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

N.C. Leppla

K.A. Bloem

R.F. Luck

UNIVERSITY OF FLORIDA

IPM Integrated Pest Management Florida

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Edited by

Dr. Norman C. Leppla
University of Florida
Institute of Food and Agricultural Sciences
Department of Entomology and Nematology
Gainesville, Florida 32611

Dr. Kenneth A. Bloem
USDA-APHIS-PPQ-CPHST
National Biological Control Institute
at Florida A&M University Center for Biological Control
Tallahassee, Florida 32307

Dr. Robert F. Luck
University of California, Riverside
Department of Entomology
Riverside, California 92521

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PREFACE

Quality Control for Mass-Reared Arthropods was written to document the two most recent workshops of the International Organization for Biological Control of Noxious Animals and Plants (IOBC), Working Group on Quality Control of Mass-Reared Arthropods (WGQC), now the Working Group on Arthropod Mass Rearing and Quality Control (AMRQC). These workshops were held at Santa Barbara, California (1995) and Cali, Colombia (1998). The book perpetuates a determined effort to assure that insects for use in integrated pest management (IPM) are reared properly and maintained at the highest possible level of quality. Great strides have been made in incorporating quality control into large scale mass rearing systems, such as those used to support sterile insect releases and augmentation biological control. However, quality control is not yet institutionalized in ways that satisfy consumer expectations for insects as industrial products. This is particularly important for product efficacy in international marketing of natural enemies and the associated harmonization of plant protection regulations. Consumers increasingly demand to know that the insects they purchase will meet their expectations.

The information from the two workshops is suitably integrated into one set of topics. The comprehensive introduction provides a context for quality control of mass-reared arthropods within the mission of IOBC and the role of AMRQC in arthropod mass rearing and commercial biological control. Reports are organized into the following sections: Organizational History, Status of Quality Control in North America and Europe, Quality Management, Nematode Quality Control, Quality Control of Predators and Parasitoids, Field Performance of Natural Enemies, Host Rearing and Artificial Diets, and Quality Control for Sterile Insect Technique. Reports are followed by a list of participants for each of the two workshops and Association of Natural Bio-control Producers (ANBP) Product Profiles for selected commercial natural enemies.

The rationale for the workshops at Santa Barbara and Cali was to address current issues and ongoing activities from previous meetings at Wageningen, The Netherlands (1991), Horsholm, Denmark (1992) and Rimini, Italy (1993). The Santa Barbara meeting focused on quality control for commercial natural enemies and impending plant protection regulations in Canada, Europe and the U.S. Significant progress was made in achieving the associated objectives, but the industry remained fragmented. European companies continued to establish IOBC guidelines and those in the U.S. began to develop American Society for Testing and Materials (ASTM) standards. Some individual companies adopted the International Organization for Standardization (ISO-9000) system and others used independent proprietary methods. The subsequent workshop at Cali emphasized the essential linkages among arthropod rearing, quality control standards, and product efficacy in the field. To better capture this more comprehensive approach to product quality assurance, the IOBC working group changed its name to AMRQC.

This expanded working group was revitalized under new European and North American leadership, increased its collaboration with global commercial biological control organizations, and assumed a more central role in harmonizing international regulations for marketing natural enemies. It is independent of commercial and national interests, and has access to the

scientific expertise needed to help develop and implement species-specific quality control tests. Future AMRQC workshops will build on the information documented in this book and the foundation provided by the working group during the past 20 years.

ACKNOWLEDGEMENTS

The workshop at Santa Barbara was sponsored by the IOBC; University of California, Riverside, Department of Entomology; UC Center for Exotic Pest Research; U.S. Department of Agriculture, Animal and Plant Health Inspection Service, National Biological Control Institute; and the Association of Natural Bio-control Producers. Lisa Forrester and Terrie Love provided local arrangements support, Jake and Mary Blehm contributed a special tour of Buena Biosystems, Tom Roberts conducted a tour of IPM in citrus, and the staff the Fillmore Protective District Insectary explained their historic augmentation biological control program. The Corporacion Colombiana de Investigacion Agropecuaria (CORPOICA), Centro Internacional de Agricultura Tropical (CIAT), Universidade Nacional de Colombia- Sede Medellin, Universidade Nacional de Colombia- Sede Palmira, Comercializadora Internacional de Insumos Biologicos (COINBIOL), and University of Florida, Institute of Food and Agricultural Sciences provided financial support for the meeting at Cali. We thank Dr. Scobie, the director of CIAT for providing excellent facilities and a thoughtful opening address, his secretary for arranging the Cali excursion and cheerfully helping with endless details, Jorge Pena for simultaneous Spanish/English translations and participation, Toni Belloti for assisting with communication, Tom Ashley for technical assistance and translation, Sherif Hassan for chairing the Trichogramma meeting with the WGQC workshop, and Fulvia Garcia Roa and staff for superb local arrangements. Pam Howell prepared the electronic manuscript. Joop van Lenteren and Luc Tirry assisted in publishing of the book.

INTRODUCTION

The International Organization for Biological Control of Noxious Animals and Plants (IOBC) was established in 1950 and now has six sections: Asia and Pacific Regional Section (APRS), Afrotropical Regional Section (ATRS), Nearctic Region (NRS), Neotropical Regional Section (NTRS), East Palaearctic Regional Section (EPRS), and Western Palaearctic Regional Section (WPRS) and about 45 working groups, including the Arthropod Mass Rearing and Quality Control Working Group (AMRQC), formerly the Working Group on Quality Control of Mass-Reared Arthropods (WGQC). The overall goal of IOBC is to promote Biological Control and it publishes the journal, BioControl. AMRQC has about 250 members, equally split between the NRS and other regions, 32 states in the U.S. and 39 countries.

At a recent IOBC conference in Montpellier, France, "Technology Transfer in Biological Control: From Research to Practice," resolutions were adopted to develop new technologies for augmentation, including the mass production, formulation and delivery of natural enemies. Regulatory authorities were encouraged to develop systems that are science-based, if they plan to register biological control products, and take into account the relative importance of market niches, intrinsic specificity of each organism, and long history of safe use of biological control. A final resolution encouraged governments to develop laws, procedures and support for biological control that will maintain its safety record, increase public involvement and continue its contributions to human welfare.

The IOBC, WGQC (AMRQC) workshops have been forums for determining the status of important issues, such as IOBC resolutions, and recommending action for the future. WGQC has encompassed both plant pests and natural enemies since it was established in 1982. However, tropical fruit flies, the screwworm and certain Lepidoptera have consumed most of the effort because they were used in expensive, large-scale IPM programs. It has always incorporated research on artificial diets and rearing techniques as they relate to product quality; however, very limited attention has been given to strain development and virtually none to promising new genetic techniques. At Wageningen, The Netherlands (1991), and thereafter at Horsholm, Denmark (1992) and Rimini, Italy (1993), much of the emphasis shifted from quality control principles and practices across several taxonomic groups to regulation of natural enemies.

At Rimini, recommendations were made for improving rearing systems, including data acquisition, analysis and exchange; communication, coordination and review; technology transfer and training; education, information and promotion; quality management; and research and development. Insect mass production managers of the U.S. Department of Agriculture (USDA) met subsequently to discuss and implement these recommendations. Most of the tests for "Quality Control Guidelines for Natural Enemies" in the Rimini proceedings were accepted in Europe and became standards for the following species: *Encarsia formosa, Diglyphus isaea, Dacnusa sibirica, Aphidius* spp., *Aphelinus abdominalis, Trichogramma brassicae, Aphidoletes aphidimyza, Orius* spp., and *Amblyseius cucumeris*. Quality control tests were also being developed for two additional species of predators and seven species of parasitoids. A meeting of the European natural enemy producers was convened at Evora, Portugal (1994)

at which QC guidelines for about 20 species were prepared for practical testing. Simultaneous work on QC guidelines was being contributed by the IOBC Working Group on Integrated Control in Glasshouses and the European Union (E.U.) project, "Designing and Implementing Quality Control of Beneficial Insects: Towards More Reliable Biological Pest Control."

