PRODUCTION OF INSECT DIETS FOR COMMERCIAL USE

Allen Carson Cohen¹,²,³

¹Insect Diet and Rearing Institute, P.O. Box 65708, Tucson, AZ 85728-5728, idri@insectdiets.com
²Cooperator, Carl Hayden Honey Bee Research Laboratory, USDA, ARS, 2000 E. Allen Rd., Tucson, AZ 85719
³Adjunct Associate Professor, Department of Entomology, University of Arizona, Tucson, AZ 85721

ABSTRACT

Despite large expenditures for development of mass-rearing/artificial diet-based technology over the past five decades, successful commercialization of biological control systems based on such technology has been sparse. Using mass-rearing based sterile release programs such as those used with pink bollworms, screwworms, and tephritid fruit flies as models of successful mass-release potential, the accomplishments of augmentation biological control are disappointing, including those of the commercial sector. The complex reasons for the disappointing results are much less attributable to legal restrictions on technology transfer or insufficient public investment in research efforts than they are to the inherent difficulties in developing mass rearing programs for entomophages and failure to appreciate these difficulties by researchers, program managers, and stakeholders. An important source of failure is the misconception by diet researchers that most diet failures were nutritionally-based rather than failings of other diet functionalities such as sensory qualities, availability, and stability. Related to this problem is an absence of any kind of education or training to do diet and rearing research. Also, many of the shortcomings of diet development are attributable to failure to recognize the tremendous difficulty in the problems of diet and rearing system development.

INTRODUCTION

Where did we go wrong?

Worldwide, a large amount of public and private sector funding has been and continues to be committed to development of programs of augmentation biological control and other biologically based strategies of pest management. Overall, substantial research efforts are dedicated to advancing information so that the private sector can succeed in commercial use of biological control. However, documented commercial successes in such programs are disappointingly rare. This presentation is an effort to explain why these programs have fallen short of expectations and what is needed for augmentation programs to succeed. The emphasis is especially on development and usage of artificial diets (AD) and AD-based mass rearing systems.

A barrier to the development and transfer of technology from the public sector to the private sector was overcome by such legislation as the Bayh-Dole Act of 1980, the Small Business Innovation Development Act of 1982, and the Federal Technology Transfer Act of 1986 (Appendix I Anonymous 2004). Although it could be argued that the zeal of directors of public research efforts to demonstrate success in technology transfer injects some conflicts of interest, exaggerations of research product quality, and other obstacles to successful, seamless technology flow, the laws cited in Appendix I certainly remove the legal obstructions. If retarded progress is not due to legal obstruction of technology development and flow, is lack of funding the primary fault? Most researchers and research managers would argue that more money would improve problem solving. However, there are substantial public and private funds dedicated to the research on biological control and related science and technology. In this author’s opinion, how the money has been managed is more of a problem than how much money has been appropriated. For example, at least ten USDA, ARS laboratories have considerable programs in diet and mass rearing system development. Likewise, the USDA Forest Service and APHIS each have substantial programs in this field, and state institutions have their own research facilities, part of which are supported by federal as well as state funds. Many of the 135 North American biological control suppliers and 100 butterfly companies have some degree of in house research on diets and rearing. Some of the research and development in private companies is supported by Small Business Innovation Research (SBIR) grants. A conservative estimate of all these research efforts would be $80-90 million per year in North America alone. World-wide, there are also substantial efforts in this field in Brazil, Europe, Israel, South Africa, India, China, Japan, Indonesia, and Australia. Yet despite the size and the impressive resources dedicated to them, these programs have a great deal of overlap and lack of coordination, and in this author’s opinion, the direction in these efforts is not focused on the most potentially productive questions or lines of inquiry.
If the barriers to implementation of commercially feasible large scale augmentation biological control are not legal or financial, then what are the real reasons for this failure? In this author’s experience, the most substantial single factor holding back desired progress in commercialization is the underestimation or lack of appreciation by researchers, program managers, and stakeholders of the complexities involved in diet and rearing system development. When we realize that what we are trying to accomplish is a simulation of systems that developed over hundreds of millions of years, we begin to see the difficulty imposed upon diet/rearing researchers. The failing that results from underestimating the difficulty of diet and rearing system development is that the researchers who plunge into efforts at diet development are remarkably under-educated in the complexities of diet and rearing system science and technology; and they are expected by their managers and the stakeholders who “support” the research to get the job done in an unrealistic time frame, under inhospitable conditions (inadequately trained support staff and improperly designed facilities).

