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10TH Workshop of the IOBC Global Working Group on

Arthropod Mass Rearing and Quality Control

Agropolis International, Montpellier, France, 21-25 September 2003

10ème Réunion du Groupe de Travail de l'OILB

Élevage de Masse des Arthropodes et Contrôle Qualité

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Abstracts of the 10th Workshop of the IOBC Global Working Group

Résumés de la 10ème Réunion du Groupe de Travail de l'OILB Mondiale

Arthropod Mass Rearing and Quality Control

Élevage de Masse des Arthropodes et Contrôle Qualité

at / à
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Preface

In September of 1996, the International Organization for Biological Control (IOBC) held the first IOBC International Conference at Montpellier, the subject of which was "technology transfer" in biological control. In his opening remarks, IOBC President Dr. Jeff Waage addressed the gap between research and implementation, and posed the following question: "Yes, but does it work in the field?" This question remains relevant today, particularly with respect to augmentative biological control.

The goal of the IOBC Global Working Group on Arthropod Mass Rearing and Quality Control (AMRQC) is to "facilitate and advance cost-effective rearing of high-quality arthropods in support of biological control and integrated pest management." As the current IOBC President, I would like to paraphrase Jeff Waage's question: "But what about field performance?" Should this not be part of the equation? I suggest that it should be, such that the end user can be assured that a "high-quality" arthropod:

- ✓ Was produced through precise control of the proper inputs,
- ✓ Has met appropriate guidelines for product or output control, and
- ✓ Can be expected to perform satisfactorily in the field.

Otherwise, how can we expect commercially available, biological control agents to compete in major markets with emerging technologies, such as reduced risk pesticides or transgenic crops that express insecticidal proteins? As Jeff Waage noted in 1996, "making it work in the field" is the major challenge for augmentative biological control, and one that will require new research and partnerships in the future. Otherwise, the commercial insectary industry may struggle to survive, except in specialty or "niche" markets.

This tenth workshop of AMRQC is timely indeed, and on behalf of IOBC, I am pleased to welcome the participants to Montpellier. I also thank the members of the Organizing Committee for their efforts over the past several months to make this meeting a success.

L. E. Ehler
President, Global IOBC

Foreword

Workshops of the International Organization for Biological Control (IOBC), Global Working Group on Arthropod Mass Rearing and Quality Control (AMRQC), formerly the IOBC, Global Working Group on Quality Control of Mass-Reared Arthropods (WGQC) have been forums for determining the status of important issues in the production and use of arthropods for pest management and recommending a course of action for the future. These meetings have helped to increase the assimilation and transfer of knowledge primarily among the producers of natural enemies or sterile insects and the research community. They identify and attempt to fill crucial information gaps in an effort to improve the effectiveness of mass-reared arthropods.

Traditionally, the workshops have shifted back and forth between the western hemisphere and Europe, including in order the U.S.A., Switzerland, Guatemala, Canada, The Netherlands, Denmark, Italy, U.S.A., Colombia, and currently France. Recent ones have addressed two general purposes: 1. To expand our knowledge of successful quality control systems used in insect mass rearing, and 2. To develop, refine, and finalize quality control systems that incorporate appropriate tests for natural enemies and sterile insects. At the Wageningen, The Netherlands meeting in 1991, emphasis was shifted from quality control principles and practices across several taxonomic groups to regulation of natural enemies. This emphasis remained through the Horsholm, Denmark; Rimini, Italy; and Santa Barbara, California workshops. At Santa Barbara, the goals were to: 1. Institutionalize quality control in arthropod mass rearing, 2. Rationally regulate commercial natural enemies and 3. Assure the efficacy of artificially reared natural enemies. The subsequent Cali, Colombia workshop furthered the development of self-regulation capabilities for the international biological control industry, as well as returned to some general principles and practices in large-scale insect rearing as they relate to quality control.

The working group has always had an appreciation for supportive research on developing artificial diets and rearing techniques but has recently shifted its focus from phytophagous species with relatively simple rearing requirements to entomophages that depend on more complex diets, in many cases another trophic level. The behavior, as well as survival, of mass-reared entomophages is determined by their diets and, therefore, quality control standards are directly dependent on related mass rearing procedures. Consequently, in 1998, the IOBC, WGQC was combined with a newly formed group on artificial rearing of entomophagous insects and renamed the IOBC, AMRQC. Both groups have benefited by shifting the quality control emphasis from regulation back toward research and technology, and linking quality control more closely to the underlying rearing techniques. The IOBC, AMRQC maintains an appreciation for research on artificial diets, rearing techniques, and quality control. However, there has been very limited emphasis on strain development and virtually none on promising new genetic techniques. The IOBC, AMRQC provides a forum for scientists and practitioners to develop quality control standards in relation to rearing processes and achieve a balance between phytophages and entomophages, while incorporating more genetic and other research advances.

The 10th workshop of IOBC, AMRQC is being held at the Agropolis International in Montpellier, France on 21-25 September 2003. The organization's renewed mission is to facilitate and advance cost-effective rearing of high-quality arthropods in support of biological control and integrated pest management. The workshop is focusing on all issues related to the rearing of entomophagous and phytophagous insects and mites, and to principles and practices of quality control. The program consists of keynote addresses by Allen C. Cohen, Karel Bolckmans and Joop van Lenteren presenting views from the industry and academia on the mass production and quality control of arthropod natural enemies and pests. The following five sessions of contributed presentations treat the different aspects of arthropod rearing as it relates to quality control: Artificial and Factitious Foods, Tools for Quality Control, Innovative Methods for Quality Control, Production Techniques, and Post-Production Quality Assurance. Papers in these sessions serve as a basis for discussion and exchange, with the final aim of improving collaboration among scientists, practitioners and regulators.

Norman C. Leppla Simon Grenier Patrick De Clercq

Chairmen AMRQC

Controlling certain insect pests in Egypt

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The egg parasitoid *Trichogramma evanescens* has been utilized in Egypt since 1988 as a biocontrol agent against the sugarcane borer, *Chilo agamemnon*, infesting sugarcane. Percentage of infestation has been found to be reduced between 50 and 79% at the end of the season in an area of 40,000 ha. This year (2003) the parasitoid was released against the peach bud borer, *Anarsia lineatella* (100 ha), the olive moth, *Prays oleae* (80 ha), and the grapevine moth, *Lobesia botrana* (30 ha) and the results seemed promising.

T. evanescens is released at a rate of 50,000 / ha only once in sugarcane fields as the parasitoid occurs naturally in such fields but with low rate of parasitism early in the season (May to July) and with high rate (up to 90%) in October to November. However, the parasitoid was released, at the same rate, 3 times against the other mentioned pests.

T. evanescens is mass produced on the eggs of *Sitotroga cerealella*. The latter is mass-produced using the method adopted by S. A. Hassan, Darmstadt, Germany. Each rearing cage produces 400-600 g of *Sitotroga* eggs within 2 months. Factors affecting the efficiency and quality of *T. evanescens* include host plant, cold storage, and successive rearing in the laboratory.

Mass rearing of sunn pest egg parasitoid, *Trissolcus grandis*: a demographic framework

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Mass production of most parasitoid species requires host rearing. Therefore one of the broad objectives for insect production is to optimally balance the number of hosts available for parasitization with the number of adult parasitoid females held for renewal. In this study, periodic mass rearing and harvesting of both the host eggs of *Graphosoma lineatum*, and the parasitoid, *Trissolcus grandis*, are analyzed based on the demographic method. Thus life history data of the host and parasitoid were used to estimate the proportion that can be harvested from a rearing colony, so as to keep population size constant. The harvest rates for host (h_h) and parasitoid (h_f) are 0.94 and 0.99 respectively. The per female production rate of the host target stage (P_h) is 7.4 (eggs/female), and the per capita adult female parasitoid rates for production of harvestable daughters (P_f) and sons (P_m), respectively, are 13.2 and 3.96 (females/day). The use of these findings in the harvesting schedule of the sunn parasitoid is discussed.

Key words: discarding age, harvesting rate, *Graphosoma lineatum*, Pentatomidae, Scelionidae, *Trissolcus grandis*,

Challenges in the development of artificial diets for insect predators

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During the last five decades some progress has been made in developing artificial diets for predators. Attempts have only been successful for a limited number of – especially polyphagous – predators, including some Coccinellidae (e.g. *Harmonia axyridis*), Heteroptera (e.g. *Podisus maculiventris*, *Geocoris punctipes*) and Neuroptera (e.g. *Chrysoperla* spp.). Moreover, biological parameters of *in vitro* reared predators are often inferior to those of insects cultured on their natural or alternative prey. Little has been reported on the effects of continuous multi-generation artificial culture and the field potential of artificially reared predators. Consequently, biocontrol companies in Europe and North America have hardly incorporated artificial diets in their production process. Much of the research for developing artificial diets has been based on trial-and-error work. We believe that further research must focus on an holistic approach, where the biochemical composition of the (un)natural prey should be copied and the feeding habits of the insect considered. This approach was followed in a research project funded by the European Commission (FAIR 6-CT98-4322), where artificial diets were developed for a number of predators, including *Orius laevigatus*. Here, composition of artificial diets was based on biochemical analyses of *Ephestia kuehniella* eggs and carcass analyses of the predator were used for tracing deficiencies in artificially reared predators. The availability of fast and reliable screening methods for assessing the quality of *in vitro* reared predators will contribute to a greater efficiency of artificial diet research. Commercial application of diets may be greatly enhanced by automation of diet preparation (e.g., via the use of microencapsulation systems). Besides making insect rearing more profitable, research on artificial diets should yield a greater knowledge of a predator's nutrition, physiology and behavior.

Interaction of *Verticillium lecanii*, and *Adonia variegata* (Col.: Coccinellidae), pathogen and predator of aphids

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Aspects of pathogenicity of the hyphomycete, *Verticillium lecanii* (DAOM 198499), were evaluated to ladybeetle, *Adonia variegata*, under laboratory conditions. Third larval instars of ladybeetle *A. variegata* were inoculated by sub-lethal, LC50 and lethal concentrations (estimated for *Myzus persicae*) of *V. lecanii*. Data analysis indicated that mortality rates in various treatments including control, 10^3 (sub-lethal), 1.4×10^4 (LC50) and 10^7 (lethal) conidia/ml were significantly different after 12 days. Minimum and maximum mortality were 4.24 and 30 percent, which related to 10^3 and 10^7 conidia/ml respectively. Effect of *V. lecanii* was evaluated on adults of ladybeetle, *A. variegata*, with the same concentrations as above. Results showed significant difference between treatments. Mortality rates related to treatments 10^3, 1.4×10^4 and 10^7 conidia/ml were 6.67, 38.1 and 36 percent, respectively. The effect of *V. lecanii* on ladybeetle adults also was verified by feeding them infected aphids (aphids treated by 10^7 conidia/ml). Data analysis indicated that there were no significant differences in mortality rates of neither adults nor female fecundity in treated ladybeetles compared to control. On the basis of results, we suggest that a biological approach to integrated management of green peach aphid using *A. variegata* and *V. lecanii* is possible with more studying.

