

***In vitro* rearing of entomophagous insects - Past and future trends: a minireview**

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Abstract

In vitro rearing of entomophagous insects (IVREI) can be conducted with two main aims: firstly to develop an efficient tool for researches on insect biology, physiology, and behaviour, secondly to mass-produce insects for applied use. It was conceived in the 1940's, but really investigated from the 50's. Several teams, including less than one hundred main researchers, distributed among about a dozen countries, were implicated in these investigations. Hymenoptera, Diptera, Rhynchota, and Coleoptera are the principal orders of insects studied. The best results were obtained with egg or pupal hymenopterous parasitoids, tachinid larval parasitoids and some polyphagous predators. The future research structures will need the constitution of national or supra-national consortiums between public and private institutions to share financial means and research teams. The species that are expected to be developed in artificial conditions for biological control are on one hand species-specific parasitoids to reduce unexpected side effects, and on the other hand, polyphagous predators to simultaneously control several pest insects. The future of the production will go through more automation and quality control, to reduce the costs of the insects produced and guarantee reliable efficiency. To reach these objectives, the investigations need development towards innovative tools to define more adapted diets and to find new efficient quality criteria.

Key words: artificial diet, artificial medium, parasitoid, predator, research tool, mass production.

Introduction

Insects represent the main group of animals in the world, and their study is well developed, especially because of their ecological, economical and medical impacts. To facilitate this study their rearing was developed in a very practical way for many species (Singh and Moore, 1985). The knowledge, or better, the control of the nutrition is a key factor for their rearing, and consequently for any study on insects.

As underlined by Allen C. Cohen (2001), a specialist of insect nutrition, except of some disciplines, such as systematics and field-oriented subjects, most insect studies are dealing with laboratory-reared insects, and most of these studies incorporate insects reared on artificial diets. In fact, the rearing conditions and the diet composition are critical parameters in any case. To draw reliable conclusions from experiments, the nutrition of the insects tested has to be perfectly known and suitable, because the answers will depend on this. Probably the vision of Knipling E. F., 1979 (in Cohen, 2001) is not completely realistic, but could be a goal for specific applications: "Mass rearing of insects is still a young science. With the help of insect geneticists, insect nutritionists, and insect behaviourists, insects might be reared under conditions that will make them equally, if not more, vigorous and more adaptable to the environment than the wild population. These improvements are likely to occur after further experiences and research." Artificial rearing entails to fulfil nutritional needs but also the other physiological needs, such as suitable physico-chemical environment, respiration, excretion, especially for parasitoids (Grenier *et al.*, 1986).

All these introductory remarks are true for any insect reared outside natural conditions, but the paper will be restricted to *in vitro* rearing of entomophagous insects (IVREI). After a short explanation of the different uses

for insects produced in artificial conditions, and a brief survey of the historical steps of the researches in this field, some possible trends for the future will be presented.

The two main aims for *in vitro* rearing

In fact the artificial rearing could be used for many purposes that could be grouped into two general objectives: academic research and practical use.

An efficient tool in academic research

This method (IVREI) represents a very efficient tool for biological, physiological and behavioural studies because we can act directly on the parasitoid or predator itself, without interaction with the host or prey, or any uncontrolled environment; this is especially true for endoparasitoids (Grenier, 2000). Here are some examples of investigations already carried out or possible, thanks to IVREI. Thus, it is possible to conduct precise studies of development allowing determination of the size of each stage, the number of larval instars (Grenier *et al.*, 1975). Studies of the food intake and of the nutrition have been developed, generally speaking (Jarjees *et al.*, 1998), using well balanced versus deficient or suboptimal diets (Yocum *et al.*, 2006). Chemicals or any molecule incorporated into the diets could be easily tested, either to evaluate their effects as toxic compounds or transgenic products (Bell *et al.*, 1999), even as conditioning factor for behavioural learning (Greany and Vinson, 1984), or to investigate pesticide resistance and strain selection (Li L. Y. and Liu W. H. personal communications). Also sensitivity versus tolerance to antibiotics, antifungal agents, and any antimetabolite could be assessed (Grenier and Liu, 1990; Alverson and Cohen, 2002; Büyükgüzel and Icen, 2004). Studies of

the different steps of the egg laying and possibly stimulation by chemicals for enhancing oviposition behaviour were conducted (Grenier *et al.*, 1993; Grenier, 1994; Marchetti *et al.*, 2008). More recently, many analyses of symbiotic relationships between some bacteria or rickettsia and entomophagous insects were developed to evaluate the role of the symbionts on the host physiology, possibly by transfer of these symbionts between species. For example in the study of *Wolbachia*, it was shown that these symbionts affect the reproduction status and the fecundity in *Trichogramma* (Grenier *et al.*, 1998; 2002). This point is important and is supposed to have a great impact on the "quality" and efficiency of entomophagous insects as discussed below. It is well known that artificially reared insects are used for purposes other than research papers.