The Eighth Global Workshop of the IOBC, WGQC was held at Santa Barbara, California, October 9-12, 1995. An excursion was made to Buena Biosystems, Ventura, California; the Fillmore Protective District Insectary, Fillmore, California, one of the first insectaries worldwide to mass-produce biological control agents and use them for augmentation in IPM; and an organic lemon orchard under the advisement of T. Roberts of Integrated Consulting Entomology, a licensed self-employed pest control advisor and IPM specialist. Prior to the meeting, the Association of Natural Biocontrol Producers (ANBP) QC Committee canvassed the producer members for topics and received the following suggestions: lacewing QC, fact sheets for products, and QC guidelines for beneficials not presently on the IOBC standards list, e.g., *Hypoaspis miles*, status of biological control regulations in various countries, parasite conditioning, molecular biology techniques, the lady beetle and related purity issues, product profiles, practical aspects of QC, and QC for beneficial nematodes. Having received the highest priority, product profiles were developed as models of the minimum amount of information that should be sent with every shipment of natural enemies. Consequently, the meeting had two general purposes: first, to expand the knowledge of successful quality control systems used in arthropod mass rearing, and second, to develop, refine, and finalize quality control systems that incorporate appropriate tests for natural enemies and sterile insects. The participants addressed questions, such as how many and what tests should be conducted and how frequently, what form should the data take and how can it be used for feedback into decision making about production and application, and what is the cost/benefit ratio for QC systems in terms of field success and associated profits? Assistance was provided at Santa Barbara in developing Canadian guidelines for regulating natural enemies, particularly the possibility of substituting QC for labeling to assure efficacy. ANBP product profiles were distributed, information supplied to the end-user specifying what quality attributes to expect from the natural enemy and how to test for them (Appendix).

The workshop consisted of four sessions, the first focusing on general quality control procedures based on Total Quality Management (TQM) and the International Standards Organization (ISO-9000). Both TQM and ISO-9000 provide a structure for developing mass production processes with a defined level of quality, as determined by the producer and end-user. They are proactive rather than reactive, emphasizing the need to identify criteria for judging quality, defining stages in which quality is evaluated as part of production, and involving the production workers in the development of production protocols, quality criteria, and evaluation procedures. TQM identifies needs, establishes the quality process, decreases costs, improves products, stabilizes production, facilitates change, virtually eliminates the need for inspection and post-production or service standards, and maintains reliability. ISO-9000 is a voluntary program for developing production protocols and setting standards against which quality can be measured. It can also evolve into a process that certifies the attainment of specific producer/user defined quality criteria. ISO-9000 is one of several programs used in industry to insure that the quality of the sub-contractors (suppliers) meets the standards required by the contractor. It reduces the probability of risk, particularly for regulated

industries, and enables internal versus external control of an industry. Both TQM and ISO-9000 must involve everyone in the enterprise, especially top management, and often require a change in the work culture.

The second session focused on regulation of commercially produced biological control agents. Representatives from the USDA, Animal and Plant Health and Inspection Service (APHIS) and Health Canada, Pest Management Regulation Agency presented the status of current regulations within the U.S. and Canada, respectively, along with some idea of future regulatory developments. An overview was also provided of the current status of regulations within Europe and of the United Nations Food and Agricultural Organization, Code of Conduct for the Import and Release of Exotic Biological Control Agents. Substantial discussion ensued on questions of practicality, efficacy, purity, identification, and international shipment. Canada regulates both microbials and invertebrates, requiring permits for importation and domestic movement. Under a new proposal, permit applications would be required 90 days before shipment and risk assessments would be conducted only for first time introductions. Notification for precedented organisms was being considered, requiring no permit for movement within bioregions. The four basic categories of risk criteria used in Canada are human safety, environmental effects, predictability, and efficacy. The goal was to register effective agents and place use requirements on the label.

The USDA, APHIS was attempting to improve regulatory procedures by expediting permits, listing precedented organisms, and increasing customer service. As in Canada, criteria for reviewing permit applications are risk-based. However, how can foreign shipments to the U.S. be inspected if no one can identify the organisms, i.e., predatory mites and *Trichogramma* spp.? There are no guidelines for nematodes. In Europe, *Trichogramma brassicae* is reared on several hosts, including *Ephestia* spp., except for the generation before release when it is switched to *Ostrinea nubilalis*. Factitious hosts could also be used to reduce risk during shipment for parasites of fungus gnats, white grubs, vine weevils, mole crickets, fleas, armyworms, and others. APHIS planned an advance notice of proposed rulemaking for the non-indigenous species regulations.

In Europe, most countries do not regulate macro-organisms, but Switzerland, Austria, and Hungary are exceptions, and Sweden and France are preparing new legislation. The E.U. has been developing a proposal for regulating macroorganisms during the past five to seven years. The UK, Germany, Denmark and The Netherlands do not exclude non-indigenous organisms but they require safety testing. Microorganisms are registered according to risk, as with pesticides, but some countries have no regulations. The E.U./German system requires that microbial pesticides be effective and have no harmful effects on humans or the environment. The commercial biological control industry should be proactive in developing statutory certification.

The working group members expressed great interest in the current and developing regulations in various countries. The regulatory goal is low risk use, and benefits are not always considered. Consistent categories of risk and data requirements should be developed. What is an acceptable level of risk? The zero risk paradigm now in use is obsolete. What is the risk to biodiversity? What is the legal liability in regulatory decision-making? Rapid risk

analysis is limited by staff size, the absence of data, environmental uncertainties, minimal scientific involvement in decision-making, and so forth. However, permits can be expedited by classifying organisms as widespread, commercially marketed, free from contamination and otherwise of low risk. Genus-level environmental assessments and an approved precedented organism list would be helpful. Is a technical advisory group similar to the one for weeds needed for natural enemies? It is important to focus on the process rather than individual cases. Perhaps producers should be certified rather than licensing products to address the consumer issue of efficacy.

The third and fourth sessions of the workshop addressed field efficacy, thus emphasizing quality in terms of economic suppression of pest populations. They considered the potential differences between mass producing a viable organism and controlling the pest. Generally, conditions in a mass production facility rapidly select for traits that maximize the reproductive success of the organisms in that context, usually within about ten generations. This selection typically manifests itself as decreased fitness of the organisms when they are released in the field, i.e., a reduction in sexual competitiveness or reproductive success. As a recommendation, methods can be used to minimize adaptation to the rearing conditions, i.e., remote food sources that force females to fly and/or variable temperature regimes. The organisms could be subjected to natural conditions periodically, i.e., reared for one or more generations in a semi-protected field cage or recaptured from the field. Strategies for maintaining colonies include intermittent selection, careful control of the rearing environment to retain genetic diversity, rearing supplemental stocks, periodic replacement, and use of inbred lines. The efficacy of *Trichogramma brassicae* for controlling the European corn borer, *Ostrinia nubilalis*, was presented as a successful model. In another example, it was recommended that high numbers of *Phytoseiulus* spp. be used to start a new colony.