Effects of different host plants on life-history parameters of *Trialeurodes vaporariorum* (Hom.: Aleyrodidae) and its parasitoid, *Encarsia formosa* (Hym.: Aphelinidae)

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The whitefly (*Trialeurodes vaporariorum* Westwood) is a polyphagous insect, attacking many different crop and ornamental plant species, thus it can be a very serious problem in greenhouse. Different host plants had various effects on herbivores and their natural enemies. Fitness of the whitefly and its parasitoid (*Encarsia formosa* Gahan) was studied when the whitefly host was reared on four host plants under laboratory conditions (25±1°C, 60% RH and 16L: 8D photoperiod). The plants tested were the tomato (*Lycopersicum esculentum* Mill), cucumber (*Cucumis sativus* L.), bean (*Phaseolus vulgaris* L.) and sweet pepper (*Capsicum annuum* L.). The results indicated that host plants variously affected most biological characteristics of both whitefly and parasitoid. Immature whitefly mortality from oviposition to adult emergence was significantly affected by host plant, varying from 18.1% on cucumber to 92.9% on sweet pepper. In contrast, parasitoid immature mortality was not significantly affected. Host plants significantly influenced development time up to adult eclosion for whitefly and *E. formosa*. For example, the shortest development time for whitefly was on cucumber but parasitoids reached the adult stage faster on this plant. Fecundity of *E. formosa* reared in whitefly on different host plants was significantly different. Parasitoids were more fecund on cucumber than other plants. The sex ratio of progeny did not vary significantly on the plants fed upon by the whitefly. There was no interaction between host plant and sex for developmental time. The results of this study showed that life history parameters of greenhouse whitefly and its parasitoid, *E. formosa*, are influenced by host plants differently. These effects were complex but generally interpretable in terms of host whitefly quality variation relative to host plants used as food by the whiteflies during their development.

Are entomopathogens safe for parasitoids? *Verticillium lecanii* as a model of entomopathogens

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Parasitoids and pathogens that attack insects are key components in biological control. Recognizing both deleterious and beneficial aspects in the host-parasitoid-pathogen complex can improve the efficiency of biological control agents.

In our research model, some possible deleterious effects of DAOM 198499, a strain of *Verticillium lecanii*, were studied on adults of *Aphidius nigripes* and *Encarsia formosa,* parasitoids of aphids and whitefly, respectively.

All experiments contained lethal, sub-lethal and/or LC50 concentrations of the pathogen (obtained on aphids and whitefly) and were done in controlled conditions.

Possible ways for *A. nigripes* infection by *V. lecanii* were put in evidence. Parasitoid mortality was higher when aerial contamination happened via spraying of the pathogen. Fewer deleterious effects were observed when conidia of the pathogen were received from foliage or when transmission to the emerged adult took place via the mummy. The fungus provoked mycosis phenomena on parasitoid adults less than 4 days after the infection. The pathogen influenced the sexual behavior of the parasitoid at the time of mating.

Adults of *Encarsia formosa* suffered similar mortality as *A. nigripes* when receiving different concentrations of fungi via cucumber foliage. Mortality rate of parasitoid increased from 10/48% at 10^4 conidia/ml to 70/93% at 10^8 conidia/ml. The LC_{50} value was 1/9 x 10^6 conidia/ml.

Integrated programs recommended for control of aphids and whitefly using both entomopathogens and parasitoids must be fully efficient to minimize the risk of interference between auxiliaries.

Diseases and disease management in mass-rearings of beneficial insects and mites

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More than 120 natural enemies are mass-reared for biological control programs and several species are used on a routine basis throughout the world. The success of a biological control program is dependent on many factors, including the quality of the beneficial arthropods that are used. In recent years, reports of pathogens in mass-reared natural enemies have raised questions regarding their quality and efficacy.

Several types of microorganisms have been reported in both mass-produced and field-collected natural enemies, including viruses, bacteria, protozoa, and fungi. Some of these microorganisms are known pathogens, whereas others seem to cause no harm or may even be of benefit to their host. Furthermore, some natural enemies may become infected with nematodes or be affected by diseases of unknown origin.

In many cases, it is difficult to determine if pathogens originate from field-collected natural enemies or arise in mass-rearing systems as a result of intense and continuous rearing under optimal conditions. Temporary starvation and localized overcrowding are often unavoidable in mass-production systems and these conditions place stresses on individuals, presumably making them more susceptible to disease.

Several techniques have proven successful for managing pathogens, including the isolation and rearing of healthy individuals from colonies of mixed infection, rearing arthropods at elevated temperatures, and the use of antibiotics. These treatments may prove useful for the management of disease outbreaks; however, consideration must be given to the type of pathogen present and the host that is infected.

Problems and challenges in commercial mass-rearing and quality control of invertebrate biological control agents

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More than 30 different invertebrate biological control agents are currently commercially available for biological pest management in greenhouse crops.

Once a biological control agent has been selected, a mass-rearing system needs to be developed. A (commercial) mass-rearing system has to be able to produce large numbers, to be able to assure availability upon demand (= predictable, reliable, flexible and short reaction time), to produce consistent product quality and to be cost-effective.

Because development of mass-rearing systems is seldom addressed in scientific research programs, a producer of invertebrate biological control agents has to develop his own mass production systems. In general mass-rearing technology cannot be protected through patenting.

Typically mass-rearing systems can be divided in systems with and without a host plant and systems with and without the use of a life host, totaling 4 different combinations. Therefore mass-rearing systems can be very diverse.

Development of a mass-rearing system includes development of a mass-rearing system for the biocontrol agent, a mass-rearing system for the host (if needed), a harvesting technique, a purification technique, a storage technique, a formulation technique, a counting or dosing technique, a packaging technique. While developing and managing a mass-rearing system attention needs to be given to automation, genetic aspects, disease management, contamination management, risk management, safety, process control and quality assurance.

Invertebrate biocontrol agents have a very short shelf life. Therefore correct storage conditions, a suitable formulation and packaging, fast logistics to the end-user and a quality assurance system are essential.

The market for invertebrate biological control agents can be rather volatile and influenced by many factors. Therefore an adequate planning and forecasting system can have great influence on the production cost of biological control agents.

Mass-rearing beneficial arthropods requires multiple skills : entomology (biology, ecology, ethology, taxonomy, genetics,...), insect pathology, plant production (greenhouse growing, fertilization, irrigation, climate control, growing systems, …), plant protection (plant pathology, pesticides, IPM,…), climate control systems of greenhouses and of climate rooms, mechanization, engineering, automation, management skills, total quality management, understanding of economics (cost-effective), ...

During the presentation research questions and future challenges for the biological control business will be identified.

Wolbachia: a novel tool for the control of insect pests and modification of beneficial insect species

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Wolbachia are a group of intracellular maternally inherited bacteria that are able to invade and maintain themselves in an enormous range of invertebrate species, including insects, mites, spiders, crustaceans and nematodes. Recent PCR surveys suggest that perhaps over 20% of the arthropod species may be *Wolbachia*-infected, rendering this bacterium the most ubiquitous intracellular symbiont yet described. *Wolbachia*-infections in arthropods are associated with a number of reproductive alterations including induction of parthenogenesis in Hymenoptera, feminization of genetic males in isopod Crustacea and Lepidoptera, male-killing in Lepidoptera, Coleoptera and Diptera, and induction of cytoplasmic incompatibility (CI), in a range of insects, as well as mites and isopod Crustacea. It has been proposed that *Wolbachia* infections can be used in an applied context (Beard *et al.* 1993; Bourtzis & Braig 1999). For example, *Wolbachia*-induced CI can be used in several ways: a) to directly suppress natural arthropod populations of economic and health importance, b) as a tool to spread genetically modified strains into wild arthropod populations (e.g. a whitefly strain which can not transmit a virus in plants) and c) as an expression vector, once a genetic transformation system for this bacterium is developed. We will present data about the potential applications of *Wolbachia* (strain optimalisation, SIT, etc.) for biological control but also about the potential problems which *Wolbachia* can cause in insect mass-rearing.

Artificial rearing of the mirid bug *Dicyphus tamaninii* on a meat-based diet

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An artificial rearing method based on a meat diet was evaluated for the rearing of the zoophytophagous mirid bug *Dicyphus tamaninii,* a polyphagous predator of horticultural pests native in the Mediterranean basin. Several continuous generations were completed on this food source which does not contain any plant material. Meat-reared insects had a high egg hatching and nymphal survivorship (>70%). They had a smaller size and weight, a delay in immature development time but similar fecundity as conventionally reared individuals (with tobacco plants and *Ephestia kuehniella* eggs as prey).

When comparing their predation efficiency, nymphs of *D. tamaninii* produced on the meat diet consumed a similar number of greenhouse whitefly pupae after 24 and 48 hours to nymphs reared by the conventional method, but diet-reared females consumed significantly more whitefly pupae after 24 and 48 hours than control females. When the cotton aphid was offered, diet-reared *D. tamaninii* nymphs and females consumed similar numbers of prey to control predators. There was no significant increase in cannibalistic behavior of diet-reared predators after 3 and 5 days of interaction. These results show that the predation efficiency and the tendency to cannibalism of meat-reared *D. tamaninii* are similar to those of conventionally-reared individuals.

The method is an important improvement in the rearing of this predator because the need for insect prey and a living plant is avoided as a food source.

Recent advances in mass rearing new world screwworms using artificial diet

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One of the largest mass rearing projects in the world is conducted by the United States Department of Agriculture in collaboration with the government of Mexico in the effort to eradicate the new world screwworm, *Cochliomyia hominivorax* (Coquerel). The mass rearing plant situated in Mexico, currently produces about 150 million flies every week. The majority of the pupae are sterilized and used in the screwworm eradication programs currently ongoing in Jamaica and Panama. To produce such a vast number of insects, the plant uses about 35,000 kg of dry food products per week.