For example, although diets are incredibly dynamic and complex mixes of multiple, interactive functional components, diet development is often treated in the most simplistic manner. Cohen (2003) has explained that there are four equally important functional domains of diets: 1) nutritional value, 2) sensory qualities, 3) availability, and 4) stability. However, too often, diet researchers pay exclusive attention to the nutritional functions of diet components without considering phagostimulation, textural factors (hydrocolloid characteristics, particle size, particle shape, viscosity, and other rheological matters), meta-nutritional antioxidant functions, anti-nutrients, bioavailability, water activity, antimicrobial potential, over-all stability, microbial deterioration, and numerous other features that must be juggled skillfully to achieve development of fully functional, successful diets (Cohen 2003, Cohen and Crittenden 2004, Inglis and Cohen 2004). In this vein, a most unfortunate confusion has developed regarding the terms “insect nutrition” and “insect dietetics,” with an even more unfortunate tacit assumption that nutrition is a “real science” and dietetics an extension of fast food cooking. As a result of this confusion and the prejudice against dietetic inquiries, researchers neglect most of the key issues that would explain how diets and certain diet components work or fail to work.

Fig. 1. This figure shows the similarity of profiles of amino acid compositions of various foods that may be used by entomophagous insects (synthesized from Cohen 2003). The ranges of total protein concentrations in each food should be noted.
Diet researchers have often pursued the “Holy Grail” of cryptic nutrients or “nutrient factors.” The nutrient factor hypothesis was derived from a few remarkable successes and nutritional advancements such as Hobson’s (1935) discovery of the absolute requirement for sterols in insect diets and similar works (reviewed by Cohen 2003) in which the essentiality of some unexpected nutrients were discovered. This line of thinking often prompted undue attention to nutrients. For example, though they are certainly key nutrients, amino acids have received disproportionate attention by researchers trying to develop artificial feeding systems (Cohen 2003). Figure 1 illustrates that the various sources of protein (casein or milk protein, wheat germ, broccoli, pea aphids, and egg yolk) each contain all the essential amino acids in relatively similar profiles. Figure 2 illustrates the similarity of profiles of essential amino acids in Geocoris punctipes (Say) (Hemiptera: Geocoridae) derived from different diet sources (field, tobacco budworm eggs, and artificial diet described by Cohen 1985). What is evident from these two figures is that 1) insects have a broad latitude in their metabolic capability of regulating amino acids and that a wide variety of crude, and 2) most natural foods offer useful profiles of these all essential nutrients. These types of observations have led this author to conclude that a too many finite research resources have been exhausted in quests nutritional solutions to meta-nutritional problems.

Fig. 2. Profiles of amino acids from whole carcass hydrolyzates analyzed by ion exchange chromatography of samples of five insects from each group replicated in triplicate (Cohen unpublished data).
The quest for an artificial diet for lacewings, big-eyed bugs, and *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) are examples of how asking the wrong questions can lead to disappointing results. The hopes and promises for having mass scale production and release of predators and parasites such as those offered in the 1960s (e.g. Ridgway and Jones 1969) never reached the desired fruition. It is this author’s opinion that much of the failure to realize these achievements stems from insufficient understanding of the basics of predator and parasite feeding systems. Early thinking about these entomophagous insects was that they were “liquid feeders” adapted to ingest the fluids of their prey/hosts. In keeping with this incorrect thinking, earlier researchers persisted in offering these insects liquid diets whose nutrients were dilute and were restricted by the requirement that they be water soluble or water dispersible (Cohen 1981, 2003). Although for well-over three decades, researchers have attempted to devise diets for *Trichogramma* spp. (Hoffman et al 1975, Nordlund et al. 1997), it was only recently that it was recognized that these egg parasitoids were using host egg solids rather than liquids as their natural dietary mainstay (Wu et al. 2000). Evidently, the partial successes in *Trichogramma* spp. rearing on artificial diets that were reported over the years came from the presence of suspended solids from chicken egg yolk, hemolymph, or “egg liquid suspensions,” (Nordlund et al. 1997, Wu et al. 2000).