The mass production for practical use

The mass production for utilization of parasitoid or predator insects in biological control strategies is the most evident aim. There are some advantages such as simplification of the production line, higher flexibility of the production, reduction or suppression of allergy problems generated by moth scales (from factitious hosts or prey), lower costs expected with scaling up effect. But, insect colonies are also used by many commercial companies for pesticide testing, production of insect derivatives (pharmaceutical products). Of course there is a growing sector concerning the multiplication of beneficial insects. Especially the bio-control strategy using inundative releases needs huge quantities of insects.

The basic food chain for entomophagous insects is the "natural" tritrophic system, with plant, pest insect (host or prey), and parasitoid or predator. One of the first ideas to improve this production line was to try to reduce the number of compartments. A first step consisted in using bitrophic systems, with two possibilities of substitutes. Artificial food could replace plant to feed host or prey insects: there are many examples with lepidopterous or coleopterous larvae used as hosts for parasitoid rearing. Also alternative host/prey, easier to produce in laboratory conditions than natural ones, could be used, such as coleopterous larvae of *Tenebrio molitor* L. and lepidopterous larvae of *Galleria mellonella* (L.), or eggs of *Ephesia kuehniella* Zeller and *Sitotroga cerealella* (Olivier). Most of the current commercial productions of entomophagous insects in the world involve a tri- or bi-trophic system.

The final solution to shorten the production line would be the availability of an artificial medium/diet allowing larval and pupal developments up to adult emergence, and possibly egg laying for parasitoids. Many examples could be given at a laboratory level, but very few as an actual mass production. *Trichogramma* spp. (Hymenoptera Trichogrammatidae) produced on artificial host eggs in China were widely tested on 1600 ha sugar fields, 1500 ha litchi trees, and 1000 ha of various other crops with good control of pest insects (Liu *et al.*, 1995; Zhang Z. L., personal communication). *Catolaccus grandis* (Burks) (Hymenoptera Pteromalidae) is another example of parasitoids produced *in vitro* in USA and tested in cotton fields (Morales-Ramos *et al.*, 1998).

In vitro rearing in the past - a brief survey

Long time ago, scientists started to study the possibility to rear entomophagous insects *in vitro*, on artificial diets. The first attempts concerned very simple artificial diets (piece of insects or crushed insects, pet's food, processed meat) to feed polyphagous or non-strictly entomophagous insects (such as Sarcophagidae). In fact, there are many kinds of diets that could be classified according to their composition. Nowadays, the presence or absence of insect component is a main discriminatory criterion. Here are some main steps of the research progress.

The possibility to use unnatural hosts or artificial diets for rearing entomophagous insects was proposed as early as 1944 by Simmonds. The first attempts for hymenopterous and sarcophagid parasitoids were reported in the 1950s, (House and Traer, 1948; House, 1954; Bronskill and House, 1957). Many other papers were published by House on *Agria affinis* (Fallén) / *A. housei* Shewell (Sarcophagidae) between 1960 and 1970. In 1970, Yazgan and House published the first chemically defined artificial diet for rearing an hymenopterous parasitoid *Itopectis conquisitor* (Say). In the following years, 1970' and 1980', efforts increased considerably to develop artificial culture methods, for parasitoid as well as for predator insects. Again Hymenoptera were concerned, including larval, pupal and egg parasitoids (mainly Braconidae, Chalcididae, Ichneumonidae, Pteromalidae, Trichogrammatidae), but also Diptera (Tachinidae), Coleoptera (Coccinellidae), Neuroptera (Chrysopidae), Heteroptera (Anthocoridae, Lygaeidae, Miridae, Pentatomidae). Generally speaking, the main successes were obtained for hymenopterous egg and pupal parasitoids, tachinid larval parasitoids and some polyphagous predators.

Many successes representing partial or complete development from eggs (or first instar larvae) to adults were obtained in different countries. Numerous people working in about a dozen of key-countries were implicated. Several reviews on this topic were published, among them: Mellini, 1975; Campadelli and Dindo, 1988; Grenier *et al.*, 1994; Thompson, 1999; Thompson and Hagen, 1999; Riddick, 2008.