The ninth IOBC, WGQC workshop was conducted at the International Center for Tropical Agriculture (CIAT) in Cali, Colombia, March 2-4, 1998. Excursions included CIAT; a *Trichogramma* spp. mass production facility, "Biofabrica;" demonstrations at *Trichogramma spp.* release sites; and a tour of the "Valle." Cali is a beautiful city and CIAT, the site of many important international meetings, is known for excellence in biological control research and the practice of augmentation by local growers. The meeting was held in conjunction with the IOBC Working Group on *Trichogramma* and Other Egg Parasitoids. The papers on egg parasitoids were published by S. Hassan in "Egg Parasitoids, 5th International Symposium, International Organisation for Biological Control, Cali, Colombia, Marsh 1998." The WGQC sessions defined the status, needs and action for the following topics: Quality Control in Sterile Insect Technique Programs, Quality Control in Nematode Production and Utilization, Biological Control in Protected Cultures, New Markets for Efficacious Natural Enemies, Quality Control of *In Vitro*-Reared Natural Enemies, ANBP Quality Standards Based on the American Society for Testing and Materials (ASTM) Program, International Biocontrol Manufacturer's Association (IBMA) Quality Standards- Status and Needs, and QC for *Trichogramma* spp. that overlapped with the egg parasitoid working group. Reports indicated that specific product control tests are important for predicting field performance. Self-regulation capabilities for the international biological control industry were advanced, as well as some general principles and practices in large-scale insect rearing as they relate to QC. An effort was made to: 1. Institutionalize QC in arthropod mass rearing, 2. Encourage rational

regulation of commercial natural enemies, and 3. Assure the efficacy of artificially reared natural enemies.

The workshop provided a forum for determining the status of important issues and recommending courses of action for the future. The IOBC workshops at Santa Barbara and Cali perpetuated the WGQC efforts to advance quality control for mass-produced arthropods. Augmentation biological control was emphasized, particularly regulations for importing natural enemies, along with the primary concerns and needs of the international biological control industry. QC systems were evaluated and tests proposed for commercial natural enemies. Industry leaders and regulators agreed that science-based risk assessments are needed to assure that beneficial arthropods are introduced into new environments safely. QC testing standards are directly dependent on related mass rearing procedures, i.e., production control is the primary determiner of quality as in TQC and ISO-9000. Product and process control tests are expensive and can be minimized, if rearing procedures are optimal. Although many researchable issues remained unresolved, guidelines were provided for establishing and maintaining genetically viable colonies. Moreover, since the performance and survival of mass-reared entomophages depends on adequate diets and related mass rearing procedures, the IOBC, WGQC was combined with a newly forming group on artificial rearing of entomophagous insects. The amalgamated group will shift the QC emphasis from regulatory issues back toward research and technology.

QUALITY CONTROL FOR MASS-REARED ARTHROPODS

CONTENTS

HISTORY

History of Quality Control in Mass-Reared Insects

E. F. BOLLER

Swiss Federal Research Station, CH-8820 Wädenswil/Switzerland

Extended Abstract of the Introductory paper given at the 3rd Biannual IOBC Workshop on Quality Control held in Guatemala-City, August 25-28, 1986

This outline of the history of quality control in mass-reared insect will be incomplete, subjective and heavily biased toward fruit flies being my own field of activities.

Early traces of quality control considerations in insects can be found decade ago but there are a few important key events or periods that produced break-throughs. Hence, I divide QC history into 4 distinct periods:

-- before 1969: Period of little concern
-- 1969 - 1975: Growing awareness, ideas and concepts
-- 1976 - 1979: International collaboration and prototypes
-- 1980 - now: general acceptance and practical application

Before 1969

The work of Wilkes (1942) is probably the first attempt to employ artificial selection in the laboratory to produce better adapted strains of parasitoids. The widely used ratio test to measure the competitiveness of sterile and fertile insects was probably developed in the 1950s by Hawaiian fruit fly specialists. An important source of information about international developments in the field of SIT and mass-rearing operations are the IAEA/FAO Panel Proceedings on SIT problems (e.g., 1962, 1964, 1966, 1967). The analysis of these documents and especially of the list of participants at the given meeting and their recommendations to the IAEA/FAO and governmental agencies reveals that this was the period of big screw-worm success stories that left little room for concerns about deteriorating quality and needs for quality control.

Period 1969 – 1975

I consider the IAEA/FAO panel on SIT in fruit flies held in 1969 in Vienna one of the important turning points in QC history. Not only did the vast majority of presented papers address the aspect of competitiveness (i.e., ratio-test data), but a paper presented by Chambers et al. (1969) covered for the first time research activities in the field of product quality assessment (flight mill, assortative mating experiments in field cages). Haisch (1969) presented at that meeting a formula for the calculation of a competitiveness-index.

The 1971 symposium of I0BC in Rome on implications of mass-rearing operations was probably the first international event addressing genetic (Mackauer, 1972) and behavioral

aspects (Boller, 1972) of insect mass-rearing. Boller proposed at that occasion a division of quality assessment and monitoring into production and product QC.

Another relevant IAEA/FAO fruit fly panel took place in 1973. Papers presented by various authors covered the aspects of genetic variation in insect populations as measured by the isozyme technique (Bush, 1975), mating propensity tests (Boller and Remund, 1975), comparative behavior of laboratory-reared and wild type fruit flies (Prokopy et al., 1975) and practical problem analysis in SIT programs (Butt, 1975). Prominent among the papers was the one given by Chambers (1975) on definitions and evaluation in quality control that are still valid today.

Many laboratories and individual scientists were now generating methods and concepts to measure and control insect quality with the inherent risk of creating a Babylonian confusion and of even further complicating the issue. These tendencies culminated 1976 in two independent symposia on quality control held at the 15th International Congress of Entomology in Washington, D.C.: A symposium on "Characterization and Evaluation of Insect Colonies" moderated by N. Leppla et al., and a symposium on "Natural Enemies" with contributions by M. Mackauer and E. Boller on "Genetic Aspects" and "Quality Considerations" in mass-rearing, respectively. This was the alarm signal that indicated that further developments in the field of quality control could be hampered if the groups split into diverging competing "schools".

Period 1976 – 1979

After the Congress an intensified exchange of letters between various persons led in November 1976 to the important decision to join forces. In retrospect, one could conclude that this was the ***point of departure of the quality control idea that is the working platform of this IOBC Working Group.***

Efforts were undertaken to bundle the diverging components and to melt them into mutually accepted concepts, terminology and methodology.

An invitation went out to 52 fly specialists to prepare short contributions for a compilation of concepts and methods to measure and evaluate quality in fruit flies, and Boller and Chambers (1977) were able to publish and distribute the IOBC/WPRS Bulletin No. 5/1977 entitled: QUALITY CONTROL: An Idea Book for Fruit Fly Workers, 10 months later. The booklet was 162 pages and introduced, among other items for the first time, elements of statistical quality control techniques utilized by industry (such as the Shewhart Control Charts and the process capability analyses).

These techniques were applied the first time for the monitoring and evaluation of a medfly mass-rearing facility in 1977-78 and led to the development of the RAPID Quality Control System (Boller et al., 1981). This research and developmental phase also covered phenotype/genotype studies in this *Ceratitis* laboratory strain and various wild and lab strains collected all over Western Europe and Northern Africa. The selection of experiments showed that it is possible to repair and improve medfly lab colonies that have apparently deteriorated

during long-term mass-rearing (Boller and Calkins, 1984).

