg yolk can successfully be employed in lieu of the *Galleria mellonella* larva homogenate. Likewise, it was found that bovine serum, an expensive ingredient as it requires microfiltration, can be substituted with sterile water on condition, in this case, that the amount of nutrients be increased. Indeed, it was found that the homogenate and the serum could both be replaced in the same diet thus giving a much more simplified artificial diet for this tachinid than what would have been thought possible, comprising only three nutrients (yeast, egg yolk and saccharose).

The present study proceeded further along these lines in order to investigate the possibility of producing even more economic diets in view of *in vitro* mass production of this parasitoid, a polyphagous species antagonist of Lepidoptera larvae which are harmful to crops and forestry. As the ingredients of the diets were diluted in sterile water or wholly skimmed milk so that the diets were in a liquid state, they were agarized as usual.

The possibility of replacing trealose with a less expensive sugar such as glucose, was first of all investigated. It was found that the use of either of these two ingredients did not lead to any significant variations in the viable parameters considered. Indeed, the number of puparia tended to be greater on the saccharose diet, yield actually being found to be higher for both sugars when they were employed at the lower dose of 2% instead of 4%.

Next, given that yeast extract is a particularly expensive ingredient, three variously priced commercial preparations were tested. The least expensive ones, however, gave a poorer puparia yield both in terms of quantity and quality. The most appropriate yeast was found to be the most expensive one (Sigma code y-

0500) as, given its high aminoacid content, it was seen to considerably accelerate larval growth especially during the first instar stage. The essential importance of aminoacids in the diets of certain Tachinids has in fact already been reported by Nettles (1990). Moreover, Bonnet et al. (1991) have found that the total aminoacid content of the larvae of three species of the aforementioned family makes up 10% of their weight.

The progressive replacement of yeast with the very cheap soy flour caused a dramatic drop in the number of puparia, their mean weight and emergence rate. The number of puparia dropped to a third and their mean weight and emergence rate to about half when yeast was completely eliminated.

The gradual replacement of yeast with powdered milk had less dramatic consequences. In fact, mean puparia weights and emergence rates did not change for all diets. Only the number of puparia dropped considerably in the pabulum in which the dose of yeast had been reduced from 8% to 4% and 2%.

The use of skimmed milk in lieu of sterile water as a diluent for considerable doses of yeast (8% → 4%) and of fresh egg yolk (14% → 18%) gave good results for all diets. The highest number of puparia and the highest mean weights were in fact obtained under these conditions. Given these findings, the use of bovine serum (which is relatively rich in proteins) as a diluent of solid nutrients in the preparation of artificial diets for *Exorista* was subsequently replaced first with sterile water (which has no nutritional value) and then with skimmed milk (which is relatively rich in nutrients but much less expensive than bovine serum, the latter requiring considerable time for its extraction from blood and especially costly sterilization filters).

Attempts were also made to replace egg yolk with the much less expensive soy flour. The progressive increase in the doses of the latter associated with a corresponding decrease in those of egg yolk, however, led to a rapid decline of all the three viable parameters examined by us.

It may be observed that soy flour, which has a high protein content, has been successfully used by other researchers (see, for instance, Nettles, 1986). Notwithstanding, it must be noted that in such cases it was used as an addition and not as a substitute. The present authors (Mellini and Campadelli, 1994b) have themselves obtained good results by adding this ingredient in doses of 5% to bovine serum-based diets in order to enhance their nutrient content. It is worth stressing again that egg yolk, added by us in doses of up to a maximum of 20%, always proved to be an excellent ingredient. Other researchers, such as Stand et al. (1988) have even used it in doses of up to 35% for an oophagous species, the Scelionid *Telenomus heliothidis* Ashm.

Finally, experiments were conducted by us to determine the optimum yeast to egg yolk ratio in simplified diets containing only these two nutrients (in addition to small doses of saccharose) diluted in sterile water. Similar experiments had already been conducted by us (Mellini and Campadelli, 1995) but using smaller doses of the two ingredients (a total of 20% instead of 28%) and greater ratio intervals. In terms of puparia numbers and mean weights, the best results (at least for the doses tested) were obtained by increasing the dose of egg yolk and correspondingly decreasing that of yeast. As far as adult emergence rates are

concerned, these were found to be rather low so that integration of the above diets with other nutrients, even if only in small doses, is advisable.

Summing up, puparia and adults were obtained in all the thirty tests conducted in the course of the present experiment, albeit with considerably different results. Moreover, depending on the diet used and on its degree of compactness, growth rates were identical or slightly greater than those reported for *in vivo* rearing. In the majority of cases, mean puparia weights were sensibly or even much greater than those obtained on the *Galleria* host. These results show that the parasitoid is highly adaptable to a variety of artificial diets. Tolerance to the physical characteristics of the pabulum, especially to its compactness, however, was not as good. In fact, if the pabulum is too soft, the newborn larvae tend to sink into it, growing with difficulty and finally dying from asphyxia, while if it is too compact the larvae have difficulty in feeding and tend to dig long galleries on the bottom of the Petri dishes, which delays their development into pupae and leads to the growth of undersized puparia. As is known, the larvae of Cyclorhaphous Diptera feature an oesophagus with a very small diameter so that they can only feed on fluids (see, for instance, Gardenghi and Mellini, 1995); albeit having a partial extraintestinal digestion, if the substrate is too compact and cannot be sufficiently fluidified, feeding is hindered so that all biological parameters are adversely affected.