The first artificial rearing medium for screwworm larvae was developed in late 1930s. Since then, the medium had been modified several times to improve the rearing system and to reduce the cost of production. The diet that is currently used for mass production consists of spray dried bovine blood, spray dried poultry egg product, a milk substitute, water, and a gelling agent to solidify the medium and to provide bulk and texture. Recent research advances were made to use less expensive dietary ingredients. An alternative source of blood protein was found to have potential for use in the mass rearing of larvae. Also, recycled paper products such as terra-mulch and cellulose fiber were tested to replace expensive and non-biodegradable gelling agents. Results indicate that recycled paper products can effectively replace the gelling agent. Significance of these findings in terms of cost-effectiveness and environmental safety is discussed.

The past and present of insect diets and rearing as precursors to a bright future

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I. Where rearing and diets are now

A. The major roles of insect rearing programs. Rearing-based technology is central to most efforts to manipulate insects, ranging from small-scale basic research, to true mass production systems that serve multiple functions. Examples of mass-production systems are the sterile insect techniques for suppression of tsetse flies, screwworms, and tephritid fruit flies as well as large scale programs in production of entomophages (such as *Trichogramma* and lacewings) and pathogens (such as gypsy moth viruses). Large-scale rearing is also involved in development of host plant resistance and pheromone-based systems; and mass-rearing is even being used in production of pharmaceutical and nutraceutical products via genetically modified baculoviruses. In fact, virtually all biologically-based management systems rely heavily upon rearing technology. In keeping with the demands and needs of the entomological community for insects that are available conveniently and economically, yet in abundance and of high quality, there have been numerous advancements made in rearing technology over the past six decades.

B. The status of rearing specialists. Given the high level of dependence of entomologically-related projects on rearing technology, it would seem intuitively obvious that rearing specialists would enjoy a high level of status and recognition for their essential contributions. However, as a group, rearing specialists including researchers, production workers, and support staff have little recognition and support for their efforts compared to fellow professionals in other scientific and technological endeavors which require parallel levels of competence. The reasons for this disparity and the means of correcting it will be discussed in this address.

C. Where are the rearing efforts being made? It is impressive how extensive rearing efforts are and what a central role they play in various insect-based programs. Well over half of publications and other communications about insect research are based on subjects that have been obtained from rearing facilities, and most of these insects have been reared on artificial diets. In fact, except for insect systematics and field ecology, the vast majority of all research on insects is done on specimens that are of laboratory origin. It can logically be concluded then that most of what we have learned about insects is derived from laboratory subjects. Insect rearing is also the mainstay of biologically-based programs in insect control, including sterile insect techniques that are used to suppress or eradicate screwworms in Tuxtla Gutierrez, Mexico, tsetse flies in Africa, pink bollworms in Arizona (USA), and tephritid fruit flies in Hawaii, California, and Florida (USA), as well as in many facilities whose products are biological control agents such as *Trichogramma* spp. and *Chrysoperla* spp. In North America, alone, there are 135 private companies listed by Hunter (1994) that are dedicated to the rearing and sales of various biological control agents that are laboratory-reared. Similarly, there are 100 North American companies that produce butterflies for education, conservation, and even novelty purposes. Many private companies, large and small, maintain insectaries for product development. In the US, government-run laboratories make up a substantial portion of rearing programs, including the USDA's Forest Service, Animal and Plant Health Inspection Serviced (APHIS), and Agricultural Research Service (ARS) laboratories with large-scale rearing, medium-scale, and many small-scale programs, and most other countries, have substantial rearing programs. Universities make up a sizeable portion of the small to moderate-scale rearing efforts, often with specialty rearing programs. For example, the University of Arizona keeps a living reference collection of *Drosophila* fruit flies comprising nearly every known species, world-wide. Finally, novelty and sundry uses of insects promotes a substantial and highly varied sector of the universal rearing domain. For example, production of insects as foods for pets and zoo animals is a thriving business as is production of insects such as crickets and wax moth larvae for fishing baits. Rearing insects for filth control, such as soldier flies in the chicken-waste management industry is on the increase.

D. How many rearing specialists? Because of the scattered nature of the field, it has not yet been possible for me to gain an accurate count of the people who work entirely or part-time in insect rearing, but based on estimates of how many of the various facilities such as those described above, I estimate that there are at least 5000 rearing professionals throughout the world. They are distributed among facilities that are staffed by a range of 1 through 30 or more people.

E. How much money is being spent on rearing? Again, the scattered nature of rearing and the fact that costs are not a matter of public record in most insectaries, it is impossible to project better than a general estimate. Conservatively, I estimate that between 0.5 and 1.0 billion dollars per year is spent on insect rearing, world-wide. The Tuxtla facility alone spends over $2.2 million on larval diet ingredients, and generally, the total cost of rearing for labor, materials besides diets, and facilities equals about 10-20 x the cost of the diet ingredients. Projecting this formula to the composite of insects reared worldwide for the various programs mentioned above, an estimate approaching one billion dollars per year is probably not exaggerated. In the USDA alone (ARS, APHIS and FS), there are well-over 30 major-sized facilities (staffed by more than 15 people each and with budgets in excess of one million dollars each) devoted to rearing pink bollworms, Gypsy moths, corn earworms, tobacco budworms, fall armyworms, beet armyworms, corn rootworms, coddling moths, boll weevils, tarnished plant bugs, stink bugs, southwestern corn borers, and many other species of pests, as well as their parasites, predators, and pathogens. Because they are generally encompassed in over-all budgets, it is difficult to parse out rearing costs, but in my experiences as research leader for the Biological Control of Insects Laboratory in Tucson, AZ and the Biological Control and Mass Rearing Research Unit at Mississippi State, MS, I observed that more than 50% of the total research budgets at each location was devoted to rearing. The total budgets at those two facilities were 0.7 and 1.8 million dollars, respectively.

II. Where rearing and diets have been? (historical perspectives)

Considering domestication of silk moths in Asia and honey bees, insect husbandry has a history dating back by millennia. Excluding these ancient practices, insect rearing has been practiced as a serious and widespread technology and science for well-over a hundred years and has been especially robust over the last 60 years. The decades of the 1940s through the 1970s were the "golden age" of work with classical addition-deletion studies with defined diets, and it was during this time frame that the foundation of most of what we know about dietary requirements of insects was developed. The addition-deletion and defined diet studies gave way, to some extent, to the radioisotope tracer experiments in efforts to determine the fundamental nutritional needs of various species. While these studies provided a considerable body of information about the basics of insect nutrition, the diversity of insects, the variation in methodologies, and especially self-criticism all contributed to a lack of a complete and comprehensive picture of insect nutrition. Compounded by the inherent difficulties and labor intensity of such study programs, insect nutritionists struggled to overcome the burden and specter of not being able to provide unambiguously pure diet ingredients, especially not in forms that were palatable to the insect targets. For example, casein, which has been a well-used base of protein nutrition, was always subject to ambiguous interpretations because of impurities such as vitamin and mineral residues. Purified amino acids always carry with them traces of other organic compounds and minerals that raised questions about complete deletion studies. Pronouncements of insects that required no iron or zinc are examples of likely failings of such deletion studies. Injected into this time frame were alternative efforts at understanding the basics of nutrition supported by studies of digestive enzymes, nutrient self-selection, extensive analyses of insect foods and whole carcass compositions. Rarely, if ever, were these efforts coordinated in an ‘eclectic’ approach to understanding nutrition.

While all of these struggles were being played out, there were always rumblings in the insect community that such basic studies were not solving the practical problems of mass rearing or production for research. Most funding for diet and rearing research was directed at solving practical problems. This phenomenon prompted basic researchers to make grandiose promises of the potential applications of their work. “Once we know that a given amino acid or vitamin is present in the natural diet of a target insect, we can make an inexpensive, practical diet more useful by simply supplementing that nutrient in an otherwise nutritionally poor diet.” This was the thinking generated by the supporters of the “basic nutrition” approaches. However, there remained a tension between the basic and applied nutritionists, and this tension promoted unfortunate rancor and criticisms of the works of the members of each school. Even today, there remains misunderstanding between the basic nutrition community and the dietetics community. It seems that no such chasm ever developed in the communities of human food science and technology community and human nutrition. This model and its applications are explained here.

III. Where we are (or should be) going

It’s appropriate to start this meeting with a consideration of where the field is going and what future opportunities and challenges will look like. As this audience knows, insect rearing has been and continues to be a core component in virtually every program that is entomologically based. Yet, as I pointed out in my paper in the 2001 *American Entomologist* and in my book on *Insect Diets: Science and Technology* (2003), insect diet and rearing specialists witness frustrations in terms of under appreciation, manifested as difficulties in publishing and lack of recognition. The reasons for the under-appreciation are complex, and they are attributable both to some causes for which we are responsible and other causes that are the domain of the entomological community and society at large. Sometimes, in our rearing/diet community, we have conducted and reported studies that were not of the highest scientific quality. Often, the entomological community has considered advancements made by the rearing/diet community as minimal or trivially incremental, even when the work was a valuable contribution. As a result of the lack of recognition and respected status, recruitment of high quality scientists and support staffs has lagged behind the influx of quality people into other scientific domains such as biotechnology.

After having given this issue a great amount of thought and discussion with many colleagues, I conclude that the path to recognition and respect must include sweeping changes in 1) education of diet and rearing professionals within the complete recognition of the fact that this is a specialized, highly technical field, 2) restructuring the communications processes for reporting scientific and technological advancements, 3) reorganization of the processes used to attack research and development projects, and 4) revision of the hiring, promotion, and funding policies of the customers of the rearing and diet specialists.

Change 1) amounts to improvement of the diet and rearing specialists per say. It calls for recognition of those entering such a specialized field that they will need to acquire a background not only in the fundamentals of entomology but also biochemistry and food science. This is certainly one of the most cross-disciplinary fields, and the special knowledge required includes insect physiology, morphology, pathology, and behavior; multiple aspects of biochemistry; and many of the sub-disciplines of food science and technology, including food chemistry and physics, food processing technology, and food microbiology. Furthermore, the emerging insect diet/rearing specialist will be required to have a grasp of the existing background in this field, which is unfortunately highly dispersed throughout the literature and within the hidden recesses of company and the “cultures” of individual laboratories.

