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Invited Paper

Artificial Rearing of Entomophagous Insects, with Emphasis on Nutrition and Parasitoids - General Outlines from Personal Experience

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Abstract

The disposability of high numbers of entomophagous insects for their use in biological control strategies is an old objective carried on, between others, by the rearing in different kinds of artificial diets. The definition and the perfecting of these diets are at first based on nutritional studies. Food or carcass analyses, nutritional balance studies, and deletion/supplementation methods allow to evaluate the different nutritional needs. Nitrogen sources are the most critical needs especially with fast growing entomophagous insects. Lipids and carbohydrates mainly constitute energy sources with storage possibility. Other nutrients such as salts and vitamins are also needed, and sometimes some specific polyunsaturated fatty acids. There are other important physiological requirements, concerning the digestion process, the respiration, or the hormonal balances. Physico-chemical factors such as Osmotic Pressure and pH often show critical values for the normal development, mostly of endoparasitoid insects. For egg parasitoids and predators, the presentation of the food is of prime importance for a normal food intake, and finally for the success of the rearing. Sterilization of the diet or incorporation of preservative agents is often necessary for preventing bacterial or fungal contaminations. Many successes were obtained for artificial rearing of entomophagous insects, but ultimate efforts are still needed for crucial improvements and supports that may lead to the extension of the use of this biological control strategy.

Keywords: Artificial rearing, Entomophagous insect, Parasitoid, Predator, In vitro, Nutrition, Food, Diet, Medium, Nutriment, Nutritional need

1. Introduction

The artificial rearing of parasitoid insects started a long time ago, with the main goal to try obtaining a mean to multiply and produce parasitoids to be released in biological control strategies. But it is also a powerful tool to conduct studies on biology, physiology and behaviour of entomophages, especially endoparasitoid species (Grenier 2000).

There are different appellations for the preparations used in artificial rearing. In this paper, "medium" will be preferably employed for parasitoids, and "diet" for predators. Food will represent the general term. Different kinds of foods have been tested, from very simple preparations (crushed lepidopteran pupae, piece of beef liver or meat…), to chemically defined media. Several categories of food could be described, and different nomenclatures were proposed. One of the most known classifications is from Dougherty 1959, with the terms of holidic, meridic and oligidic, mainly based on the presence or absence of complex components. The latter being not easy to typify, the distinction between the 3 terms is not very relevant. In fact, only a complete description of the composition would be able to characterize an artificial food. Nevertheless for practical considerations, the presence or absence of insect-derived components (hemolymph, tissue homogenates or extracts, egg juice) is a critical characteristic, leading to operate a simple distinction between food with or without insect components. The type of diet does not prejudge of its performance for insect rearing, but may influence the accuracy of the experiments (Grenier 1986). For a complete success in rearing entomophagous insects in artificial conditions, all their physiological requirements have to be fulfilled, but nutrition is one of the most critical functions. Generally speaking, any non-natural food used to produce an insect for laboratory studies, could be well known in its composition to draw reliable conclusions of the achieved tests.

This paper is mainly based on a 40 years' personal experience on the rearing of entomophagous insects in artificial conditions for preimaginal development. Thus, most of the bibliographic references are from the author. For covering a subject, as far as possible, the most recent references, preferably review papers were given. For other general data, the readers have to consult these key references: Mellini 1975, Thompson 1999, Thompson and Hagen 1999, Cohen 2004.

After a brief presentation of the various ways to determine the nutritional requirements, these latter will

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be described, as well as some other key physiological requirements. Then, important parameters of the food, its presentation and preservation will be described, before to conclude.

There are different types of nutritional needs, and they are variable according to the stage and the physiological state, usually higher for larvae than for adults, except during reproduction. We can discriminate, general needs for basic metabolism, needs for development and needs for reproduction. In this paper, only the preimaginal development will be considered.

2. Evaluation of nutritional needs

Various analytical approaches were employed to try to determine the different requirements and define the food composition for entomophages.