An international team of fruit fly specialists cooperated in 1978 during several weeks of field-experimentation in November-December 1978 in Guatemala and developed the first prototypes of field tests for the medfly to complement the laboratory-based RAPID system (Chambers et al., 1983). This action was followed in September 1979 by an international workshop and training course on quality control in fruit flies organized by the IOBC/WPRS Working Group on Fruit Flies of Economic Importance in collaboration with various national and international organizations (USDA-ARS, IAEA/FAO, Spanish Plant Protection Service, Swiss Federal Research Station Wädenswil) in Castellon de la Plana, Spain. During the 10-day program three different medfly strains of various quality levels were evaluated by the simultaneous application of the RAPID Quality Control System in the laboratory and the field tests that had been developed in Guatemala (Chambers et al., 1983).

After that workshop in Spain a manual on standardized laboratory and field tests was produced (Calkins, unpublished) and the conclusions were reached that the time was ripe and the stage set for the formal establishment of a standing international body pursuing systematically quality control matters.

Period 1980 – 1986

The formation of an international working group on quality control in mass-reared arthropods under the umbrella of the Global IOBC was proposed by Boller and Chambers after the workshop in Spain. This proposal was accepted by the Global IOBC Council in a letter dated October 13, 1980. D. L. Chambers and E. F. Boller agreed to act as co-chairmen of this new group and to initiate activities according to the terms of references negotiated between chairmen and Council. High on the priority list was the organization of international biannual workshops on quality control, where scientists, plant managers and administrators involved in mass-rearing operations could meet, exchange information and develop standardized technologies and concepts in quality control. Another item was the availability of the group to participate in the planning, organization and conduct of training courses in quality control.

The first meeting was held at Gainesville, Florida, in 1982, gathering for the first time people working in mass-rearing of fruit flies, the screwworm fly, biting flies and lepidopteran pests used in SIT programs. At this meeting, common terms of references, concepts and approaches were discussed and standardized methods for the most advanced medfly programs agreed upon. This meeting was followed by a tour of the screwworm factory at Tuxla Gutierrez and the medfly factory at Metapa, Mexico. Several members of the working group participated as instructors in an IAEA/FAO training course on SIT technology in *Ceratitis capitata* after the Gainesville meeting covering the quality control part of the program.

The second meeting held at Wädenswil, Switzerland in 1984 broadened its activities to include for the first time specialists working in the field of entomophagous species.

The third meeting held in Guatemala in August 1986. In retrospect, this meeting again focused on the fine-tuning of quality control technologies applied in large fruit fly and

screwworm programs now expanding over the entire Central American area. Visits to the medfly factory in San Miguel de Petapa near Guatemala City provided an interesting view on the state-of-the-art quality control had reached at the level of factory implementation. The high output of quality assessment data every day and the difficulty in making the information digestible to plant managers and administrators at various levels called for the development of an efficient data management system. This important work is now in progress in Guatemala and will provide important service to other large-scale rearing operations. Another new aspect covered during this meeting was the specific requirements at higher administrative levels to be fulfilled by quality control departments within major eradication programs. This topic was of particular relevance to a considerable number of persons carrying responsibilities at various levels of the on-going medfly and screwworm programs in Central America.

Conclusions

Quality control has progressed considerably since its necessity has become obvious in the major mass-rearing operations and field use of mass-produced insects. The evolution of concepts and methods has also found its precipitation in papers presented at international and national meetings and symposia and as parts of recent textbooks (e.g., Calkins et al., 1982; King and Leppla, 1984; Moore et al., 1986).

Whereas dipterans (especially *Ceratitis capitata* and *Dacus* spp. mass-reared in Japan) face a stage of "fine-tuning" and coordination, emphasis of research and development will be placed on quality control technology in entomophagous arthropods. Future workshops might address this topic area that has found the interest of the IOBC Working Group on *Trichogramma* and of governmental and private agencies involved in the mass-production, handling and commercialization of parasitoids and predators. Another traditional field of activity might also face a period of increased attention, namely the participation in training courses on quality control concepts and methods that might emerge in the wake of expanding SIT operations.

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Roots and Traditions of the IOBC Global Working Group on Quality Control of Mass-Reared Arthropods

N. C. LEPPLA

University of Florida, Institute of Food and Agricultural Sciences, Central Florida Research and Education Center, Apopka, Florida 32703-8504, U.S.A.

A brief history of the International Organization for Biological Control (IOBC) Working Group on Quality Control of Mass-Reared Arthropods (WGQC), along with some of its roots and traditions, was included in the preface to the Proceedings of the VII Workshop on Quality Control of Mass-Reared Arthropods (Nicoli et al., 1993). It suggested that QC of mass-reared arthropods began to take shape as a coherent subject following presentation of formative papers by Ernst Boller and Manfred Mackauer at an IOBC symposium in Rome, Italy (Boller, 1972; Mackauer, 1972). These papers, published in the same volume of Entomophaga, were widely read and discussed relative to their applications in sterile insect technique and augmentative biological control.

During most of the 1970s this emerging field advanced as Ernst Boller and Derrell Chambers organized and conducted a series of international conferences and training courses, and published many scientific papers and the book, "Quality Control, An Idea Book for Fruit Fly Workers" (Boller and Chambers, 1977). In October 1980 the official working group was sponsored by IOBC and approximately biannual workshops were established (Boller, unpublished). IOBC has supported every one of these workshops both financially and through periodic participation of the President and Secretary of the Global Body. The workshops and their leadership were as follows:

Year	Location	Chairman
1982	Gainesville, Florida	E. F. Boller and D. L. Chambers
1984	Wadenswil Switzerland	E. F. Boller and D. L. Chambers
1986	Guatemala City, Guatemala	C. 0. Calkins
1988	Vancouver, Canada	C. 0. Calkins
1991	Wageningen, The Netherlands	F. Bigler and J. C. van Lenteren
1992	Horsholm, Denmark	F. Bigler
1993	Rimini, Italy	M. Benuzzi and N. C. Leppla
1995	Santa Barbara, California	R. F. Luck and N. C. Leppla
1998	Cali, Colombia	N. C. Leppla and T. R. Ashley

The workshop held in Gainesville, Florida in 1982 focused on pests of man and animals, i.e., biting flies, such as the screwworm, *Cochliomyia hominivorax* (Coquerel), fruit flies and Lepidoptera. A post-meeting excursion was conducted to Tuxtla Gutierrez, Mexico (screwworm) and Metapa, Mexico (medfly). Tours of operational pest management programs based on mass-produced arthropods were important to maintain a practical orientation. Based on the pioneering research of Manfred Mackauer (Mackauer, 1976), natural enemies were featured for the first time at the workshop held at Wadenswil, Switzerland in 1984. At

Guatemala City, Guatemala in 1986, the meeting was organized by subject rather than taxonomic group: insect colonization and strain development, colony maintenance, QC of production and products (laboratory bioassays), irradiation, shipment and distribution, field assessment and management of QC systems. Colonization and strain development, critical subjects that receive minimal attention, were also emphasized. After the meeting, Tom Ashley developed a computerized QC system for medfly production at Guatemala (Ashley, 1987). The Vancouver, Canada workshop in 1988 was divided into two sections, Quality Control of Pestiferous Insects and Quality Control of Entomophagous Arthropods. Along with the topics previously discussed at Guatemala, behavior and colonization of entomophages were respectively added by Joop van Lenteren and Manfred Mackauer. IOBC was represented by the Secretary of the Global Body, John Paul Aeschlimann who later published an important book on technology transfer in biological control (Aeschlimann, 1996). This and the previous meeting at Guatemala led to a synthesis of concepts into total QC. The V workshop held at Wageningen, The Netherlands in 1991 was co-chaired by Franz Bigler and Joop van Lenteren. It concentrated completely on entomophagous arthropods and resulted in a very important book that described specific QC tests for natural enemies (Bigler, 1991). This workshop produced the special subject meeting at Horsholm, Denmark in 1992 that reviewed and improved guidelines for product control of natural enemies, drafted product control methods for additional organisms, discussed QC costs and resources, and determined QC information for European Community labels. At Rimini, Italy, in 1993 the VII workshop again brought together experts in augmentative biological control and arthropod colonization and mass production. Regulation of the fledgling international biological control industry was debated extensively. The VIII workshop held at Santa Barbara, California in 1995 and the 1998 meeting at Cali, Colombia are described in the introduction to this volume. At the conclusion of the Cali meeting, the IOBC, WGQC was combined with a newly formed group based on artificial diets for rearing entomophagous insects. This amalgamation was named the Arthropod Mass Rearing and Quality Control Working Group (AMRQC) and is under the leadership of Simon Grenier (France), Patrick DeClercq (Belgium) and Norm Leppla (U.S.).