From the 1990's, it appeared a stabilization of the works about artificial diets, but many teams are still working on the subject in present time. Meanwhile, the development of reliable methods for *in vitro* rearing of beneficial insects using artificial diets or media is a demand of producers more and more clearly expressed these last years. Perhaps as a consequence, we observed nowadays a change in the objectives of the researches about artificial rearings.

In the past, of course, even if one of the final applied objectives was the production of entomophagous insects for biological control releases, the short term objective was mainly academic results aimed to obtain a better knowledge of the conditions required for the artificial rearing. Probably in relation with the difficulties for obtaining public supports, some researches are now more applied oriented, and in USA for example the results are sometimes patented, even if very rarely used at the industrial level.

Moreover, the demands from producers' companies are insistent even if, may be because of, the companies have not so much money to develop researches by themselves. In fact, many companies selling insects are small and miss an actual R & D branch. We have now about one hundred species of predators and parasitoids that could be reared on artificial diets or media.

Trends for the future

There are several directions in which the studies about IVREI could be put forward. Some perspectives have already been considered (Consoli and Parra, 1999). Critical fields concern the framework of the research, the choice of the species to promote, and the production process.

Future research structure

In the future, as it is already working for other research fields, the main way for developing researches on artificial rearings will be the constitution of "consortiums" including universities, public research institutions and private companies to elaborate research programs in the view to share their means (financial, research staff) and to obtain support from government or supra-national commissions (i.e. the European Commission). As an example, in the context of successive research programmes, in a European contract and thesis projects, some partners from France, Belgium, Italy, and Spain have developed a methodology which combines an iterative analytical approach of the composition of foods with biological (duration of different developmental stages, weight, longevity, fecundity) and biochemical (composition in amino acids and fatty acids) evaluations of the insects thus produced (FAIR6-CT98-4322).

Nevertheless, it is regrettable that the protection system of the knowledge (competition, secrecy, licences, patents...) seriously hampers the exchanges between actors and slows down the improvements. Moreover, it has to be pointed out that some research fields needing high investments but with non-immediate and regular returns must be supported by public governmental institutions. A clear example is the biological control by acclimatization of an entomophagous species to control a new non-indigenous pest. It is also true for any biological control strategy of local importance not attractive enough for industrial business. Farmer associations could also support such a program in critical situations, as it was done in the past in California with the coccinellid *Rodolia cardinalis* (Mulsant) against the scale *Icerya purchasi* Maskell.

Future species of entomophagous insects

Concerning the most promising species to be reared there are two approaches. On one hand, the demand concerns species-specific insects to limit the side effects of biological control. These beneficials are not necessarily easy to rear. The examples are mainly parasitoid insects, such as *Trichogramma* (or other egg parasitoids) or some Hymenoptera parasitizing aphids (Aphelinidae Aphidiidae). Tachinidae are also good examples even if some of them are polyphagous. On the other hand, the

producers are looking for more polyphagous predator insects that could manage several pest insects at the same time. Coccinellids are good examples, but also chrysopids (Neuroptera) or some Heteroptera. Bugs are interesting in protected cropping systems, because the zoo-phytophagy of some Mirids is favourable to a good survival of the insects in early season even in the absence of prey. They have also a good tolerance to low temperatures. In any situation, under close protected cropping systems, unexpected side effects of biological control are very limited.

Future of the production

Mass rearing and production of entomophages is hampered by different bottlenecks (Cohen *et al.*, 1999). Two main trends could be put forwards for the next years.

A u t o m a t i o n

To reduce the cost, the process for multiplication of insects needs to be simplified, for example with less plant utilization, and more mechanised steps to make easier the automation of the production line. In China, the mass production of *Trichogramma* started long time ago (Hubei Coop. Research Group, 1979) and is now feasible thanks to an automatic machine designed by the Guangdong Entomological Institute (Guangzhou, China) and manufactured in 1997. This improved model "Auto-Ova-Make 2" for making artificial host-eggs automatically for *in vitro* rearing of *Trichogramma* spp. and *Anastatus* spp. could produce up to 13 millions *Trichogramma* or 0.3 million *Anastatus* per day. Production of artificial preys for Chrysopidae was developed long time ago in the former USSR. Some techniques and processes, already available for phytophagous insects, could be transposed and adapted to entomophages. Great efforts have to be done to develop automation, with more efficient cooperation between researchers and engineers.

Q u a l i t y C o n t r o l

Besides the classical considerations for improving IVREI, it appears more and more important to take into consideration the "Quality Control" and "Quality Assurance". These concepts were first developed for insects in general, and progressively adapted to entomophagous ones (Leppla and Ashley, 1989). To develop any use of insects and especially in biological control strategies, the products (entomophagous insects) delivered to the end users (companies, technicians, farmers...) must show stable and well defined characteristics to ensure a high reliability in their use.