2.1 Food analysis

For example, the biochemical analyses of host or prey could be conducted in their amino acid contents after total or partial hydrolysis. Fatty acids or carbohydrate analyses are also often achieved. The results have to be expressed in total amounts per weight or in percentages as relative values (patterns) useful for establish the quantity for each component of the food. Examples could be found for Tachinidae (lepidopteran larvae), Trichogrammatidae (lepidopteran eggs), predatory bugs and coccinellids (aphids, lepidopteran eggs, *Artemia* cysts).

With parasitoids, the main difficulty lies in the necessity to know which exact part of the host is ingested in the course of the development (hemolymph, body fluids, whole body or egg content...). To make fruitful comparison, host and non-host species could be studied as well (Barrett and Schmidt 1991, Yazlovetsky 1992).

2.2 Carcass analysis of whole parasitoid/predator body

Instead of host/prey, the same type of analysis could be done with the entomophagous species itself. There are many examples with Tachinidae, *Trichogramma*, *Macrolophus*, *Dicyphus*, *Orius,* and *Harmonia*... (Bonnot et al. 1976, Grenier et al. 1989, Bonnot et al. 1991, Grenier et al. 1995, Specty et al. 2003, Vandekerkhove et al. 2009). But, to establish the exact composition of the food, it is necessary to take into account the intermediate metabolism. Moreover, the existence of catabolism and inter-conversion metabolism impede from knowing the exact form (mono or polymers) of each component to incorporate into the food. One advantage of these carcass analyses is also to give an evaluation of the quality of an insect grown on artificial food by comparison with insect grown on control food, and to allow improving the composition of this food (Grenier and De Clercq 2003, Zapata et al. 2005, Dindo et al. 2006, Sighinolfi et al. 2008).

2.3 Nutritional balance sheet

The evaluations of food intake, weight gain, faeces and wastes rejected during a given time, allow calculating some indexes (nutritional rating of digestibility, conversion efficiency ... of the food). With this method, specific compartments could be studied separately (nitrogen or lipid compartment). The determination of the enzymatic activities of the insect to be reared in artificial food gives some information about its possibility of digestion and assimilation. Complementary studies could be conducted *in vitro* for digestion of nutriments by enzymatic extracts of the insect concerned. Fine experiments on digestion, metabolism processes and inter-conversions could be conducted thanks to NMR spectroscopy or radiotracer methods (Thompson 1990b, Grenier et al. 2005).

2.4 Classical dietary deletion/supplementation studies

Many nutritional studies for free living insects are dealing with the omission/deletion or supplementation of one component to verify if it is essential or not. But this method is less powerful for parasitoids of which growth is fast and because components balance, as well as pH and osmotic pressure stability, could be key parameters. For predators the method gives some good results (Arijs and De Clercq 2002).

3. The nutritional requirements

It is generally admitted that the basic qualitative nutritional requirements for parasitoids and predators are not different from those of free-living insects. Contrary to the species developing at the expense of growing stages (koinobionts), idiobiont parasitoids and especially egg parasitoids develop in closed systems, for a short time, without external nutritional supply (Mellini 1986). Consequently they need very rich and concentrated food.

The nutritional plasticity of some predator species is a positive characteristic regarding their possibility to be reared on artificial food (Specty et al. 2003). Sometimes, a diet designed for a species can be appropriate for another species, possibly with small adaptations, as observed with *Harmonia axyridis* diet used for *Chrysoperla carnea* (El Arnaouty et al. 2006).