**Scientific/intellectual needs**

The research community is charged with the delivery of several specific scientific products: 1) specific knowledge of which biological control agents are going to be effective (e.g. works such as those of Rosenheim et al 1993 done before rearing operations are undertaken), 2) how these organisms are best applied [pre-release conditioning, life-stage for release, method of application, field preparation such as “nursery crops,” cover crops and host plant/ prey phenology (Bugg 1992, Daane and Yokota 1997)], 3) how these organisms are to be reared (including artificial diets, diet presentation systems, other aspects of rearing such as physical conditions, sanitation, harvesting of useful life stages, colony propagation, and automation for all of these component processes).

**A.** The first requirement is a comprehensive and well-documented plan of identification of target agents (species and biotype) which will be useful, how many are required, how they will be delivered to their site of biological impact, and how the agents will be disposed of once they have done their job. It is essential to have a thoroughly documented and convincing basis of understanding of the biological control agents that are targeted for use in our programs. The considerable costs of development of diets, rearing systems, and release/applications technology are wasted when the resources are applied to the wrong species, and these resources are withheld from potentially feasible projects. Also, it is defeating news for researchers to learn after years of dedicated study that their target species has been relegated to the trash bin as ineffective because it is now regarded as ineffective due to concerns for entomophage specificity, intraguild predation, incompatibility with the intended environment, etc.

The type of coordination required for development of these requirements and those that follow must come under the auspices of an organizing superstructure such as the community of funding organizations and appropriate advisors. Unfortunately, the leadership in organizing efforts to develop coordinated programs in augmentation biological control has not been effective and has not provided a coherent master plan. The “five year plans” used extensively by the Agricultural Research Service are based on the sound premise that complex scientific efforts require careful planning and a reasonable time frame to allow programs to develop to fruition; however, too often, political agendas or other non-science based distractions cause planned programs to veer from their course. Organizations and working groups such as IOBC or W84 have access to most of the scientific expertise that could provide the structure for effective and complete augmentation programs, but they lack the funding and administrative authority that would lead to the desired accountability.

**B.** Once a viable master plan is wrought and appropriate target organisms are selected, a thorough understanding of the biological requirements must be developed (including the feeding biology, as well as the reproductive and environmental requirements). For example, the **exact** feeding target of the species in question must be determined. One of the most important tools that a diet development researcher can have is an exact knowledge of the crude composition of the exact feeding target so that the researcher can design the artificial diet to match the texture, “mouth feel,” macro-nutrient content (moisture content, proteins, lipids, carbohydrates, inert ingredients, minerals, and vitamins), and bioavailability of the natural diet. A thorough knowledge of natural feeding stimuli, including token stimuli, will also greatly enhance the chances of success in diet development. All this knowledge must be developed through careful analysis of feeding habits and recourse to chemical analysis of the natural foods or access of a data base of the specific food composition. For example, if a cotton boll feeding specialist is using only the endosperm of seeds, then the specific composition of the endosperm is most useful.

**C.** Once the researcher has a clear picture of the physical and chemical nature of natural foods and the specifics of feeding mechanisms, the researcher can begin to use available crude food substitutes such as wheat germ, soy products, meat products, chicken eggs, or yeast products, to mention a few. One of the most important
concepts—one that has evaded many diet researchers—is that once an assemblage of crude components is designed to match the crude composition of insects’ natural diets, the remaining questions of adequacy of artificial diets is tied to diet consumption (phagostimulation), bioavailability of nutrients, and stability of diets as a whole.

D. Contrary to popular belief, the problem of artificial diet-based rearing system development is not solved once diets that are capable of meeting feeding, nutritional needs, bioavailability requirements, and stability are developed. Some of the most challenging problems of commercializing artificial diets for mass rearing systems can be the scale-up of large quantities of diet, the packaging or presentation of diets, the stabilization of diets (including preservation), and the development of rigorous, responsive quality control systems.