Change 2) would come from the improvement in channels of communication between the current leaders in diets and rearing and the editors of journals and leaders in various entomological societies. What must be understood is the essentiality of setting aside places in journals and other communication domains where advancements in diet and rearing science and technology are not merely tolerated but welcomed. In this vein, there must be a place for incremental advancements. Not every advancement can be monumental, but at the same time researchers must not try to oversell their accomplishments or avoid proper reference to and attribution of the related, background works that set the stage for the current topic. It would relieve a great deal of the pressure on emerging scientists if they did not have to feel that their works had to be a cure for cancer, a huge improvement of the environment, and an elimination of racial prejudice—all in one paper. However, it is the responsibility of the rearing specialists to make sure that each study is carefully planned, well-designed, and adherent to strong principles of science and technology. In other words, if we are to expect our works to be better accepted by the scientific community, we must upgrade the overall quality of these works.

Change 3) expands the scope of topics that are to be considered the legitimate domain of scientific inquiry in diet and rearing research. Traditionally, diet and rearing research authorities have insisted on direct application of insects in all research topics. In contrast with the liberal range of experimental latitude that characterizes studies of human foods (and those of domesticated vertebrates), where studies of food processing strategies and their consequences can be reported in respected publications where these advancements are welcomed. In contrast, the pervasive attitude of the entomological community is that however far-reaching the implications of a diet or rearing study may be, if results are not presented showing direct improvements of target insects, the study is not worth publishing. I provide here an example of the type of study, which should become acceptable where we measured the effects of various kinds of storage treatments on the antioxidant content and peroxidation potential of three artificial diets.

Another restriction on the content of research results is that for results to be considered publishable, they must be considered as making a large, saltatory advancement over the previous state of the field. The causes for this problem are complex—a combination of underestimation of the difficulties inherent in diet and rearing system advancements along with a tendency for researchers reporting their works to exaggerate the scope of their findings. The correction for this situation is a simultaneous realization on the part of editors and reviewers that diet and rearing advancements are very difficult and demanding and compliance on the part of researchers with high scientific standards, which must include proper, honest explanation of the problem being solved, the rationale for the approaches, and proper attribution of credit for the past and supporting works.

Change 4) (revision of the hiring, promotion, and funding policies of the customers of the rearing and diet specialists) would evolve more readily if the first three changes were made. However, what is also essential to promote the credibility that is needed for this change is for entomologists and other scientists who depend upon advancements in insect science to become convinced of the inherent worth and difficulty of rearing advancements. Insect diet and rearing progress will continue to progress slowly and not keep up with the needs of the customers of the rearing community until there is a clear recognition of the value of our contributions and potential of rearing professionals. Only when it is realized by the entire domain where reared insects are used that the applied and basic programs can be no better than the insects they depend upon and that they cannot function if the insects are not available will necessary support for rearing professionals be put in place.

Performance of several insects on a liquid or powder form of a zoophytogenous diet

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Cost, performance, presentation and utility of food are critical factors to the success of an insect rearing program. Factitious food sources and artificial diets are commonly used to lower the cost or increase the availability of a food source. However, non-natural food sources may lower the performance of an insect or may pose a challenge for the method used to present the food to the insect. Additionally, different food sources, for each insect species or developmental stage, elevate the cost of maintaining insect colonies. Consequently, the value of a zoophytogenous diet, originally formulated for the beneficial predator, *Podisus maculiventris*, has increased as our awareness of the competitive costs and utility associated with the diet has extended to include several beneficial and pest insects. Thus far, the zoophytogenous diet has supported the rearing of six beneficial insect and four pest insect species, has a realized cost of rearing that is competitive with natural food for two beneficial predators, and can be presented in liquid, gel or powder form. Currently, research and industry stakeholders are testing the zoophytogenous diet in mass rearing and high through-put bioassay systems.

What can genomics and the other "-omics" contribute to arthropod mass rearing and quality control?

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New technologies enable scientists to measure alterations in global gene expression patterns among living organisms and that capability holds promise of providing information that is valuable to insect mass rearing efforts. A short-term goal for the application of these technologies is the development of molecular markers that can be used to evaluate the health and performance of insect colonies. A long-term goal is to use the biomarkers to accelerate the rate at which we enhance the health of insects in culture and the performance of insects used as biological control agents. The immediate challenge is to integrate the new technologies into a research process that will result in useful biomarkers. Because an insect is capable of compensating for some physiological or environmental stimuli, not every stimulus will result in a net change in the expression of a gene or protein. Additionally, biological and technical variations, as well as rigorous dataset analyses can complicate this type of work. However, the potential for developing universal probes that are rapid and sensitive indicators of the health and performance of insects warrants an investigation into the exploitation of global gene expression patterns.

Quality control guidelines of commercially available natural enemies: current status and future

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From 1991 to 1997 researchers and producers of beneficials worked together to develop guidelines for quality control of natural enemies under the umbrella of IOBC. This resulted in a set of guidelines for the most important natural enemies, giving minimum standards and specifying methods to assess different aspects of quality. Some of the guidelines are still under development. Since 2000 the IBMA is responsible for the guidelines.

Today the guidelines are used by most of the European producers. A discussion is ongoing about parameters, statistics and test methods. Improvement of the guidelines has to be a continuous action, so the guidelines will always be subject of change.

This paper will discuss about certain aspects of the guidelines and will give some new ideas for improvement.

Mass production of arthropods for the biological control of weeds: a Florida focus

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Biological control of weeds involves the planned use of undomesticated target-specific organisms (usually arthropods) to regulate host plant densities. Mass production of healthy individuals is an essential component of successful biological weed control projects, but rearing these plant-feeding arthropods can be a time-consuming and labor-intensive task. In addition, developing the techniques for laboratory or insectary rearing of arthropod weed biocontrol agents can be a challenging endeavor because many of the arthropods are undescribed and their biologies are unknown. However, the importance of establishing and maintaining a vigorous colony of the candidate arthropod for host range testing in quarantine or mass rearing of an approved arthropod for field release cannot be overstated. Factors contributing to the success of the mass production program include selection of the rearing medium (host plant or artificial diet), biotype matching (host plant variety), maintenance of genetic and behavioral fitness (quality control), general hygiene (disease management), pest control on host plants (pesticide selection), the biology/behavior of the weed control agent (mating requirements, oviposition and pupation sites, diapause conditions, and supplementary adult nutrition), and ingenuity of the investigator. Several examples of mass rearing arthropods from aquatic and terrestrial weed biological control programs in Florida, USA, will be presented.

Factitious foods for the production of arthropod natural enemies

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Costs may be reduced when natural enemies can be produced on unnatural or factitious hosts that are easier and less expensive to rear than the natural host. Factitious hosts are organisms that are not normally attacked by the beneficial, mostly because they do not occur in its natural habitat, but do sustain its development and/or reproduction. Eggs of the lepidopterans *Ephestia kuehniella* and *Sitotroga cerealella* are routinely used in the commercial production of various natural enemies including coccinellid beetles, lacewings, predaceous heteropterans, and egg parasitoids of the genus *Trichogramma*. Hatching of the eggs is prevented by UV- or gamma-irradiation or by freezing, but processing may affect the nutritional value of the food for some natural enemies. Although flour and grain moths are easily produced on inexpensive foods (wheat flour or grains), there are substantial investments for mechanization of rearing procedures and health care of workers. Because of a continuously high demand, this has led to high market prices especially for *Ephestia kuehniella* eggs, amounting to 800-1200 Euro per kilogram by the end of the 1990s. Further, long-term rearing of some parasitoids on *Ephestia* eggs (e.g., *Trichogramma brassicae*) or on other factitious hosts has led to significant shifts in host acceptance. Other insects that are frequently used as factitious food in commercial insectaries and research labs include larvae of the greater wax moth, *Galleria mellonella* (for several ichneumonid, braconid and tachinid parasitoids and for predatory stink bugs) and of the yellow mealworm, *Tenebrio molitor* (e.g., for reduviids and predatory stink bugs). The predatory mites *Neoseiulus cucumeris* and *Hypoaspis* spp. are routinely reared on mould mites (*Tyrophagus putrescentiae).* Some non-insect materials may also hold promise for use as foods in insect mass culturing. The anthocorid bug *Orius laevigatus* can be reared on cysts of the brine shrimp *Artemia franciscana* with similar developmental and reproductive success as on *Ephestia kuehniella* eggs. Given that *Artemia* cysts are at least an order of magnitude cheaper than flour moth eggs, they may be an economically viable alternative food for the mass propagation of *Orius* bugs and possibly other predaceous insects.

Evaluation of biological and biochemical traits of the parasitoid *Exorista larvarum* cultured *in vitro* and *in vivo*

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Exorista larvarum (L.) (Diptera : Tachinidae), a polyphagous larval parasitoid, is an important antagonist of forest lepidopterous pests, including *Lymantria dispar* (L.). This tachinid was reared *in vitro* on different artificial diets composed of crude materials with adult yields comparable or even higher than those usually obtained *in vivo* in the factitious host *Galleria mellonella* (L.). *E. larvarum* was produced on two of the most effective diets and in the host *G. mellonella* to make a quality comparison of *in vitro* versus *in vivo*-reared parasitoids. The two diets were respectively based on skimmed milk and veal homogenate and were both integrated with chicken egg yolk, yeast extract, sucrose, gentamicin solution and agar. Significantly higher puparium and adult yields were obtained in the diets than in the host. The diet based on skimmed milk produced the highest yields. The parasitoid development time from egg to adult was significantly longer *in vitro* than *in vivo* and no difference was found between the two diets. The puparia reared in the diets weighed significantly more than those formed in the host. The heaviest puparia were obtained on the veal homogenate-based medium. The longevity of the female flies emerged from puparia of uniform weight was similar in the diets and in the host. The females obtained in the skimmed milk-based diet however oviposited a significantly lower number of eggs than the *in vivo*-reared ones. The biochemical analysis showed that the total amino acid content was lower in the larvae reared in both diets than in those obtained in the host. It cannot be excluded that the lower number of eggs laid by the *in vitro*-reared females is related with this amino acid deficiency. Moreover the tyrosine level was lower in the parasitoid larvae produced *in vitro* than in the *in vivo*-reared ones, probably due to the low tyrosine content of the two diets. It is well-known that this amino acid is important, especially for Diptera, for the melanisation and tanning process occurring at pupation. The tyrosine level found in the *in vitro*-reared parasitoid larvae was however sufficient to promote pupation. We can conclude that the quality of *in vitro*-reared *E. larvarum* may be improved. It is however confirmed that this parasitoid is to date one of the most promising beneficial insects for mass production on artificial diets.