The accomplishments of the IOBC, WGQC extend far beyond its initial goal of institutionalizing QC in arthropod mass rearing programs. Today, unlike prior to about 1980, QC is an accepted practice throughout the world for both sterile insect technique and biological control. The WGQC has published QC guidelines, tests and standards for more than 20 natural enemies. Additionally, the proceedings of workshops and allied publications form a useful body of literature on the subject.

QC of mass-reared arthropods is evolving from product control (monitoring the outputs) to production and process control (precisely controlling the inputs). Production control has the greatest effect on the quality of mass-produced organisms, arthropods or otherwise, relative to process and product control. Production control is the precision with which all rearing operations are performed, including quality of materials and maintenance of adequate environments. This is often combined with process control, monitoring arthropod development along the production line, as a set of standard operating procedures.

In the future, it is absolutely essential that the new AMRQC remain broadly based in arthropod QC and flexible to address opportunities in the production and use of mass-reared

arthropods. The pioneers of this field never imagined the excessive amount of time that would be spent on regulatory issues, such as risk assessment and efficacy requirements. Nor did they anticipate regulations for the international shipment of natural enemies and the advent of genetically modified organisms. The IOBC, WGQC, now AMRQC, has strong roots and traditions, and remains zealous in its search for improvements in the rearing and QC of mass-reared arthropods.

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STATUS of QUALITY CONTROL
in NORTH AMERICA and EUROPE

Status of Quality Control for Natural Enemies in Europe

J. C. VAN LENTEREN

Laboratory of Entomology, Wageningen Agricultural University
P.O. Box 8031, 6700 EH Wageningen, The Netherlands

Abstract. Inundative and seasonal inoculative biological control are based on regular introductions of natural enemies. Mass-rearing of natural enemies often takes place at small companies with little know-how and understanding of conditions influencing performance, which may result in natural enemies of bad quality and failures of biological control programmes. This makes robust quality control programmes a necessity. In such programmes not only natural-enemy numbers but also natural-enemy quality (performance in the field) should be determined. Companies producing natural enemies and scientists working on quality control in Europe have agreed to cooperate intensively during the coming years to develop quality-control programmes. To date, only simple guidelines for laboratory tests have been developed, and much work still has to be done on designing relevant performance tests to show whether the present criteria do properly test field performance. Implementation of quality control tests may be augmented by the recent activities in the field of regulating importation of non-indigenous organisms that are taking place worldwide.

In this paper I will first explain the state of affairs concerning the quality control guidelines and certification of natural enemies in Europe. Then, I will describe the developments with regard to importation and legislation of natural enemies.

Introduction: quality control and legislation of biocontrol agents in Europe

Although biological control of pests in Europe is known since around 1900, large-scale use of natural enemies of pests started only recently (van Lenteren, 1990). Commercial application of biological control spans a period of less than 30 years and started in 1968 with the use of a predatory mite to control spider mites (van Lenteren and Woets, 1988). In some areas of agriculture, such as apple orchards, corn, vineyards and greenhouses, it has been a very successful environmentally and economically sound alternative for chemical pest control (van Lenteren et al., 1992). National agricultural policies in Europe are presently strongly promoting non-chemical control strategies, including biological control. One reason for this is the decreasing number of active ingredients available for arthropod control. Of the 300 active ingredients currently on the market for insect and mite control, 120-180 are on the blacklists of several European countries.

Inundative and seasonal inoculative releases of natural enemies are commercially applied mainly in corn and greenhouse cultures in Europe and have increased considerably over the last two decades. Success of biological control in these crops is primarily dependent on the quality of the natural enemies that are produced by commercial mass rearing companies and sold to farmers. In 1968, when commercial biological control started in Europe, two small commercial producers were active. Today, Europe has about 30 natural enemy production companies. Only three have more than 100 people employed, the others are very small, often not having more than 10 persons

contracted (Ravensberg, 1994). Total employment in Europe is circa 750 persons and growing. Only the three large companies produce the whole range of natural enemies (> 25 species) and bumblebees. Chemical companies also show some interest in biological control and one company has started to sell natural enemies. As biological control is a strongly developing market influenced by small competing companies, product quality and prices are continuously under pressure. This may in the short-term be profitable for growers, but in the long run it could lead to biological control failures. Although natural enemies were properly evaluated some 20 years ago, nowadays some species of natural enemies are sold without tests under practical cropping situations showing that they are effective against the target pest. Total sales of natural enemies in Europe amounted to some 15 million US $ (end user value) in 1987 (with the largest producer having a market share of 65%) and to 60 million US$ in 1991 (market share of largest producer 50%, market share of three largest producers 85%). In addition to biological control, bumblebees used for pollination accounted for 10 million US$ in 1991. It is only at the larger companies that some control of quality of natural enemies takes place. The rise and fall of small companies and the poor quality of natural enemies they produce results in negative advertisement for biological control (van Lenteren, 1991).

The reliability and visibility of biological control would be improved considerably if standards for acceptable quality could be developed for all marketed natural enemies. Quality standards and efficacy data are also essential to obtain registration of natural enemies in several European countries, such as France, Switzerland, Austria and Hungary.

The issue of quality control for natural enemies was discussed for years, but no concerted actions were taken until the end of the 1980s to develop standard procedures. The fifth workshop of the IOBC global working group "Quality control of mass reared arthropods" (Bigler, 1991, Wageningen, the Netherlands) formed the starting point for a heated discussion among producers of natural enemies and scientists on how to approach quality control in the commercial setting of Europe at that time. Workshops funded by the IOBC and the European Union followed in 1992 (Horsholm, Denmark), 1993 (Nicoli et al., 1993; van Lenteren et al., 1993; Rimini, Italy) and 1994 (van Lenteren, 1994; Evora, Portugal), and as a result quality control guidelines were designed for about 20 species of natural enemies for practical testing in 1994/95. Also, fact sheets about natural enemies and pests are being composed for training purposes. At this moment the guidelines comprise only characteristics that are relatively easy to determine in the laboratory (e.g., emergence, sex ratio, lifespan, fecundity, adult size, predation/parasitization rate). Work is now focused on development of (1) flight tests and (2) a test relating these laboratory characteristics to field efficiency. Besides the IOBC global working group "Quality control of mass reared arthropods", two other working groups play an important role in developing quality control criteria: the IOBC/WPRS working group "Integrated Control in Glasshouses" and an EU funded working group "Designing and implementing quality control of beneficial insects: towards more reliable biological pest control".