But, what means quality?

The quality is not an absolute concept, but different criteria could be defined in relation to the objectives for which insects are produced. The degree of difference between insects produced *in vitro* and *in vivo* that can be accepted, will depend on the goals of the production. For example, in inoculative releases (acclimatization) for which the establishment of the insect colony in the field is required for a long time, the quality parameters are evidently different from those for inundative releases

with expected effects of the first generation mainly: well adapted strain, high genetic variability, long term survival... on one hand, vs. high immediate reproductive potential, good dispersal, high predation/parasitization efficiency, on the other hand. In Sterile Insect Technique as well, the compatibility with the local population of the males released in the field, and their sexual competitiveness are key parameters. Thus, different criteria with various importances according to the final use of the "insect product" could be defined to control the quality.

Which critical parameters to check?

As said above, it is necessary to have a biological material (insects) of good quality and in "good health" for any study, for any use. Illness is not easy to detect in insects, but an estimation of the morbidity of a population could be done by the evolution of the mortality rate during a given time period. To verify the "value" of the insect, besides some evident characteristics, such as right species identity and stages as announced on the label, right number of insects in the box, other qualities have to be checked. For this purpose it is necessary to find and define different reliable criteria as far as possible easy to measure or evaluate. Some criteria were already defined and some of them are in a current use.

A general survey of the criteria for Quality Control of parasitoids and predators produced in artificial conditions were given by Grenier and De Clercq (2003). We can distinguish several sets of parameters. The morphological parameters mainly include size or weight of different stages, and also percentage of abnormalities. Among the development and reproduction parameters, we could find survival rate, durations of the different stages (egg, larva, pupa, adult), and sex ratio, fecundity, longevity, durations of egg laying phases in adult. There is a special mention for presence/absence of symbionts playing roles in reproduction or nutrition. The biochemical parameters are not so commonly checked, but should be important, such as protein, lipid, carbohydrate contents, even hormone titre. The behavioural parameters are often integrative criteria and thus critical for practical use in biological control, such as predation or parasitization efficiency. Host or prey localization capability, as well as locomotion or flight activity, are also of prime importance. The genetic parameters, not yet currently used, concern genetic variability and homozygosity rate. In the present days, the quality control or quality assurance of entomophagous insects is an unavoidable step in the use of biological control strategies for crop or animal protection. The producers themselves are demanding regulation to dictate some kinds of ethics inside insect trade, which is the only way to ensure a good image of the method, and an increased use of biological control strategies. Scientists are also asked to conduct some researches on this topic, to try to improve the parameters already used for evaluation, or define new ones.

Future directions for researches

Among different topics needing investigations, the incomplete knowledge of nutrition is a key point that hampered rearing and production of entomophagous insects. As already explained by many scientists, and con-

trary to the thinking of some obstinate decision-makers in research institutions, insect rearing is not only a technical application but a true basic research field to settle at a front place. Both academic and applied studies are required to guarantee success in IVREI, and contribute to development of biological control. For improvement of artificial diets, the nutrigenomics, a very innovative tool already in progress, has to be developed in a near future (Coudron *et al.*, 2006). For quality control of entomophagous insects produced, some biochemical criteria such as protein/lipid contents or level in specific protein, need to be extended by chemical analyses or immunoassays (Shapiro and Ferkovich, 2002; Zapata *et al.*, 2005; Dindo *et al.*, 2006; Sighinolfi *et al.*, 2008). The development of new quality control parameters, such as asymmetry (Ueno, 1994; Hewa-Kapuge and Hoffmann, 2001) could be suggested. Extensive investigations about the symbiont-host relationships, and their subsequent effects on nutrition, reproduction and quality parameters would be expected.

Conclusions

Lot of works have been done on artificial rearing of entomophagous insects, but essential improvements are needed to develop their use mainly outside the protected crops towards open fields and orchards. To preserve the huge quantity of results already brought for years by many governmental and some private institutions in this field, research efforts need to be carried out. The current thinking favourable to organic agriculture and more and more suspicious to chemical pesticides should be a powerful incentive to push forward IVREI and biological control. Moreover this Insect Pest Management method perfectly fits the sustainable development, nowadays a key concept for all human activities.

Inside the International Organization of Biological Control, the global Working Group named AMRQC "Arthropod Mass Rearing and Quality Control", is coordinating the common efforts of investigators, insect producers, regulators, and end users, in order to try to improve production and quality of insects (website: <http://www.amrqc.org>).

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