Engineering solutions for insect pest management decision-making in grain - including improved handling of beneficial insects during shipment

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Scientists at the USDA ARS Grain Marketing and Production Research Center are developing technologies to (1) protect grain from quality loss resulting from insect attack during storage, (2) measure biological parameters of pest and beneficial insects, and (3) maintain quality of beneficial insects during shipment from producer to customer. Near-infrared spectroscopy (NIRS) was used to detect hidden insects in wheat kernels, to identify the infesting species, to determine the age of major Coleopteran pests of stored wheat, and to detect the presence of parasitized in-kernel insects. In tests to determine if NIRS could detect internally infested kernels, we collected spectra from single kernels infested with rice weevils, *Sitophilus oryzae* (L.), lesser grain borers, *Rhyzopertha dominica* (F.), and Angoumois grain moths, *Sitotroga cerealella* (Olivier). For all 3 major pest species, we were able to differentiate uninfested kernels from infested kernels. Larval size was a factor in the sensitivity of the system, with 3rd and 4th instar rice weevil larvae being detected with 95% confidence. By analyzing spectra collected from 11 species of stored-grain Coleoptera, we could correctly identify >99% of insects as primary or secondary pests, and correctly identified >95% of insects to genus. Similarly, chronological age rice weevils, lesser grain borer, and the red flour beetle, *Tribolium castaneum* (Herbst), were also well correlated to NIR spectra. In addition, NIRS could differentiate between wheat kernels containing weevil larvae and those containing parasitized weevil larvae. In total, biological information measurable by NIRS during grain storage, i.e. the detection of an insect infestation, pest identification, age structure of pest population, and presence of biological control organisms can provide expert systems with decision making information regarding control options.

Application of NIRS to determination of chronological age of Diptera of veterinary importance was also successful. Confidence limits of predicting age for house flies, *Musca domestica* L., stable flies, *Stomoxys calcitrans* (L.), and face flies, *Musca autumnalis* De Geer, were smaller for NIRS than when using the pteridine fluorescence age-grading method. Young flies could be readily differentiated from old flies. Puparia containing parasitoids could also be detected by NIRS with 80-90% success rate. Detection of viable hymenopterous parasitoids within puparia could assist insectaries in delivering known quantities of parasitized puparia for use in biological control of pests and in rapidly determining levels of parasitization in the field.

We have also developed improved specifications for shipping containers to deliver healthy beneficial insects for IPM practitioners. Heat transfer through the container walls was evaluated to determine the amount of insulation and natural refrigerant (such as ice) necessary for maintaining the internal temperature in the desirable range during shipment. An energy balance on the shipping containers was used to provide the needed design equation to specify the insulation level. Containers with temperature-monitoring sensors were shipped by overnight delivery from a cooperating supplier's laboratory to GMPRC to obtain validation data. Also, standard frozen gels and other potential natural refrigerants were compared in laboratory tests of the containers for the times and temperatures comparable to those measured in the experimental shipments.

Pathogenicity of *Verticillium lecanii* Zimm. Viegas (Deut.: Moniliaceae) on *Euproctis chrysorrhoea* L. (Lep.: Lymantriidae) larvae

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Euproctis chrysorrhoea is an important defoliator of oak and a number of hardwood tree species with irritant hairs which makes the species an important sanitary insect. Introduction of a suitable biological agent together with other control measures is important especially in the case of population outbreaks.

Verticillium lecanii strain DAOM 198499 grown on a solid culture medium was used for pathogenicity and bioassay procedures. Suspensions of 10^4, 10^5, 10^6, 10^7 and 10^8 conidia/ml with a control (distilled water) containing Triton X-100 were used on third instar larvae in a completely randomized design with three replications. In other experiments, treatments included two suspensions (10^6 and 10^8 conidia/ml) and two durations of 97±2 RH% supply (24 and 48 hours after spore application), with or without desiccant material on third instar larvae.

Analysis of the first experiment revealed that suspensions of 10^7 and 10^8 conidia/ml were significantly different from the others ($p=0.0001$). LC_{50} was determined by probit analysis at a concentration rate of 7.2×10^6 conidia/ml. Over 50% larval mortality was observed only in 10^7 and 10^8 conidia/ml suspensions, and LT_{50} values were measured by life test procedure at the rate of 7±0.31 and 5±0.22 days respectively. Treatment of 48 hours duration of RH supply with average mortality rate of 90.95% was significantly different compared to 24 hours RH supply with 40.44% median mortality ($p=0.0006$).

Strategies for improving artificial diets with insect-derived components

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The improvement of artificial diets for many beneficial insects with the addition of insect components is well documented in the literature. Our strategy for diet improvement is based on the premise that yet undiscovered factors are present in insect tissues that could be used to supplement artificial diets and thus promote better growth and development of immature stages and increased of fecundity of female entomophages. Research on the identification of factors, however, is very limited. Specific factors were first reported for certain parasitoids but are incompletely characterized. Insect cell lines that were found to display *in vivo* properties through production of specific products were found to substitute for tissues (e.g. fat body) in promoting development of parasitoids grown in tissue culture. Only in recent studies have factors been implicated in predators. Our studies have indicated the existence of factor(s) in prey tissues, yet to be defined, that promote the fecundity of a predator, *Orius insidiosus* (Say) upon addition to a basal insect-free artificial diet. Research is needed on both parasitoids and predators to provide supplements that may be used to improve suboptimal diets and to better understand the physiological and biochemical roles of such agents.

Quality control of field trials

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Field trials require more effort in “production control”, “process control” and “product control” than basic insect production. The researcher’s greatest difficulty is in performing functions that have not become familiar routines and accomplishing them in a timely manner. Difficulties are compounded by the unpredictability of crops and pests due to weather. Producers can help by offering researchers protocols for accomplishing quality control tasks essential for interpreting the outcome of the field trials. The speaker will describe two routines that she has offered to researchers for gathering quality control data essential in interpreting field trial results.

Development of ASTM quality specifications for invertebrate biocontrol products, complementary to IOBC guidelines

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In 1998, North American producers joined with customers, researchers and government representatives to create a subcommittee of ASTM International with the goal of developing quality assurance standards for commercial beneficial organisms. ASTM is a standards organization that was founded in 1898 for the purpose of creating voluntary and full-consensus standards for such industries as steel and railroads. The subcommittee agreed to develop ASTM specifications that focused on end-product quality control. Driving forces behind this effort were reports of poor quality products in academic publications and a desire to harmonize quality assurance protocols used by producers, distributors and end-users. The ASTM organization was chosen for it's low cost to join and it's built-in administration and publishing capabilities. ASTM uses a full consensus approach, balloting to approve standards and a mandatory five-year review. Existing IOBC guidelines were used as models for new ASTM Specifications, sparking debate over the necessity of developing parallel documentation with implied duplication of effort. In 2002, an informal committee of both ASTM and IOBC coordinators was formed with the intent to harmonize efforts of both groups. This committee resolved to work together to complete IOBC guidelines as a first step, then extract and re-format basic counting methods for publication as ASTM Standard Specifications. ASTM task group leaders are identifying 'critical quality parameters' for each bio-control product and recruiting experts to test practical methods and review draft specifications prior to balloting.

Overview of artificial diets for parasitoid insects, and new prospects

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The artificial rearing of parasitoid insects started a long time ago, and different kinds of diets have been tested, from very simple preparations to chemically defined media. Many kinds of diets could be defined, but the presence or absence of insect components (mainly hemolymph or holotissue extracts) is a critical characteristic for practical considerations. Very few diets are completely defined; most of them include complex components such as hen's egg yolk, cow's milk, liver or meat extracts, yeasts, proteins... The performances of the diets were in close relation with the parasitic status of the parasitoids, showing generally more success with idiobiontic than with koinobiontic species, which usually show closer hormonal interactions with their hosts. The Tachinidae family should be an exception, with 10 species grown in artificial diets including 6 with successful development from egg/first instar larva to adult. Out of 49 species of Hymenoptera (belonging to 9 families) grown in artificial diets, 33 were grown from egg/first instar larva to adult. Complete development was obtained in 45% of koinobiontic species vs. 90% in idiobiontic species grown in artificial conditions, most of them belonging to the Trichogrammatidae family.

The production of insects, even in artificial conditions, has to be linked with quality control. The quality control of parasitoids produced in artificial conditions is still in its infancy. Besides traditional characteristics (mainly morphological, biological or behavioral) it is worthwhile to consider biochemical parameters. The amino or fatty acid composition of parasitoids produced in artificial conditions could be compared with that of control insects developed in natural conditions, to evaluate their quality. Moreover the biochemical parameters (excess or deficiencies in some elements) could be used to deduce modifications in the composition of the diet, possibly leading to improvement of the insects produced. With *Trichogramma*, an increase of the protein content of the diet allowed us to obtain a higher protein content of the insects developed in the modified diet. The lipid / carbohydrate content of the larva is also a good parameter to estimate the capability to develop up to the adult stage, and to give an indication of its life potential or fitness. Key parameters linked with reproduction are especially pertinent, such as vitellin content to estimate the fecundity. Recently nutrigenomics, which applies to nutrition the recent progress of molecular biology, opened a new way for improvement of artificial diets for entomophages. The final criterion for estimating the quality of a parasitoid is probably the parasitization rate under field conditions, however laboratory tests are necessary in a first step.

Evaluation of native aphid parasitoids for biological control of aphids infesting sweet peppers grown in unheated plastic greenhouses in Algeria

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The present contribution is a part of a larger study of some natural enemies of important aphids infesting sweet peppers crops in Algeria. In our opinion, local natural enemies may succeed better than the imported ones.

In the past years an inventory of native parasitoids of aphids was made in an IPM project. *Aphidius colemani* and *Lysiphlebus fabarum* are the two indigenous species selected.

In laboratory experiments, *Aphidius colemani* seems to be the best parasitoid to use for control of *Aphis gossypii* in local conditions during the spring season.

The objective of this study was to test the quality of the parasitoid reared on both *Aphis gossypii* and *Myzus persicae*.

Laboratory experiments on fecundity, female longevity and emergence rate of the parasitoids are reported.

In the greenhouses experiments we attempt to better evaluate the performance of the parasitoid by determining the best host to be used for producing wasps of good quality for inoculative or inundative releases.