Until recently, the use of beneficial arthropods was exempt from registration in most European countries, but several countries are implementing (very different rules for) registration now. For microbial pesticides quite strict registration procedures are followed in many European countries, often similar to those for chemical pesticides, but also here differences in procedures among countries exist. Uniform European legislation would prevent confusion and might help

implementation of biocontrol, but such ruling is not expected to be developed in the near future. Importation and use of non-indigenous microorganisms is covered by the registration procedure. For macro-organisms, European countries have very different criteria to allow importation and releases, varying from no rules to rather strict criteria including the need to provide information on possible environmental impact. Rules for importation of natural enemies are on the agenda of several European countries. The FAO code of conduct (in preparation) might help standardization of ruling in the EU and worldwide. Several of the quality control criteria could, in combination with other information, be used for registration procedures.

Quality control guidelines for natural enemies

The guidelines developed until now refer to **product control** procedures, not to production or process control. They were designed to be as uniform as possible so they can be used in a standardized manner by many producers, distributors, pest management advisory personnel and farmers. These tests should preferably be carried out by the producer **after all handling procedures just before shipment**. It is expected that the user (farmer or grower) only performs a few aspects of the quality test, e.g., percent emergence or number of live adults in the package. Some tests are to be carried out frequently by the producer, i.e., on a daily, weekly or batch-wise basis. Others will be done less frequently, i.e., on an annual or seasonal basis, or when rearing procedures are changed. For each test two coordinators were appointed to follow up the application of quality control tests by the producers and, upon their feedback, to reassess the technical and economic feasibility of those tests. If necessary, coordinators will contact relevant scientists or producers in order to design and carry out further studies that are essential for the completion of the quality control guidelines.

Most of the tests for the species listed below were accepted and will now function as initial guidelines. This remarkable success is the effect of very positive cooperation between commercial producers and scientists active in the field of biological control of pests. In addition to the tests it was decided that fact sheets on natural enemies and pests were needed to inform new quality control personnel and plant protection services on biological details.

In the near future, flight tests and field performance tests will be added to these guidelines. Such tests are needed to show the relevance of the laboratory measurements. Laboratory tests are only adequate when a good correlation has been established between the laboratory measurements, flight tests and field performance.

Quality control guidelines have been developed for the natural enemies listed below:

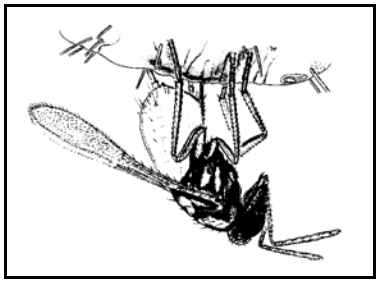
Amblyseius cucumeris (Oudemans) (Acarina: Phytoseiidae)
Amblyseius degenerans Berlese (Acarina: Phytoseiidae) (provisional)
Aphelinus abdominalis Dalman (Hymenoptera: Aphelinidae)
Aphidius spp. (Hymenoptera: Braconidae)
Aphidoletes aphidimyza (Rondani) (Diptera: Cecidomyiidae)
Chrysoperla carnea Steph. (Neuroptera: Chrysopidae)
Dacnusa sibirica Telenga (Hymenoptera: Braconidae)
Dicyphus tamaninii Wagner (Hemiptera: Miridae) (provisional)

Diglyphus isaea (Walker) (Hymenoptera: Eulophidae)
Encarsia formosa Gahan (Hymenoptera: Aphelinidae)
Leptomastix dactylopii Howard (Hymenoptera: Encyrtidae) (provisional)
Macrolophus caliginosus Wagner (Hemiptera: Miridae) (provisional)
Orius spp. (*O.laevigatus, O. insidiosus, O. majusculus, O. aldibipennis*) (Hemiptera: Anthocoridae)

Phytoseiulus persimilis Athias-Henriot (Acarina: Phytoseidae)
Trichogramma brassicae Bezd. (=*T. maidis*) (Hymenoptera: Trichogrammatidae)
Trichogramma cacoeciae Marchal (Hymenoptera: Trichogrammatidae)
Trichogramma dendrolimi Matsumura (Hymenoptera: Trichogrammatidae)

Full descriptions of the tests can be found in van Lenteren (1994). An example of the test for *Encarsia formosa* is given below.

***Encarsia formosa* Gahan (Hymenoptera: Aphelinidae)**



Test conditions
22°C±2°C

Temperature:
RH: 60-90%
Light regime: 16L:8D

Quality control criteria

Emergence rate: ≥ the number of adults specified on the label which will emerge over a two-week period; n=1000; a weekly or batch-wise test

Sex-ratio: ≥ 98% females; n=500; a four-weekly test

Adult size: Head width ≥ 0.28 mm; n=30 females; an annual test; ***this may be replaced for hind tibia length measurements next year***

Fecundity: ≥ 7 eggs/female/day for days 2, 3 and 4 after emergence of the adult; n=30 females; an annual test

Description of testing methods

Emergence: Specify the number of adults that should emerge before conducting the test. Take 3 sub-samples that make up 1,000 or more full black pupae in total. Put the samples in a closed container for two weeks and then determine the number of emerged adults. This can be done by counting the number of emerged adult parasites or by comparing the number of empty pupae at the start and at the end of the test. A combination of both counting methods will give the most reliable results. The quantity of emerged adults should achieve the number specified on the label.

Sex-ratio: Take a sample of 500 of the adults from the Emergence-test and count the number of male wasps. These are completely black and easily distinguished from the females, which have a yellow

	abdomen. The number of females should be ≥ 98%.
Adult size	To measure the size of adult parasites, freshly emerged parasites, no more than 24 hours old, are collected from a container and killed with ether. The head width is measured between the outer edges of the eyes. Position the adults on their back and measure head width from the ventral side with an ocular micrometer. Make sure that the head is in a horizontal position. A magnification of 80x is recommended. Convert the ocular micrometer units into millimeters. Provision of food does not affect head width. Measure 30 females. All measured females should have a head width of ≥ 0.28mm. This test should be performed in the period August to October, when population numbers are lowest.
Fecundity	
Day 1	Put an ample amount of black pupae that are close to emergence in a container. Remove all adult parasites at the night before the day on which the test animals will be collected from the container.
Day 2	Collect 30 freshly emerged females at about 10 o'clock; put each into a small container with a droplet of honey until the following day. This is to feed them and to get them through the pre-oviposition period.
Day 3	The test is conducted on individual females in small round plastic "petri dish type" trays (φ 32 mm; height 15 mm) that can be closed very tightly. A nylon mesh is incorporated into the lid to facilitate air exchange. Trays are filled with agar solution (1%) to a depth of 5 mm. Just before the agar solidifies a leaf disc is placed with its upper surface in contact with the agar. The fecundity test can be performed using tobacco leaf discs with whitefly larvae. Care should be taken to insure that the leaf contains enough whitefly larvae. Provide each female with at least 25 whitefly larvae (*Trialeurodes vaporariorum*) in the 3rd and 4th instar. Use 15 females in total.
Day 4	Provide the female with a new supply of whitefly larvae by placing her in a new tray. Do this around 10 o'clock in the morning, again.
Day 5	Repeat day 4.
Day 6	Remove the parasites from the whitefly larvae. Keep all whiteflies that were exposed to *E. formosa* in closed containers to prevent unwanted parasitism after the test. Count all black pupae after 10 and 14 days. The average number of black pupae per female per day should be ≥7. This test should be performed in the period August to October.