3.1 Nitrogen sources

The sources of nitrogen are a very important parameter in the nutrition of entomophagous insects, because of the very fast growth of many species (Grenier et al. 1974). For example, the weights of newly hatched and mature larvae of the tachinid *Lixophaga diatraeae* are respectively 12 µg and 33 mg. The larval growth is completed in 8 days with a weight doubling time of about 17 hours (Grenier 1980). The supply in amino acids (aa) has to fit the needs, to avoid a lost of time and energy in conversion between amino acids, and the production of toxic metabolites. Parasitoids and predators are carnivorous species needing a protein-rich diet, with some specific requirements in aromatic amino acids especially in parasitoid diptera at the end of their larval development for cuticle tanning (Bonnot et al. 1976). Nevertheless some free aa like phenylalanine, may be toxic at high concentration, or have a very low solubility like tyrosine, enforcing to deliver the aromatic aa as tyrosine-rich peptides or proteins. The 10 "essential" aa are required, but some other ones are highly beneficial for a normal growth (Grenier et al. 1994). Many species need a complementary supply of several non-essential aa because they fail to develop normally in diets containing only these 10 essential aa. The modification of the balance between the different aa of a medium that only permits the larval survival of the tachinid fly *Phryxe caudata*, could induce the start of its growth (Grenier et al. 1975). For the first time for a tachinid, the complete development from egg to adult was obtained with *L. diatraeae*, in a medium containing 19 aa in well-balanced proportions (Grenier et al. 1978). To maintain the osmotic pressure (OP) within acceptable values, part of the aa could be provided as proteins, protein hydrolysates or peptides, but free aa could be required for some species (Nettles 1987, Thompson 1980, 1986, Thompson et al. 1983). Casein, lactalbumine, ovalbumine, serumalbumine, soybean extract and yeast are the most common used proteins (Grenier 1994) (see § 4.1).

3.2 Lipids

The similarity of the composition in total fatty acids of many parasitoids with that of their host, suggests they may copy to a certain extent the host composition (Thompson and Barlow 1972, Delobel and Pageaux 1981). It could be the same for predators (Sighinolfi 2008). *Itoplectis conquisitor* (Yazgan 1972) can develop in a diet without any fatty acids, but their addition improves the yield and the fecundity of the adults obtained, but conversely *Pimpla turionellae* requires a mixture of fatty acids to produce normal adults (Yazgan, 1981). Polyunsaturated fatty acids may be required for normal growth of several entomophagous insects (Sighinolfi 2008). Dietary sterols are required by a great number of parasitoids and predators, such as *Exeristes roborator* (Thompson 1990a), *P. caudata* (Grenier et al. 1975), or *Geocoris punctipes* (Cohen 1985, 1992). Fatty acids may be supplied as free fatty acids or triglycerides, and need the use of emulsifying agents to obtain their homogeneous dispersion in the aqueous phase. The most employed emulsifying agents are Tween 80 (polyoxyethylenesorbitan monooleate), lecithin (phosphatidyl-choline) or lauryl sulphate. Egg yolk frequently incorporated into artificial media for egg parasitoids provides highly well emulsified concentrations in fatty acids, cholesterol, and lecithin. Free fatty acids are toxic for the tachinid *P. caudata* (Grenier et al. 1974). The degree of toxicity may depend on the emulsifying agent used for *E. roborator* (Thompson 1977). Emulsion process could have a detrimental effect on larval respiration.

Eicosanoids (derived mainly from the arachidonic fatty acid) were recently recognised to mediate different functions in insects, such as reproduction and immunity. For example, the inhibition of eicosanoid biosynthesis strongly reduced the production and hatchability of the eggs in the ectoparasitoid *Bracon hebetor* (Büyükgüzel et al. 2011). These molecules also exert a role in melanotic nodulation reactions to viral infection in the endoparasitoid *P. turionellae* (Durmus et al. 2008).

3.3 Carbohydrates

Carbohydrates are often considered as energy sources, as well as some lipids. It is usually admitted that there are no specific needs for carbohydrates, but glucose promotes growth and lipogenesis, increasing the level of unsaturated fatty acids in *E. roborator* (Thompson 1982).

Trehalose, the most common non-reducing disaccharide in insects, plays an important role in metabolism and stress resistance (Qin et al. 2011). It could be used instead of sucrose or glucose, and also partly replaced hemolymph in media for *Trichogramma* (Lü et al. 2011) (see § 4.1). To reduce the OP in medium/diet it is recommended to replace oligosaccharides by polysaccharides, like glycogen, but OP being not so critical for many predators, sucrose has been used in place of glycogen to reduce the cost of the diet (Cohen 1985, 1992). Moreover, sucrose may act as a feeding stimulant on parasitoid, as well as on predator insects.