Guidelines to successful programs

In Table 1, eight changes are suggested in our approaches to reaching the goal of commercial success in development of augmentation biological control programs. Some of these changes are elaborated by Cohen (2002) and deal with upgrading the appreciation for the difficulties and complexities of diet and rearing system development and formalizing education of diet and rearing specialists. It would also be a huge boost to the potential for progress if diet and rearing specialists had a dedicated journal section in which the standards for high quality publications are clearly delineated and the editors and reviewers were specialists in diet and rearing related disciplines. In this vein, establishment of programs of support and encouragement for mentors would help the field by setting up cooperation from the few specialists who could share their valuable backgrounds. Finally, one of the most important changes that could be made towards advancement of the problem-solving process would be the re-direction of attention to the mechanisms of how and why diets are effective and the “flip side:” how and why diets fail. This calls for scientifically based inquiries into the “meta-nutritional” aspects of diets: sensory qualities, availability of nutrients, and stability (exemplified by such studies as those by Inglis and Cohen 2004, Cohen and Crittenden 2004, and Cohen et al. 2004).

Table 1
Factors that need to be improved to facilitate application of artificial diet-based technology for commercialization.

<table>
<thead>
<tr>
<th>Past practices</th>
<th>How we should do it</th>
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<tr>
<td>1. Underestimation of the difficulty of developing diets and rearing systems that simulate all the requirements of complex organisms</td>
<td>1. Educate researchers and their stakeholders as to the complexity of understanding and applying diet &amp; rearing system dynamics</td>
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<tr>
<td>2. Target insect chosen for wrong reasons and sparse knowledge of feeding &amp; other aspects of biology</td>
<td>2. Choose best species for intended job and develop thorough knowledge of target’s biology</td>
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<td>3. Ignorance of best release methods</td>
<td>3. Develop best release methods before development of rearing system</td>
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<td>4. Attention to nutrition in lieu of attention to sensory aspects, availability, and stability</td>
<td>4. Encourage attention to sensory aspects, availability, and stability</td>
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<td>5. Diet &amp; rearing training on-the-job learning</td>
<td>5. Formal training (see Cohen 2002)</td>
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<td>6. Difficulty publishing diet/rearing works</td>
<td>6. Establish rearing/diet section of journal with expert editor and reviewers</td>
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<td>7. Poorly defined standards in rearing/diet field</td>
<td>7. Clearly define rearing/diet standards (experimental designs, etc.)</td>
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<td>8. Little mentorship for diet/rearing novices</td>
<td>8. Establish program of reward for mentors</td>
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Appendix I (Anonymous 2004)

Bayh-Dole Act of 1980 (PL 96-517)

• Permitted universities, not-for-profits, and small businesses to obtain title to inventions developed with
governmental support.

• Provided early on intellectual property rights protection of invention descriptions from public
 dissemination and Freedom of Information Act (FOIA).

• Allowed government-owned, government-operated (GOGO) laboratories to grant exclusive licenses to
 patents.

Small Business Innovation Development Act of 1982 (PL 97-219)

• Required agencies to provide special funds for small-business R&D connected to the agencies’ missions.

• Established the Small Business Innovation Research Program (SBIR).


• Made technology transfer a responsibility of all federal laboratory scientists and engineers.
• Mandated that technology transfer responsibility be considered in employee performance evaluations.

• Established a principle of royalty sharing for federal inventors (15 percent minimum) and set up a reward system for other innovators.

• Legislated a charter for the Federal Laboratory Consortium for Technology Transfer and provided a funding mechanism for that organization to carry out its work.

• Provided specific requirements, incentives and authorities for the Federal Laboratories.

• Empowered each agency to give the director of GOCO laboratories authority to enter into cooperative R&D agreements and negotiate licensing agreements with streamlined headquarters review.

• Allowed laboratories to make advance agreements with large and small companies on title and license to inventions resulting from Cooperative R&D Agreements (CRADAs) with government laboratories.

• Allowed directors of GOGO laboratories to negotiate licensing agreements for inventions made at their laboratories.

• Provided for exchanging GOGO laboratory personnel, services, and equipment with their research partners.

• Made it possible to grant and waive rights to GOGO laboratory inventions and intellectual property.

• Allowed current and former federal employees to participate in commercial development, to the extent that there is no conflict of interest.