Comments

1. New information shows a strong positive relationship between (host) pupal size, hind tibia size and head width of adult *Encarsia*, so measurement of pupal length may be sufficient as an indicator of adult size. Entomology Wageningen will perform new experiments to study these relationships. At the Antibes meeting in 1996 a decision will be made on what measure to use (pupal length, head width or hind tibia length).

2. One producer had problems with the fecundity test. Contact between coordinators and this producer will hopefully solve problem. To be discussed at next meeting. Fecundity test will be done by Bunting, BCP, Koppert and possibly by others.

3. Another method to measure fecundity, based on testing a group of females concurrently instead of determining the fecundity of individual females, will be developed by Bunting.

4. A short-distance flight test developed at Wageningen will be tested by Bunting, BCP, Koppert, Wageningen. The threshold/standard for sufficient quality has to be agreed upon at the Antibes meeting (e.g., 80% of emerged adults should reach trap). This flight test will be used for other natural enemies as well.

5. Entomology Wageningen will develop a long-distance flight test.

Fact sheets have been developed for 5 pests (*Heliothis, Ostrinian, Sesamia, Tetranychus and Trialeurodes*) and 2 natural enemies (*Phytoseiulus and Encarsia*). Fact sheets for 15 other pests and 17 natural enemies are in preparation (for details, see van Lenteren, 1994).

Regulatory issues related to biological control agents in Europe

Legislation of biological control agents in Europe

Registration of bacteria, viruses, fungi and protozoa (micro-organisms)

In general, products based on microorganisms such as bacteria, viruses, fungi and protozoa need to be registered through similar procedures as those used for chemical pesticides in most European countries, as in the USA. The problem is, of course, that the registration procedure for chemicals contains many elements that are not relevant for microorganisms. These expensive procedures do not encourage development of safer pesticides, which is at the same time advocated by authorities. Therefore, special criteria and guidelines for registration of microbials should be developed. In the Netherlands a specific application procedure has been designed for microbials, where the amount of preregistration data required depends on the risk category of the microbial, but European regulations largely overruled the procedure. Registration fees vary in Europe: some countries apply no costs for microbials (Denmark until 31 December 1995), apply a lower fee for microbials (UK: c. 25% of the fee for chemical pesticides), or apply the same costs for microbials and chemical pesticides (The Netherlands since 1994). High costs for registration form a serious barrier for implementation of microbial agents.

The present situation in Germany is used to illustrate recent developments concerning registration as they are the most rigorous in Europe and the EEC Directive on legislation of pesticides (91/414/EEC) is similar to the German provisions (Klingauf, 1995). In the EEC and German registration procedures microorganisms and viruses are included, but macro-organisms

such as arthropods are not included. Micro-organisms are granted authorization if examination of the plant protection products shows that:

1. The plant protection product is sufficiently effective in the light of scientific knowledge and technique.
2. The precautions necessary for the protection of human and animal health in dealing with dangerous materials do not require otherwise.
3. The plant protection product, when used for its intended purpose, and in the correct manner, or as a result of such use:
    a. does not have any harmful effects on human and animal health or on groundwater, and
    b. does not have any other effects, particularly with regard to the natural balance, which are not justifiable in the light to the present state of scientific knowledge.

It is clear that this description allows for different interpretations! "Any other effects" is defined as: all those effects that cannot be excluded with a probability next to security. Products will only be registered if other effects, especially those that affect the balance of nature, almost certainly can be ruled out. This particular point might create serious difficulties for registration of biocontrol agents! The EU registration procedures for microorganisms and viruses are similar to those of Germany. The requirements are extensive and demand, among others, a thorough risk assessment including determination of effects on flora and fauna (for a list of requirements, see Klingauf, 1995). It is estimated that the **preparation of a dossier** complying with the EU guidelines would require 1.6 person years per active ingredient and will contain thousands of pages.

A positive point of the EC directive is the mutual recognition of registration. A member state must refrain from requesting new submission of test results and must recognize the registration of a plant protection product by another member state so far as the conditions are comparable.

Registration of predators and parasitoids (macro-organisms)

Most European countries do not demand registration of macro-organisms such as mites, insects and nematodes. In Switzerland, Austria, and Hungary it is, however, necessary to register these kinds of natural enemies. Switzerland has no specific administrative procedure and registration is handled on a case-by-case basis. Austria applies regulations, and for Hungary official registration is required but not yet strictly enforced. France and Sweden are preparing legislation, and particularly procedures for Sweden look complicated. Other European countries are discussing the need of registration of macro-organisms. In the European Union macro-organisms are still exempt from evaluation under the new pesticide legislation (Directive 91/414/EEC). Although a uniform European legislation would prevent confusion and might help implementation of biocontrol, such ruling is not expected to be developed in the near future.

Importation of non-indigenous organisms

Use of non-indigenous microorganisms is covered by the registration procedure, where more questions are asked about likely environmental impacts than for indigenous organisms. For macro-organisms European countries have very different criteria to allow importation and releases (from no criteria to rather strict criteria including information on possible environmental impact). In the UK, Germany and Denmark existing legislation applies to import of alien organisms. In the

UK the release of non-indigenous organisms is prohibited under the Wildlife and Countryside Act, backed up by the Plant Health Order for pest species. Non-indigenous natural enemies have recently been included in the ruling, and an import license is needed for these organisms through the Department of the Environment; the procedure for granting licenses is still under review. Also for Germany, procedures are under review. Officially, non-indigenous natural enemies cannot be introduced. Denmark enforced a new Act on the protection of the environment and releases of alien organisms (including biocontrol agents) are no longer permitted. Harmonization within the EU is under discussion, and there are efforts to include the FAO Code of Conduct for the Importation and Release of Biological Control Agents into the EU (Klingauf, 1995).

Code of conduct for importation and release of natural enemies

FAO and IOBC are developing a code of conduct for import and release of biological control agents, with the aim " … to set forth responsibilities and to establish voluntary standards of conduct for all public and private entities engaged in or affecting the distribution and use of biological control agents, particularly where national legislation to regulate their use does not exist or is inadequate". The first versions of the code of conduct mainly dealt with classical biological control, now also augmentative releases are included. The 1994 draft resulted in so many reactions that a final version has not yet been produced. The way in which the 1994 version was drafted could seriously complicate augmentative forms of biological control. The goal of this code is to harmonize regulation, to prevent unnecessary and complicated national legislation and to prevent undesirable, harmful effects of releases as much as possible. The new version, when formulated properly for different types of biological control, might form a basis for worldwide harmonization of importations, which is certainly beneficial for biocontrol.

Consequences of legislation on commercial biological control

Commercial biological control in Europe has a history of some 25 years. The need to regulate biocontrol agents to conform with the use of other control compounds is felt only recently, both by ruling agencies and the biocontrol industries. For microbial agents registration is a must because of toxicological and environmental issues. The large diversity in registration requirements between countries makes it very difficult and expensive for the relatively small biocontrol companies to apply for registration. A unified procedure for Europe in the form of a EU Pest Control Agent Registration Directive would ensure that all member states will have the same requirements for registration, and also that once a biocontrol agent is registered in one country, registration in another country would be easier.

Registration of macrobials should be less demanding than that of microbials. A very general efficacy test to show that the natural enemy is capable of reducing target pest numbers, and - particularly with alien natural enemies - an estimate of environmental effects could suffice. A very important issue is here to develop a database on geographical distribution, host range and possible influence on the ecosystem in which the agent will be used.