3.4 Other needs

3.4.1 Inorganic salts

They are generally required for the normal development of insects, but their level and the balance between the different cations, especially K+/Na+ is of prime importance and varies according to the species. The predator *G. punctipes* prefers to feed on diets containing a K/Na ratio exceeding 2 than on diets with a ratio lower than 1 (Cohen 1981). Many authors introduce in the medium/diet a classical list of salts, frequently, Wesson's or Neissenheimer's salt mixture.

3.4.2 Vitamins

The accurate determination of the needs in vitamins implies very delicate experiments, requiring vitamin-free components, and taking into account the egg stocks. Very few specific investigations were conducted for entomophagous insects. It was usually admitted that their needs would be not different from those of other insects. Habitually about 12 vitamins were added in the diets, mainly hydrosoluble ones including B vitamins, as well as C vitamin, and 2 liposoluble ones (retinol-A, and tocopherol-E). Commercial preparations are available and often used by many authors, like Vanderzant vitamin mixture for insects (Vanderzant 1969).

3.4.3 Miscellaneous

Ribonucleic acids (RNA) are sometimes incorporated in medium/diet, but their dispensability is questionable. RNA could increase survival or promote growth.

Supplementing an artificial diet devoid of insect components with cells from an embryonic cell line of *Plodia interpunctella*, enhanced oviposition rates in the bug *Orius insidiosus*. Protein content or some other nutritional components of the cells might be responsible for this increase (Ferkovich and Shapiro 2004).

4. Other physiological requirements

For endoparasitoids, the medium is not only the food source, but also the environment in which they are bathed for all their larval life. Thus, besides the nutritional needs, the medium must have acceptable physico-chemical parameters, and provide for other requirements concerning essential physiological functions like respiration, excretion, and general protection (Grenier et al. 1986).

4.1 Digestion process - structure of the gut

The (temporarily) blind posterior gut of some hymenopterous species increases the efficiency of the conversion of food and protects the remaining food from contamination by waste products, but implies a high concentrated food. On the contrary, the open functional gut in several tachinid larvae has to be taken into consideration. The medium could be renewed along the larval development or supplied as an amount greatly exceeding the strict needs, in order to dilute the excretory products below a harmful level.

Extra-oral digestion could be observed in many predaceous species, and about 80% of predaceous terrestrial arthropods use this strange feature, according to Cohen (1995) (see § 6.2).

Besides the composition of the medium/diet, protein digestion and utilization, are key processes that could be investigated to assert a proper metabolization of the diet components (Gomes et al. 2000, Grenier et al. 2005). This is also true for many other food ingredients. The composition of the medium/diet nicely fitted with the needs, is necessary but not sufficient, it is also of prime importance to make sure that digestion and assimilation processes will correctly liberate the components required.

4.2 Respiration

The good accomplishment of this crucial function is closely related with the presentation of the food (see § 6). Some lipids could hamper the respiration by alteration of the characteristics of the interface larval tegument / medium.

- Generally speaking, the gas exchanges in parasitoid larvae occur through the tegument or by the spiracles. Early larval instars in hymenopterous and tachinid species mainly respire by cutaneous diffusion from host body fluids, allowing to use liquid media for their development. Nevertheless the volume of liquid has to be adapted to insure an efficient gas exchange with the surrounding atmosphere (Grenier et al. 1975). Tachinid larvae present a special respiration structure resulting of interactions with host. The host immune response usually results in a partial encapsulation forming a structure called respiratory funnel. This funnel allows the larva to be in relation with the tracheal system of its host. The larva could be attached to trachea or spiracle or sometimes directly on the integument, especially at the end of the larval development (Mellini et al. 1996, Herting 1960, Stireman et al. 2006). The last larval instar of most Tachinidae is amphipneustic and exhibits a high respiratory rate of about 1 µl O^2/mg wet weight /hour, in *P. caudata* (Bonnot et al. 1984). A gelled medium is recommended at that stage, also for some hymenopterous larvae, even those with hydrophobic tegument.