A general finding of this study which in various degrees has also been recorded in previous ones, is the relatively low puparia yield vis-à-vis the number of eggs employed, which rarely reaches 50%. This low yield is only to a very small extent due to the failure of the eggs to hatch (usually around 10%) or to larvae mortality during growth, which is practically negligible; in fact, it is largely caused by the marked tendency of the newborn larvae to abandon the pabulum on which the eggs have been deposited. The newborn larvae initially wander at length on the diet, while a considerable number of them finally climb up the walls of the Petri dish reaching the lid where they starve to death a few days later. Indeed, doubling of the dose of saccharose, which is generally considered an ideal phagostimulant for insects, resulted in this adverse effect being enhanced. The primary objective of a future study on this subject will therefore be to investigate the means for preventing this massive exodus of the newly hatched larvae so as to maximize puparia yield.

SUMMARY

The present paper is the fifth in the series of our studies dealing with the *in vitro* rearing of *Exorista larvarum* (L.). In particular, its purpose was to devise ever more inexpensive artificial diets based on the ones which had already given satisfactory results in the previous studies. It was found that trealose can be successfully replaced by the much cheaper saccharose at the same dose of 2%. As regards yeast, which, in the absence of host homogenate, as in our experiments, is an indispensable ingredient, the only alternative product which was found to give good results was the one with the highest aminoacid content which, however, is much more expensive than the others tested. Soy flour, which is a very low-cost ingredient, can only partially substitute yeast. As far as yeast substitution is concerned, better results were obtained with powdered milk. Partially skimmed milk used as a diluent of solid nutrients in place of water gave optimum results and permitted to halve yeast doses. Beyond a certain limit, the progressive replacement of egg yolk with the less expensive soy flour led

to a drop in all viable parameters, thus confirming the great importance of the former ingredient as a component of artificial diets. As regards the egg yolk to yeast ratio in diets containing only these two nutrients to a total amount of 28% (in addition to 2% saccharose) diluted in sterile water (70%), the best results were obtained with a 2:5 ratio.

In all the diets tested, more or less numerous puparia were formed, often exhibiting mean weights far greater than those reported for *in vivo* reared specimens. Moreover, albeit at different rates depending on the diets, adults always emerged, thus confirming *E. larvarum*'s considerable adaptability to artificial diets, even the most diverse. This characteristic accords well with the polyphagous nature of the parasitoid. The adults obtained even on the simple diets not containing any host material were capable of reproducing themselves and giving rise to a fully viable new generation at the expense of the factitious host, *Galleria mellonella*. The ability of the females to successfully attack this host continued for several days.

Puparia yield vis-à-vis the number of eggs employed is currently below 50%. This gap is not so much due to the mortality of the developing larvae but mostly to the fact that many newborn larvae tend to abandon the trophic substrate on which the eggs had been deposited, dying then from starvation. This tendency is to some extent favoured by the fact that the eggs are dehiscent so that the newborn larvae hatching before the corion come to find themselves directly free on the pabulum surface.

Formulazioni economiche per le diete artificiali del parassitoide *Exorista larvarum* (L.).

RIASSUNTO

Con questo nostro quinto lavoro, sull'allevamento *in vitro* di *Exorista larvarum* (L.), abbiamo tentato di rendere ancora più economiche le precedenti diete artificiali, che già avevano dato soddisfacenti risultati. Si è accertato che il trealosio può essere efficacemente sostituito dall'economico saccarosio nella stessa dose del 2%. Per quanto riguarda il lievito di birra, che è un prodotto indispensabile in assenza di omogeneizzato dell'ospite, come nel caso della presente sperimentazione, è risultato valido solo un prodotto caratterizzato da un discreto contenuto di aminoacidi; esso, però, è assai più costoso degli altri. La farina di soia, che è molto economica, può sostituire solo in parte la dose di lievito. Migliori risultati, in questo senso, ha dato invece il latte in polvere. Il latte parzialmente scremato, poi, usato come diluente dei nutrienti solidi in sostituzione dell'acqua, ha fornito ottimi risultati ed inoltre ha consentito di dimezzare le dosi di lievito. La progressiva sostituzione del tuorlo d'uovo, con la più economica farina di soia, ha portato, oltre un certo limite, ad una flessione in tutti i parametri vitali, confermando la grande importanza di quel componente nella dieta. Per quanto riguarda il rapporto quantitativo tra tuorlo e lievito, in diete costituite da questi due soli nutrienti per un totale del 28% (oltre al 2% di saccarosio) diluiti in acqua sterile (70%), i risultati migliori, nell'ambito dei rapporti sagggiati, si sono ottenuti con valori attorno al 2,5.

In tutte le diete qui sperimentate si sono formati, più o meno numerosi, i pupari, sovente con medie ponderali assai superiori a quelle degli individui sviluppatasi *in vivo*. Inoltre, sia pure in diversa misura secondo le tesi, sono sempre sfarfallati gli adulti, confermando così la grande disponibilità di *E. larvarum* ad essere allevata sulle diete artificiali più varie, in accordo con la sua grande polifagia. Gli adulti ottenuti anche dalle diete più semplici, e prive di materiali dell'ospite, sono stati in grado di riprodursi e di dare origine ad una nuova generazione pienamente vitale a spese dell'ospite di sostituzione *Galleria mellonella*. La capacità delle femmine di aggredirlo con successo si è protratta per vari giorni.

La resa in pupari, rispetto alle uova impiegate, è al momento, inferiore al 50%. Tale scarto non è però dovuto a mortalità delle larve in accrescimento bensì, in larghissima misura, alla tendenza di numerose larve neonate ad abbandonare il substrato trofico, sul quale erano state trasferite le uova, con conseguente morte per inedia. Certamente tale tendenza è favorita anche dal fatto che le uova sono deiscenti, per cui le larvette, sgusciando davanti al corion, si trovano automaticamente libere sulla superficie del pabulum.

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