Key words: Aphids, *Aphis gossypii, Aphidius colemani,* biological control, native parasitoids, evaluation of quality control, sweet peppers

The perilous journey from producer's gate to farm gate: tools for quality assurance in transit

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In theory, quality of commercial shipments of invertebrate biological control agents (IBCA's) tested at the producer's gate, should be indistinguishable from products arriving at the farm gate. In practice, this quality may be compromised by adverse conditions encountered during transit. In most cases, extremes of temperature, i.e. freezing or heat stress, are responsible for mortality or reduced performance of IBCA's. Other factors affecting quality of IBCA's during transit are mechanical damage, cannibalism and release of metabolic toxins.

Producers have developed practices and protocols to attempt to mitigate these hazards. Examples of such practices include the use of temperature-controlled delivery vehicles, packaging product in temperature-controlled rooms, choosing the best freight carrier for each geographic region serviced and packing shipments appropriately for conditions during transportation and at their destination using ice packs or heat packs in properly insulated boxes. Producers require products to arrive at the farm gate in optimum condition, both to avoid replacement cost and to ensure that the product works as intended. If a farmer releases poor quality IBCA's, the desired results will not be achieved, resulting in disappointment and economic loss. The customer will likely cease to use the product or at the very least, switch suppliers. It is important to the farmer that IBCA's arrive in good condition, both to manage the pest problem and to avoid paying labor costs to release an ineffective product.

In recent years, tools such as freeze watch indicators, and data loggers have been developed to provide qualitative and quantitative data on temperature fluctuations inside a shipping box. Farmers can use data provided to assess at a glance, whether a shipment of IBCA's should be accepted or rejected. Members of ASTM working groups have agreed to identify 'critical quality parameters' for each commercially available IBCA species to be assessed at the farm gate and to select those parameters perceived to be important for field performance.

Development and results of quality assurance testing for mass-reared and laboratory-colonized insects after storage in liquid nitrogen.

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Developing cold storage protocols to supplement rearing regimes for factory- or laboratory-propagated insects can be useful or even a required strategy to ensure that production is economical, reliable, and results in quality insects. However, there is widespread concern by first time users of insects having experienced a "cold storage bottleneck" that irreparable damage or change may have occurred. Resistance by insect rearing personnel to incorporation of a new technology such as insect embryo cryopreservation into an established rearing regime is especially common. To allay these concerns, we have devised and conducted a number of quality assurance tests on 4 species of insects that are extensively propagated for release in area-wide autocidal control programs. The insects tested for fitness after cryopreservation via vitrification were: the Caribbean (CBFF), Mexican (MXFF), and Mediterranean (MDFF) fruit flies and the New World screwworm. For the CBFF, lab determinations were made on fertility and fecundity. Adult progeny from parents cryopreserved as embryos were paired with untreated flies. The number of eggs collected from the mating pairs and the larval hatching was not significantly different from the control levels ($P > 0.01$; n = 10/sample). Male mating propensity and flight mill endurance of male and female MXFF cryopreserved as embryos was evaluated under laboratory conditions and found to be the same as that of the controls ($P > 0.0001$, n = 30 and $P > 0.5$; n = 37). Preliminary studies using RAPD analysis for measurement of a possible change in genetic diversity of MXFF recolonized after cryopreservation, suggest no overall bias in RAPD marker frequencies when compared to untreated parent colony flies. Progeny (G_3) from cryopreserved factory-reared MDFF were field-tested for survival, mating competitiveness, and fecundity. We found no significant differences in these fitness parameters when compared to those of a wild-type control strain ($P > 0.05$; n = 100/sample). Further, lab comparisons on flight ability of newly emerged MDFF adults showed that the cryopreserved line exceeded that of the wild-type ($P < 0.01$; n = 100). Screwworms recovered from liquid nitrogen storage had lower pupal weights and pupation and adult emergence rates than the untreated control flies ($P < 0.05$; n = 200-600/sample) but the sex ratios were similar. However, by the next generation these differences were no longer present. In summary, we have tested 4 species of flies cryopreserved as embryos or as progeny of cryopreserved parents for a number of indicators of fitness or quality. Based on the results, there is little to suggest that permanent damage or change occurs when using an insect cryopreservation protocol such as been developed in our laboratory.

Reference

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The history and mission of AMRQC

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Quality control in the production of biological control agents and sterile insects always has been one complex subject that combines the two applications. In the recent publication, “Quality Control for Mass-Reared Arthropods, Proceedings of the Eighth and Ninth Workshops of the International Organization for Biological Control Working Group on Quality Control of Mass-Reared Arthropods,” Ernst Boller cited work in the early 1940s on artificial selection of parasitoids followed in the 1960s by fruit fly and screwworm mass rearing programs. Boller’s “Behavioral aspects of mass-rearing of insects” and Manfred Mackauer’s “Genetic aspects of insect production,” two corner stone papers on quality control of insects and specifically parasitoids, respectively, appeared in the same volume of Entomophaga in 1972. The concepts in these papers were updated in 1976 and presented in a single symposium at the 15th International Congress of Entomology in Washington D.C. At this conference, Norm Leppla moderated another symposium on the subject, “Characterization and Evaluation of Insect Colonies”. This growing interest in the quality of mass-produced insects paved the way for Boller and Derrell Chambers to establish the Working Group on Quality Control of Mass-Reared Arthropods under the umbrella of the Global IOBC in 1980. Leppla briefly described the history of this working group in its recent proceedings. After about 20 years and nine workshops, this organization was revitalized as the Working Group on Arthropod Mass Rearing and Quality Control (AMRQC) with the mission: to facilitate and advance cost-effective rearing of high-quality insects and other arthropods in support of biological control and integrated pest management. This historic conference is the 10th and first under this new title and mission.

Development of the flight test for *Encarsia formosa*

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Whitefly control is strongly dependant on the searching ability of the parasitic wasp, *Encarsia formosa*. It locates the host through random searching between and within the plant canopy. The ability to fly and search is a fundamental requirement for its effective performance.

The objective of this study was to develop a flight test and to assess flight response to varying temperatures and storage length. Discussion includes the impact of the results on the biological control of whitefly on greenhouse crops and on the development of a performance standard through ASTM.

E. formosa can search for the host by walking, jumping or flying. Assessment of flight alone requires the experimental set up to exclude jumping or walking from being measured. The design of the flight test was determined by testing different sizes of cylinders, studying jumping behavior and by testing response of *E. formosa* to several deterrents. Although not significantly, the number of wasps caught on the sticky lid and wall was highest in the medium size cylinder (10 x 14 cm). To prevent wasps from jumping on the wall sticky surface, it was positioned 6 cm above the base of the cylinder. To prevent wasps' from walking onto the sticky surface a 1.5 cm wide strip of non-toxic chalk was added to the wall, just below the sticky surface. This cylinder was then used to test the flight response to varying temperatures and storage length.

The flight of *E. formosa* was tested at 15, 20, 25 and 30°C and 16:8 L:D photoperiod. A significant curvilinear relationship was found between temperature and percentage of wasps caught flying (df=15,1; n=16; F=138.2; $P<0.0001$; $r^2=0.95$). At 20°C, 87.7 (±2.17) percent were caught flying while at 15°C only 18.56 (±2.77) percent were caught. Most greenhouses in Canada producing vegetables maintain daytime temperatures above 20°C, suggesting that temperature is not a critical factor for the flight of *E. formosa*.

Shipments of *E. formosa* were stored for 1, 4, 8 and 12 days at 7°C (±1.0) and the flight was tested thereafter at 24°C and 16:8 L:D photoperiod. A curvilinear and negative relationship was found between the length of storage and percent of wasps caught flying. The relationship was significant (df=15,2, n=16, F=17.3, $P<0.0001$) and had $r^2=0.72$. This suggests that the length of storage before or after delivery could be a critical factor affecting performance of *E. formosa*.

The flight test is currently being tested by the *E. formosa* suppliers. A draft of the flight test will be presented at the next ASTM meeting. If approved, it will be the first standardized performance test for biological control agents.

The IAEA fruit fly manual

S.D. McCombs

The predatory stinkbug, *Andrallus spinidens* (F.): its occurrence, rearing and potential in Iran

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The generalist predator *Andrallus spinidens* (F.) occurs in the north of Iran where rice is the dominant cultivated crop. However, some reports corroborate its presence in the south of the country. Laboratory colonies of the predator and its prey, *Galleria mellonella* L., were established using techniques described for the well-known asopine *Podisus maculiventris* (Say), particularly those used at Ghent University (Belgium). The predator was collected from Amol vicinity (North Iran), and maintained at 25 ± 1°C, 70-75 % R.H. and a photoperiod of 16:8 (L:D) for about four years. Plexiglas containers of 1.5 and 4 liter were used to house different nymphal instars and adults. Several caterpillars of Noctuidae (e.g., *Naranga aenescens* and *Helicoverpa armigera*) and Crambidae (e.g., *Chilo suppressalis*) were attacked by both nymphs and adults of the predator in rice, maize and soybean fields. Since natural populations of *A. spinidens* are drastically hampered by chemical treatments, agricultural practices should be oriented towards conservation of this beneficial insect. The predator can also be a promising biocontrol agent against defoliator caterpillars in greenhouses.

Key words: *Andrallus spinidens*, Crambidae, *Galleria mellonella*, Iran, mass rearing, Noctuidae, Pentatomidae

Rearing the predatory bug *Macrolophus caliginosus* Wagner on media currently used in the mass culture of parasitoids

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Since 1995 the Laboratorio Allevamento Insetti Utili (Beneficial Insect Rearing Laboratory) has undertaken biological control programmes in Sardinian citrus and olive groves. For this purpose, the parasitoids *Leptomastix dactylopii* (How.) and *Opius concolor* Szepl. were mass reared on currently used substrates. To develop a labour saving method for culturing the mirid bug *Macrolophus caliginosus* Wagner, an attempt was made at integrating the rearing procedures of the two parasitoid species and the predator, by letting them share certain culturing media. Between 1999 and 2001 preliminary investigations were carried out to test the suitability of two substrates used in the mass rearing of the two parasitoids, bleached potato sprouts and mature larvae of *Ceratitis capitata* Wied. - host plants and prey, respectively - as substrates for the predatory bug.

The larvae of the Mediterranean fruit fly killed in hot water were accepted by all stages of *M. caliginosus*. Even though it was found that the mirid bugs reared on the unnatural prey did not perform as well as individuals fed on the natural prey *Trialeurodes vaporariorum* Westw., in terms of development time, fecundity and survival, the use of the fruit fly larvae was regarded as convenient since it greatly simplified culturing the predator. Proper methods for killing, storing and offering the prey should be studied to improve its suitability in mass rearing procedures. Tests for quality control have shown that the mirid bugs reared on the unnatural prey satisfy the quality standards defined by IOBC for *M. caliginosus*. Potatoes were found to be unsuitable substrates for the mass culture of *M. caliginosus* due to a significant reduction in predator fecundity associated with the increase of its density on the hosts and a rather short useful life of the sprouts compared with the development time of the mirid bug.