- The problem of gas exchanges is critical with egg parasitoids, because of their very high respiratory rate. The consumption of oxygen by *Trichogramma dendrolimi*, increases during larval development, reaching 7µl/h (for 100 larvae) at the prepupal stage and slightly decreases thereafter (Dai et al. 1988). To culture *Trichogramma* species *in vitro*, it will be of prime importance to ensure a normal respiration of prepupae and pupae. Some species like *Ooencyrtus pityocampae* (Encyrtidae) have a special anatomical adaptation, in a form of a stalk protruding from the host egg or plastic film, allowing them to respire through the host tegument or through an artificial membrane. But a high mortality was observed in artificial conditions when mature larvae leave their respiratory stalk before pupation (Battisti et al. 1990, Masutti et al 1992).

4.3 Hormones

Many egg parasitoids can develop in killed host eggs revealing the low host-parasitoid interactions at hormonal level. In the absence of demonstration of the role of hormones, not any was added in artificial media for egg parasitoids, though insect hemolymph was frequently incorporated. In natural situation, the hormonal changes in parasitoids may be synchronized with those of their hosts, and are key factors for parasitoid's development and species survival (Mellini 1983, Beckage 1985, Lawrence 1986, Rhamadane et al. 1987, 1988). The parasitoids allowing their host to continue to feed and develop beyond parasitization are named "koinobionts" and those, which paralyze or kill the host very soon after parasitization, usually before their egg hatches, are named "idiobionts" (Askew and Shaw 1986). Dipteran parasitoids, especially tachinids, don't however fill well into this classification, as many species show characteristics of both strategies (Dindo 2011).

Although the parasitoid's life cycle may be closely dependent upon host hormone titres *in vivo*, the dependence could be not so strict *in vitro*. Thus, the tachinid *P. caudata*, whose cycle is accurately synchronized with its host cycle in nature, does not need any hormone for the first and the second moults *in vitro* (Grenier et al. 1975). Other tachinids, *L. diatraeae*, *Eucelatoria bryani* and *Exorista larvarum* can develop from egg to adult in media devoid of hormones (Grenier et al. 1978, Nettles et al. 1980, Dindo et al. 1999). In the same way, Thompson (1980) showed that the addition of 20-OH ecdysone, neither stimulates the development, nor the pupation of the Hymenoptera *Brachymeria intermedia*. The complete development of this parasitoid has been obtained on an insect-material free artificial medium, devoid of host hormones (Dindo et al. 2001). But, for the tachinid *Pseudoperichaeta nigrolineata*, Grenier (1988) showed that the presence of 20-OH ecdysone in artificial media is necessary to trigger the first moult, and in *Pseudogonia rufifrons* the first instar larvae only moult to second instar when ecdysone is added into the medium (Fanti 1990). In the hymenoptera *Cotesia marginiventris*, 20-OH ecdysone can prevent egg hatching, but neither ecdysone, 20-OH ecdysone, nor juvenile hormone, improves larval development *in vitro* (Greany 1986). It is usually admitted that koinobiont parasitoids, either alter the host endocrine system in order to promote their own growth (called "regulators"), or depend on host hormones to synchronize their development with that of the host (called "conformers") (Lawrence 1986).

In presence of 20-OH ecdysone, lipophorin or lipophorin-transported lipids could act as a growth-promoting factor putatively involved in a pupal extract from their host *Galleria mellonell*a for the development of the endoparasitoid *Venturia canescens* (Nakahara et al. 1999).

For predators, the role of hormones present in their prey, is not well documented. However, the development of many predators on artificial diets devoid of insect components (Cohen 1985, Arijs and De Clercq 2002) indicates that they are not dependent on exogenous hormones. Nevertheless, some juvenoids added in diets could prevent the reproductive diapause of the adults of coccinellids like *Coccinella septempunctata* L. (Chen et al. 1984).

The fenoxycarb, an insect growth regulator, mimetic of Juvenile Hormones, strongly disturbs the development of the tachinid *P. nigrolineata*, by delaying or stopping the growth and reducing the yield in pupae (Grenier and Plantevin 1990). Actually, the fenoxycarb shows a high JH activity and induces deleterious effects on many parasitoids and predators (Grenier and Grenier 1993).

4.4 Teratocytes

Cells, called teratocytes, derived from the embryonic membrane of egg in some Scelionidae or Trichogrammatidae species are released into host hemocele at hatching. Their role in the successful *in vitro* culture of egg parasitoid suggests a function on digestion and assimilation of host components (Grenier 1994, Thompson and Hagen, 1999).

5. Physico-chemical factors

5.1 Osmotic Pressure (OP)

The dietary osmolarity is an important parameter and must be adapted to each species, especially for endoparasitoids for which the medium is not only the food, but also the environment in which they are bathed all their larval life. Ectoparasitoid Hymenoptera are usually more tolerant to high OP. *Itoplectis conquisitor* (Yazgan 1972) and *E. roborator* (Thompson 1976) can develop in medium reaching 1700 milliosmoles, but for *Brachymeria lasus* and *Pachycrepoideus vindemiae*, the results were improved if the OP was lowered from 700 to 400 mOsM (Thompson et al. 1983). In tachinid larvae, such as *P. caudata* and *L. diatraeae*, the optimal OP varies between 350 and 400 mOsM, and no development was observed above 450 mOsM (Grenier et al. 1975, Grenier et al. 1978). Above the same threshold, for the oophagous species of *Trichogramma,* neither normal egg hatching, nor larval development occurs, the optimal OP being near 320 mOsM (Grenier and Bonnot 1988). The degree of tolerance to OP seems in relation with the anatomical features of the larval integument. The thick hydrophobic cuticle of some ectoparasitoid Hymenoptera allows them to avoid a close contact with the medium. On the contrary, endoparasitoid Hymenoptera and Diptera may lack such integument and could undergo an osmotic shock.

5.2 pH

Usually hydrogen ion concentration in insect hemolymph varies from 6.0 to 8.2, but is more often observed between 6.4 and 6.8 (Mullins 1985). Most of the artificial media for parasitoids show a pH near these latter values in the absence of studies concerning the effect of this factor on their development. The tachinid larvae of *E. bryani* seem highly tolerant to pH because Nettles (1986) observed that there is no significant difference in pupal and adult yields between diets with pH varying from 5.5 to 8.0, although higher absolute yields (egg to adult) were obtained between 6.75 and 7.5.

With media containing holotissue of *Antheraea pernyi* pupae, egg yolk, milk and water, the optimal pH for parasitization, pupation and adult production of *Trichogramma confusum* varies between 6.7 and 6.95 (Zhong and Zhang 1989). Usually, high percentages of adults of *Trichogramma* spp. were obtained for pH between 6.6 and 7.0 by many authors.

6. Presentation of the food

The medium consistency could be a limiting factor for the larval and pupal parasitoids, mainly in relation with respiration (see § 4.2), but the presentation of the food is more crucial for egg parasitoids and predators.

6.1 Egg parasitoids

Especially for non-gregarious species, larvae need a limited quantity of food because they cannot regulate their food intake, otherwise the development will terminate mainly at pupation, or will produce adults showing abnormalities (Grenier and Bonnot 1988). Thus, for egg parasitoids the medium is presented in various ways to create an artificial host egg. The hanging drop technique consists in the deposition of droplets of medium on a flat surface in which the *Trichogramma* eggs are deposited for development. In wax eggs, paraxylylene eggs or plastic egg cards, the artificial eggshell is made of wax/paraffin mixture, polymerized paradixylylene or polyethylene/ polypropylene film, respectively (Grenier and Bonnot 1988, Grenier 1994, 1997). Egg laying occurs directly in these artificial host eggs, but the stimulation of the oviposition by the *Trichogramma* females is usually enhanced by smearing the surface of the artificial eggs by some chemicals like moth scales extracts, or polyvinyl alcohol solutions (Cônsoli and Grenier 2010, Grenier et al. 1993, Grenier et al. 1998a, Han et al. 1994). In wax and paraxylylene eggs the development usually stops at pupation, probably because of gas exchange limitations. The artificial egg shell has to be permeable to oxygen and carbon dioxide, but not to water vapour, in order to avoid desiccation. The development of *Trichogramma* from eggs to normal fecund adults was observed inside small bags constituted by a heat sealed polypropylene/ polyethylene film with a series of hemispherical cupules filled with the medium. This latter technique was greatly developed in China and in France (Li 1986, Li et al. 1988, Grenier 1994, 1997).