Development of quality control for tsetse fly mass production

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The adverse impact of trypanosomosis on human and animal health and the economy in Africa has for decades led to a variety of measures designed to control the vectors, tsetse flies, which comprise 22 separate species. For a variety of economic and environmental reasons the use of the Sterile Insect Technique (SIT) has received increasing acceptance for eliminating the last remnants of tsetse populations. Currently, worldwide tsetse fly production capacity is about 180,000 sterile males per week. The projected needs are ca. 1.5 million per week in 2004 and 3 million per week in 2006. The first large scale production facility with a capacity of 1 million sterile males per week is due to start production in 2004.

To achieve this objective it is essential the QC measures suitable for the expanded production be in place. Improvements in QC methodology will not only help to ensure the attainment of these production goals, but will also improve quality of rearing, minimize production costs and generate trained QC and production staff that are mutually responsive and aware of techniques that are required to successfully produce flies and to monitor their quality and suitability for release.

The parameters currently being regularly monitored are mortality, fecundity and pupal emergence. Changes in daily mortality can indicate problems with the holding conditions, feeding conditions or blood contamination. Fecundity (as pupae per female per ovarian cycle) reflects the nutritional quality of the blood and holding conditions, and when expressed as pupae per initial female indicates the overall performance of the colony. For sterile male release, the parameters monitored are sterility, mortality, fliers (the number of flies flying from the emergence box within 5 minutes), sexing error and marking efficiency.

The following areas will also need to be addressed for expanded production:

***Reproductive behavior**:* Sound production and detection, courtship, mate location, strain compatibility, strain competitiveness, re-mating, odour cues/attractants, and mate selection.

***Tsetse fly diet**:* Source, Quality, Storage and Handling, Additives, Artificial diet, Contamination, Feeding regimen, Adaptation to artificial feeding.

***Irradiation of tsetse flies**:* Stage of development, Irradiation atmosphere, Radiation exposure, Dosimetry.

Field release studies

***Colony maintenance**:* Strain management and compatibility, Colonization of species, Salivary gland hypertrophy virus, Sex separation, Feeding equipment and materials, Mortality checks.

Facilities, equipment, and materials for QC

Harmonization of QC Methods

Several of these quality control procedures for the expanded production of tsetse fly can be adapted from other arthropod species that are mass produced for SIT or other purposes.

The IAEA is running a Coordinated Research Project to develop protocols in some of these areas.

Artificial diets for phytophagous insects with emphasis on quality control

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The first phytophagous insect reared on artificial diet was *Ostrinia nubilalis* in the 1940s. From then until the 1960s, the qualitative requirements were well defined for the composition of artificial media, especially for Lepidoptera, Coleoptera and Diptera, and in the late 1960s studies on quantitative nutrition began, allowing great advances in pest management, plant resistance, allelochemicals, etc. In the 1980s, studies on artificial diets especially for parasitoid rearing were intensified, although the expected results have not been reached with *in vitro* production yet. Today, diets for other insect orders have been sought, and the quality of the insects produced in the laboratory is expected to be comparable to that found in nature. In order to evaluate quality, standards are established, observing along the generations quality components assessing morphological, biological, physiological, ecological and behavioral characteristics; in mass rearing, such control is to be directed to the production, process and product stages. Such evaluations are obviously to be carried out in association with the abiotic rearing characteristics, as well as with the eventual effect of pathogens, frequent in artificial diets. These evaluations evolve from the factitious host’s nutritional effect on the parasitoid produced up to problems with the development of diets for monophagous insects; strains failing to adapt to the diets, especially in tropical regions; changes in the physiology which determine changed thermal requirements of insects reared on inadequate diets; change in the dispersing capacity by the alteration of wing size along the generations; change of insect color in its different stages; or in studies of modified (transgenic) plants, the enzyme inhibitors can be dependent upon the diet quality. For such determinations, one can perform regular biological evaluations or molecular, electrophoretic, electroretinographic, olfactometric, enzymatic, chromosomic determinations, studies on resistance to chemicals, damages caused, metabolism, etc., according to the rearing purpose. Genetic characteristics including initial effective size of population, reproduction type, genetic drift, selection and inbreeding must be taken into account in laboratory rearing to produce insects comparable to those found in nature.

Effect of temperature and prey abundance on mass rearing of the spider *Lycosa pseudoannulata* (Boesenberg & Strand) (Araneae, Lycosidae) under laboratory conditions

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Quantitative relationship between the temperature and the speed of development of eggs of *Lycosa pseudoannulata* (Boesenberg & Strand) showed that the incubation time of eggs increased as the temperature rose to about 34°C. Above this temperature the speed of development decreased rapidly as the temperature was raised up to 36°C. At this latter temperature the embryo is unable to develop and hatch. The effect of prey abundance on mass rearing of *Lycosa pseudoannulata* that was reared from 1st stage to adult on the rice moth *Corcyra cephalonica* Staint. and the mosquito *Aedes aegypti* (L.) supplied at different daily rates, showed that the spider was able to capture a specific level of prey and reach adulthood. Mortality varied inversely with feeding rate but few spiders matured at a specific feeding schedule. Both sexes were matured following the fifth moult. The characteristics of growth of the spider as related to food quantity revealed that at least one *C. cephalonica* rice moth on every second day or one *A. aegypti* mosquito per day would be required in the early (I & II & III) stages, and four rice moths or five mosquitoes in the later (IVth & Vth) stages. A linear relationship was also found between the number of prey consumed per individual and the percentage of spiders surviving to adulthood.

Immunoassays for measurement of reproductive fitness

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Immunoassays (ELISAs) were developed to describe and predict responses of female predators to nutritional and environmental influences. These tests were predicated on classical models of egg development (vitellogenesis) in insects such as mosquitoes, the kissing bug *Rhodnius prolixus*, houseflies, and other insects that develop eggs as adults, following a protein meal. Characteristic of these models is a direct response of female insects to the protein meal. Feeding by adult females is followed rapidly (within hours) by synthesis of yolk precursor proteins (vitellogenins) in fat body, secretion of vitellogenin into hemolymph, and its uptake and incorporation into developing oocytes as yolk protein, or vitellin. Quantification of vitellin and vitellogenin in whole insects and hemolymph may allow us to assess the strength of early reproductive response to a meal, and to predict resulting rates of oviposition. Tests in *Podisus maculiventris* substantiated responses within days after feeding on prey. The strength of response was lower and delayed in insects fed on artificial diet relative to those fed on prey. *Orius insidiosus* also demonstrated feeding-dependent vitellogenic response. The *Orius* YP-ELISA has allowed preliminary quality control tests to determine reproductive fitness of insects received from producers in overnight shipments. Comparisons were made between vitellin contents of *Orius* upon receipt following shipment, and after 3 days of feeding. Results suggest that reproductive status in females can be readily monitored, and reflects their initial state following shipment as well as their subsequent ability to respond to diets.

Development of *Exorista larvarum* (L.) (Diptera: Tachinidae) in natural and factitious hosts at three temperatures

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The development of the parasitoid *Exorista larvarum* (L.) (Diptera: Tachinidae) was studied on three Noctuidae host species, *Peridroma saucia* (Hübner), *Pseudaletia unipuncta* (Haworth) and *Xestia c-nigrum* (L.), (Lepidoptera) common in the Azores Archipelago, at three temperatures - 15, 20 and 25ºC. The laboratory host *Galleria mellonella* L. (Lepidoptera: Pyralidae) was also used to compare the parasitoid production on factitious and natural hosts.

The distribution of eggs by classes from 1 to 3, 4 to 7, 8 to 12, 13 to 20 and more than 20 eggs laid in each host, was evaluated in percentage at the different temperatures. The percentage is higher in the class with 1 to 3 eggs, especially for *G. mellonella*, and decreases when the number of eggs increases.

The percentage of the class number of pupae obtained by host decreases when the number of pupae per host increases at temperatures of 20 and 25ºC, and at 15ºC no pupae are formed. All hosts give up to 2 pupae at 25°C and up to 3 pupae at 20ºC, except *G. mellonella*. Only *P. saucia* gives up to 5 pupae per host at both temperatures.

E. larvarum appears to be a very efficient parasitoid with the studied hosts, considering host mortality reaching 80% in most of cases, even when no parasitoid pupae are formed.

These results confirm the polyphagous status (habit) of this parasitoid, but various parasitization effects are registered in the different host species.

Key words: Biological control, parasitoid, *Exorista larvarum*, *Galleria mellonella* L., *Peridroma saucia*, *Pseudaletia unipuncta*, *Xestia c-nigrum.*

Quality assurance at the grower level

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A number of simple and easy methods have been developed by producers and growers to test the quality of biological control agents on samples of products as shipments are received. Some of these have been further developed as IOBC Quality Control Guidelines and others have been developed by the Association of Natural Bio-control Producers (ANBP) using the American Society for Testing and Materials (ASTM) platform. ASTM certification requires grower input and formal acceptance of any standards, therefore, this could become a functional Quality Control standard for use at the grower level. A variety of test methods for *Phytoseiulus persimilis, Encarsia formosa*, *Aphidoletes aphidimyza* and other common biocontrol products are described.

Fluctuating asymmetry as a tool for assessing the quality of mass-reared natural enemies

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The completely symmetrical form of a given organism represents the successful stabilization of all structures during ontogeny. Loss of genetic variation (with attendant loss in fitness) results in increasing asymmetry, measured as Fluctuating Asymmetry (FA) values. FA is defined as small non-directional departures from perfect symmetry in bilateral traits. Such departures result from the inability of individuals to buffer their development against genetic and environmental perturbations, and has become a widely-used measure of developmental stability. Stresses incurred by an organism during development would increase its FA, a value that can be recognized in the parents before any harmful changes are noticed in the progeny.

Mean asymmetry values can be calculated as the absolute differences between the left and right sides of an individual, summed across characters, and divided by the number of individuals. By monitoring a colony's variance FA has been used as a general tool for assessing quality during the mass-rearing of insects, and could be used as an early-warning system for recognizing problems before they arise. An option suggested herein is to use FA as a means for determining the optimum conditions under which to rear natural enemies, as well as for comparing their strains. An important advantage of FA is that its use requires neither costly equipment (except a dissecting microscope) nor highly trained personnel. Practitioners should however beware of statistical pitfalls in calculating FA, especially in measurement errors.