6.2 Predators

For predators, the presentation of the diet is a key parameter although the respiration is not mainly involved. Liquid diets that were fully- or semi-defined were presented within wax capsules for the neuropteran *C. carnea* and the hemipteran predator *G. punctipes*. Diets for different lacewing species (chrysopids) were encapsulated, presented on cellulose sponge or in the form of a free hygroscopic powder.

Diets for predaceous coccinellids could be presented in gelled cubes or as powder, or dry pellets. Stretched Parafilm was used to package diets with a paste-like consistency for several hemipterous predators, as well as for some species of coccinellids. Parafilm enclosing synthetic foam cubes soaked with diet devoid of insect components was successfully used to rear *Macrolophus caliginosus* (Grenier et al. 1989). For other bibliographic references, see Grenier et al. 1994, Thompson and Hagen 1999, Cohen 2004.

For the rearing of the stinkbugs *Podisus* spp., cylindrically shaped “artificial larvae”, 2-4 cm long and 0.3 cm diameter, were produced by bringing thawed or fresh diet onto a stretched Parafilm M sheet and wrapping a single layer of the Parafilm around the meat-based diet paste (De Clercq and Degheele 1992). For *Orius laevigatus*, the diets were encapsulated in Parafilm M using an encapsulation device (ARS, Gainesville, USA) forming small hemispherical domes (35µl) sealed with transparent tape. The Parafilm was stretched before encapsulation to facilitate stylet penetration by early instars of the predator (Bonte and De Clercq 2008, 2010). The same presentation with 2 artificial diets was successfully used for the development and reproduction of *M. caliginosus* (Vandekerkhove et al. 2006). Glycerol and starch are texture and consistency agents used in diets for predators such as coccinellids or chrysopids.

The presentation has to take into consideration the quite common habit of many predators to exhibit an extra-oral digestion, allowing them to attack relatively large prey compared to their own size. Thanks to the injection of specific hydrolytic enzymes in the prey and the absorption of the resulting fluids, these predators greatly increase the nutritional efficiency of a prey (Cohen 1995). A bulk of food included in a same membrane could be preferable to scattered small pieces.

7. Sterilization and Preservatives

The artificial diets are rich in all the nutrients allowing the growth of bacteria and fungi. Fungi are especially detrimental because they can spread in the entire rearing system from a unique contamination spot thanks to mycelia and spores. The sterilization of the medium/diet by filtration or heating is not always possible because of size particles or coagulation. Gamma irradiation could also modify the consistency of the diet and the structure of some components. Some classical bactericides (penicillin, streptomycin, gentamycine) are efficient to control bacteria and non-toxic for entomophagous insects (Dindo et al. 2003, Grenier 1994, Cônsoli and Grenier 2010). Nevertheless it is necessary to pay a special attention with insects harbouring symbionts, because some antibiotics, such as tetracycline used with *Trichogramma*, could remove these symbionts and deeply modify the reproductive status of the hosts (Grenier et al. 2002). The fungicides incorporated into the food for phytophagous insects (nipagine, merthiolate, sorbic acid) are often detrimental for entomophages (Grenier 1977). Specific fungicides used in cell cultures (amphotericine, nystatin) are better tolerated than more generalist products (Grenier and Liu 1990).

Sometimes, high levels of antimicrobial agents, especially antibiotics, could modify the diet consistency or cause the formation of some aggregations destroying the homogeneity of the diet. These modifications may interfere with the nutritional value of the diet and with the food intake of the larvae (Büyükgüzel 2002).

Some antimicrobial agents (penicillin, streptomycin, rifampicin, tetracycline hydrochloride, lincomycin hydrochloride, methyl p-hydroxybenzoate, cyclohexi-mide and sodium benzoate) could significantly increase the protein content of the pupae of the parasitoid *P. turionellae* while this content was decreased by nystatin (Büyükgüzel 2002).

Various effects, some positive ones, were observed for bacterial DNA gyrase inhibitors (novobiocin, nalidixic and oxolinic acids) on survival and development of the ichneumonid parasitoid *P. turionellae* reared on chemically defined synthetic medium (Büyükgüzel 2001).

8. Discussion and Conclusion

At final, during several decades, many successes were obtained in different countries around the world, mainly with idiobiontic parasitoids and polyphagous predators. Approximately 130 entomophagous species have been partly or completely reared in artificial diets, among them more than 20 species of Trichogrammatidae. Nevertheless from the years 2000, it appeared a slow down of the works concerning artificial diets, even if some teams are still working hard (Cônsoli and Grenier 2010). Besides it was observed, at the same time, that the motivation of the researchers in artificial rearing moves from academic objectives to more applied orientated works, sometimes not steadily published or even patented, as in the USA for example (Grenier 2011).

The development and especially the continuous rearing of parasitoid and predator insects are currently limited. Yet, except for laboratory tests, it is not recommended to maintain entomophagous insects on artificial diet, and even on factitious hosts or prey. In the tachinid *Lixophaga diatraeae*, a long rearing in the laboratory on *Galleria mellonella* induced some modifications of capability to develop in artificial medium (Grenier and Pintureau 1991). The nutrition is one of the main constraints to reach these objectives, but generally speaking more knowledges are required about the physiology, behaviour and genetics of the insects to be reared.

There are special lacks of basic research in functional morphology and host-parasitoid relationships for immature parasitoids (Cônsoli and Parra 1999). An analytical approach with biochemical analyses of the food and of the carcass of the insects produced is a powerful method to define and test artificial diets (Grenier 2002). A new research direction recently open concerns the nutrigenomics with some new tools, such as micro-arrays, allowing to improve a diet by comparing gene expression patterns under different nutrition conditions (Coudron et al. 2006). More studies on host/symbiont relationships would probably be profitable for better knowledge on nutrition and reproduction as well as for enhanced definition of quality criteria, possibly by transfer of symbionts between species (Grenier et al. 1998b). Many parameters control the different physiological functions, including nutrition and may interact with each other simultaneously. It is recommended to use multivariate/multifactorial analyses to take into account efficiently these multiple interactions (Grenier et al. 1986).

A lot of constraints limit the development in the rearing of entomophagous insects, mainly technical and economical ones. Thus, new directions and future trends for this field may also concern the structures of the research, the choice of the most promising species, and the development of specific production means such as automation and quality assurance (Grenier 2009). For better chances of success, it is also recommended to constitute teams with different specialists such as entomologists, engineers, and economists... (Cohen et al. 1999).

The quality of the insects produced is of prime importance to ensure success in the biological control strategies and therefore to retain the confidence of the end users. The required characteristics of the insects vary in relation with the purposes of their production. For example, the criteria retained are not the same for inoculative or inundative releases. Morphological characters, immature development parameters, sex ratio, symbiont association, fecundity, longevity as well as biochemical parameters are important cues to be considered for quality control and in addition, these latter could indicate the deficiency or excess in a particular nutritional component. In fine the predation efficiency or the parasitization rate have to be evaluated (Grenier and De Clercq 2003).

The current public opinion highly suspicious to the immoderate use of chemicals and more and more disposed towards organic products may encourage the practice of biological control strategies and consequently stimulate the development of artificial rearing, one of the way to reach economically acceptable mass production of entomophagous insects. Further efforts have to be accomplished to burst the last locks impeding the development of these approaches.

9. In Memoriam

This paper is dedicated to the memory of Guy Bonnot, Bernard Delobel, and Pierre Laviolette.

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