Research on the blowfly, *Lucilia cuprina,* has shown that FA was more consistent than any other quality control parameters that were evaluated during an examination of the effect of stress conditions on blowfly development.

Results of a preliminary study on FA in the mass-reared predatory mite *Phytoseiulus persimilis*, and its relation to standard quality control parameters, such as fecundity and longevity, are reported herein.

The development of biotechnology for a genetic control technique of codling moth

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Codling moth (*Cydia pomonella* L) (Lepidoptera: Tortricidae) is a cosmopolitan insect pest of deciduous fruits in Armenia. Apple is the preferred host of codling moth. This is the reason that the codling moth occurs where apples are grown in Armenia. Besides apples, the codling moth can develop on pome fruits such as pear and quince, on stone fruits such as apricot, plum, peaches, walnuts etc.

Different methods of control have been used against the codling moth. Chemical control has been the most extended method for a long time. Codling moth larvae damage fruit directly making it a pest that can be tolerated at only very low levels. For seven decades, chemical measures have been the mainstay of codling moth control, but insecticide use is beginning to fail on worldwide scale due to evolution of pesticide–resistant strains. Replacing conventional control tactics with safer and less disruptive controls requires a combination of tactics. Environmentally benign biological controls operating alone cannot provide sufficient suppression of codling to protect fruit. The most promising tactics include mating disruption, insect growth regulators, a granulosis virus (biological controls, sterile insect release and cultural practices most likely to be used in various combinations).

Earlier we developed the genetic method of codling moth control, based on the phenomenon of inherited sterility (HD). This modification is very simple and uses introduction of diapausing larvae into pest natural populations to synchronize the development of introduced and natural insects. To develop the biotechnology of production and release of HD material it is of strong necessity to study thoroughly the HD producing terms, the conditions of HD material storing and other problems. Our work was devoted to answer these questions. The results obtained suggest the following. Developing larvae of the first generation (F_1) were bred at 25-26^{0}C, atmospheric relative humidity 50-60% and at short day length (L: D = 8:16) to induce the diapauses and long day (L: D = 18:6) to induce the development without diapause.

HD F_1-diapausing larvae prepared during the previous season were divided into two groups and were stored under various conditions: in refrigerator (at + 5^{0}C) and under natural conditions in netted nurseries. Half of the larvae from the refrigerators were transferred into orchards at the beginning of spring reactivation of wild populations (the 1st term), the second half – at the time of the pest's second generation development (40 days later) (the 2nd term). The flight dynamics of wild and F_1-moths were compared. To this end the observations were carried out on F_1-moths in nurseries and wild ones with pheromone traps.

The results obtained may be taken into account for the development of biotechnology of HD material production.

Quality control and mass production of natural enemies: where do we go?

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There is a great need for quality control in the production and use of natural enemies, because deterioration of mass reared biological control agents leads to failures in pest management. The area of quality control is rather new for biological control workers. Therefore, a book with contributions from many IOBC partners was prepared on this topic specifically for biological control agents (van Lenteren, editor, 2003). The first section of the book is devoted to emergence of quality control for natural enemies. The need of quality control for mass produced biological control agents is discussed, and the aspects of total quality control for the production of natural enemies are described. The second section of the book provides scientific background information for quality control workers. It explains the basis of variability in foraging behavior of natural enemies and describes technologies illustrating how to manage this variation. This section makes clear that insight into behavioral variability in the foraging behavior of natural enemies is a prerequisite for proper mass rearing and efficient application of natural enemies in pest management. The third section focuses on how to cope with this variation. A population genetic perspective is given on how to manage captive populations. Examples of adaptation to captive rearing and of the trade-off with field performance are presented. Effects of a transfer of natural enemies from the field to a mass production facility are described, such as reduction of fitness and enhancing the possibility of fixation of deleterious mutations in the population by genetic drift. Also ways to prevent these negative effects are presented. Further, the possibilities and advantages of unisexual reproduction for biological control are discussed, and mass production of natural enemies on artificial media is reviewed, particularly with regard to their quality. Finally, pathogens of mass-produced natural enemies and pollinators, and the effects of these pathogens on performance of the infected organisms are discussed. The fourth section gives an overview of the species of natural enemies that are mass produced world-wide. The fifth section contains chapters that describe developments towards quality control testing of natural enemies in North America and Europe, and discusses the need of quality testing beyond the Petri dish. The sixth and final section deals with currently used quality control tests (parasitoids, predators, and microbials), and presents basic statistical methods for analysis of the data obtained with the quality control tests.

The current quality control guidelines will certainly undergo modifications in the coming years. First, it is expected that simple tests will be included to determine the flight capacity of mass reared biocontrol agents. Next, semi-field and field performance tests will be developed. Finally, based on extensive ring testing by the mass production industry and comparison of results of the current tests with those of the new flight and performance tests, a new set of criteria will likely evolve.

Reference

Lenteren, J.C. van (ed.), 2003. Quality Control and Production of Biological Control Agents: Theory and Testing Procedures. CABI Publishing, Wallingford, UK

Regulation of release of natural enemies: need or non-sense?

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In the past 100 years many exotic natural enemies have been imported, mass reared and released as biological control agents. Negative environmental effects of these releases have been reported in a few cases. The current popularity of inundative biological control may, however, result in more problems, as an increasing number of activities will be executed by persons not trained in identification, evaluation and release of biological control agents. Therefore, a methodology for risk assessment has been developed as a basis for regulation of import and release of exotic natural enemies used in inundative forms of biological control (i.e. not in ‘classical biological control’ though some of the same principles and approaches apply) (van Lenteren et al., 2003).

A general framework of a risk assessment methodology for biological control agents is proposed, integrating information on the potential of an agent to establish, its abilities to disperse, its host range, and its direct and indirect effects on non-targets. Of these parameters, estimating indirect effects on non-targets will be most difficult, as myriads of indirect effects may occur when generalist natural enemies are introduced. The parameter ‘host range’ forms a central element in the whole risk evaluation process, because lack of host specificity might lead to unacceptable risk if the agent establishes and disperses widely, whereas, in contrast, a monophagous biological control agent is not expected to create serious risk even when it establishes and disperses well. Drawing on published information and expert opinion, the proposed risk assessment methodology is applied to a number of biological control agents currently in use. These illustrative case histories indicate that the risk assessment methodology can discriminate between agents, with some species attaining low ‘risk indices’ and others scoring moderate or high. Risk indices should, however, not be seen as absolute values, but as indicators to which a judgement can be connected by biological control experts for granting permission to release or not.

This risk assessment method forms one of the elements of a guidance document for the regulation of Invertebrates as Biological Control Agents (IBCAs) within Organization for Economic Cooperation and Development (OECD) countries, which is expected to be released in September 2003. The information on which permission for use of natural enemies will be based, consists of (1) characterization of the natural enemy, (2) assessment of human health and safety, (3) assessment of environmental risks, and (4) assessment of efficacy.

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Mass-rearing beneficial insects for biological control of flies

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Filth flies have a wide array of natural enemies that can be exploited for augmentative biological control. Mass-rearing methods have been developed for several predators, including the mite *Macrocheles muscaedomesticae*, the histerid beetle *Carcinops pumilio*, and the black dump fly *Hydrotaea aenescens*. The most widely used natural enemies are pteromalid parasitoids in the genera *Muscidifurax* and *Spalangia*. Rearing methods for these parasitoids are straightforward, but require close attention to detail and the biology of the species being produced. The first requirement is an economically efficient method for mass-rearing house fly hosts. Material costs are fairly low. The USDA lab in Gainesville uses two different larval fly diet recipes; costs for these diets total $0.45 and $0.57 per 10,000 fly pupae produced. Eggs are added to media at a rate 2.5 ml eggs per 4 lbs of dry media (plus water) in an effort to maintain a target pupal weight of 20mg. Pupae are exposed to hosts for about 3 days, using host:parasitoid ratios that ensure high rates of parasitism without causing excessive mortality and male production because of superparasitism. Host:parasitoid ratios depend on parasitoid species and age, but a good starting point is 4-5 pupae/female/parasitoid/day. The period of practical fecundity of a cohort of female parasitoids varies widely among pteromalid species. Female *Muscidifurax raptorellus* are productive for about 7 days, whereas *Spalangia cameroni* is productive for 21 days. Rearing protocols also need to take into account whether the species is solitary or gregarious and the aggressiveness of parasitoid larvae in superparasitized hosts.

Most filth fly parasitoids are vulnerable to *Nosema* and *Wolbachia* infections, with the latter emerging as a recently recognized phenomenon. *Nosema* disease is common in mass-reared pteromalid colonies, where transmission is amplified by horizontal transmission within superparasitized hosts and the high efficiency of maternal transmission. Infected parasitoids have shorter lifespans, longer development times and reduced fecundity compared to healthy individuals. Such infections are easily detected, however, and can be prevented in colonies by good sanitation practices during initial colony establishment. The Pasteur method can be used to eliminate disease in colonies where infection rates are low to moderate, and heat and drug therapies are available to manage the disease when infection rates approach 100%. Differences in development times between healthy and infected parasitoids can also be exploited by using diagnostic windows of development times in which high proportions of healthy parasitoids are expected to emerge. Drug or heat treatment can be followed up by the Pasteur method to eliminate the disease.

Novel diet encapsulation / packaging methods

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Presentation of artificial diets for insects, including parasitoids and predators, must be made in a form that meets the behavioral requirements of these organisms. Practical considerations require that the presentation approach also is cost-effective, allowing rapid and convenient encapsulation / packaging of the diet. Exposure of the diet to desiccation and deleterious microorganisms must be minimized. Diet handling also should be convenient for introduction into the insects' environment and removal thereafter. We will discuss two systems that have been successfully developed to encapsulate or package insect diets to meet these objectives. A simple system for stretching, forming, filling and sealing Parafilm or other elastic films will be described, along with a novel method for production of Hydrocapsules ® , individual diet 'pills' that can be filled with a wide array of aqueous solutions / suspensions / diets. Hydrocapsules offer a completely new way to deliver food and water (or control agents) to insects and they allow unparalleled opportunities for incorporation of kairomones and phagostimulants to increase discovery, recognition and acceptance of these capsules.

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