For commercial biocontrol no extra regulations would be needed. It is in the interest of the biocontrol industry to develop a certification system that includes quality control criteria to assure reliability to its customers. The framework in which testing can be effected (e.g., voluntary by producers, compulsory by governmental registration institutes, or as a form of certification under the responsibility of an official organization) is still under discussion. It is, however, clear that

very strict registration procedures for macrobials are neither to the benefit of governments advocating pesticide poorer crop protection, nor to producers. This may be illustrated by the registration problems that were recently experienced in Japan and Morocco, two countries without any history of commercial biological control. These countries demand efficacy data for each natural enemy on every crop based on tests done by the authorities over several seasons. Test procedures appeared to be not very appropriate and lead to failures in determining efficacy and, thus a waste of time. In an IPM programme for vegetables, implementation can only start when all essential natural enemies are registered: this is expected to take a decade or more!
(The information in the above section on regulation originates mainly from Ravensberg, 1994)

Conclusions

Quality control procedures for natural enemies are presently being developed for most of the commercially applied natural enemies in Europe. The quality control criteria relate to product control and are based on laboratory measurements, which are often easy to carry out. The criteria need to be complemented with flight tests and field performance tests. Development and implementation of quality control procedures takes place in an IOBC/EU working group, which is financially supported by the European Union.

Registration procedures for biological control agents are very diverse in Europe. For microbial agents, the costs of registration are often prohibitive for the natural enemy producers. Both the high costs and the diversity in procedures hamper implementation of biological control. Standardization of registration in Europe will take about a decade. Similar problems exist for importation of natural enemies.

On the one hand, many governments worldwide advocate pesticide reduced or pesticide free production of food, while at the same time they simultaneously put up serious barriers for use of one of the most environmentally friendly and sustainable forms of pest control, biological control. If biocontrol scientists and producers are able to come up with good initiatives related to regulatory issues (registration, importation and quality control), reasonable compromises might be the result.

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APPENDIX

History of Commercial Biocontrol Europe

1920's: *Encarsia Formosa* in greenhouses
1968: 2 commercial producers of natural enemies
1970 onwards: Large-scale commercial biocontrol - greenhouses, corn, vineyards, fruit orchards
1995 onwards:

- 30 commercial producers, many distributors
- 3 have >100 persons employed and rear 20 spp of natural enemies and bumblebees, others often <10 people
- Total employment in Europe circa 750
- 1 chemical company active in commercial biocontrol

History of Quality Control (q.c.) of Natural Enemies (n.e.) in Europe

1988: Vancouver IOBC Global Q.C.: several papers on n.e. q.c.
1991: Wageningen IOBC Global Q.C.: many papers on n.e. q.c.
- discussion between industry and science on how to approach problem

1992: Horsholm: selected group: how to approach problem
1993: Rimini IOBC/EC Q.C.: start development of criteria
1994: Evora EC Q.C.: continuation of the development of criteria
- testing of initial set of criteria
- discussion on need for flight, semi-field and field tests

1996: Antibes EC Q.C.: proposal for q.c. n.e. Europe
- discussion of additional tests, training material

Regulatory Issues of Biocontrol Agents in Europe

Registration of bacteria, viruses and fungi (micro-organisms)

In general, microorganisms need registration through similar procedures as those used for chemical pesticides, but pesticide procedures have many elements not relevant for microorganisms

Registration of micro's is very different among European countries

Type of pre-registration data required should depend on the risk category of the microbial

Registration fees vary from 0 - same as those for chemicals

In the EU/German registration procedures, microorganisms and viruses are included

Registration granted if:
- agent is effective
- no harmful effects on human/ animal health or groundwater
- does not have any other effects, particularly with regard to the natural balance, which are not justifiable in the light of the present state of scientific knowledge.

"Any other effects" is defined as: all those effects which cannot be excluded with a probability next to security: how to correctly determine effects on the natural balance??
Registration of predators and parasitoids (macro-organisms)

Most European countries do not demand registration of macro's

Switzerland, Austria and Hungary demand registration, but Switzerland ad hoc, Austria difficult, Hungary not enforced

Sweden and France prepare legislation; Sweden very difficult

EU: macro's are still exempt from legislation:
- will take between 5-7 years before ruling is proposed
- many countries discuss forms of ruling

Uniform European legislation might help biocontrol, depending on complexity

Importation of non-indigenous organisms

Use of imported micro's covered by registration procedure; includes questions about environmental impact

Import and application of macro's: very different criteria

For UK, Germany, Denmark & Holland: non-indigenous organisms cannot be introduced: but no strict procedures designed!

Harmonization within EU is under discussion; ruling will be related to FAO code of conduct for importation and release of biocontrol agents.

Commercially applied natural enemies for control of greenhouse pests (after van Lenteren 1995).

Natural enemy	Target pest	In use since
Phytoseiulus persimilis	*Tetranychus urticae*	1968
Encarsia formosa	*Trialeurodes vaporariorum*	1970 (1926)
	Bemisia tabaci	1988
Bacillus thuringiensis	Lepidoptera	1972
Opius pallipes	*Liriomyza bryoniae*	1980-1983*
Amblyseius barkeri	*Thrips tabaci*	1981-1990*
	Frankliniella occidentalis	1986-1990*
Dacnusa sibirica	*Liriomyza bryoniae*	1981
	Liriomyza trifolii	1981
	Liriomyza huidobrensis	1990
Diglyphus isaea	*Liriomyza bryoniae*	1984
	Liriomyza trifolii	1984
	Liriomyza huidobrensis	1990
Heterorhabditis spp.	*Otiorhynchus sulcatus*	1984
Steinernema spp.	Sciaridae	1984
Amblyseius cucumeris	*Thrips tabaci*	1985
	Frankliniella occidentalis	1986
Chrysoperla carnea	aphids	1987
Aphidoletes aphidimyza	aphids	1989
Aphidius matricariae	*Myzus persicae*	1990
Verticillium lecanii	whitefly/aphids	1990
Orius spp. (c. 5)	*F. occidentalis/ T. tabaci*	1991
Aphidius colemani	*Aphis gossypii/ M. persicae*	1992
Aphelinus abdominalis	*Macrosiphum euphorbiae*	1992
Trichogramma evanescens	Lepidoptera	1992
Leptomastix dactylopii	*Planococcus citri*	1992
Cryptolaemus montrouzieri		
Anthocorus nemorum	Thrips	1992
Metaphycus helvolus	Scales	1992
Trichoderma harzianum	*Fusarium* spp.	1992
Amblys. cucumeris/degenerans non-diapausing strains	Thrips	1993
Delphastus pusillus	whiteflies	1993
Eretmocerus californicus	*Bemisia tabaci*	1993
Metaseiulus occidentalis	*Tetranychus urticae*	1993
Hippodamia convergens	aphids	1993
Macrolophus caliginosus	whiteflies	1994
NPV-virus *Spodoptera*	*Spodoptera exigua*	1994

* use terminated, other natural enemy available

Quality Control Activities at the Association of Natural Bio-control Producers

D. ELLIOTT and C. S. GLENISTER

ANBP Quality Control Committee

Abstract. The Association of Natural Bio-control Producers (ANBP) was formed in California in 1990 to provide a unified voice for the North American biological control industry and to focus on quality issues, research and education on the use of biological control products. The ANBP has developed a Code of Ethics to define the standards of conduct and practices that are binding on its members and is developing a number of quality control initiatives for the industry. Standards that have been developed to date include product-labeling requirements. These specify that biological control product labels should include a minimum of the following: name(s) of the beneficial species in the package, number of beneficials packaged, packing date, the level of purity of biological control agents in the package and that the number of live biological control agents is at least 100% of the quantity stated on the label. A consumer quality control guide is also being produced to enable end users to do some basic product quality evaluations.

To improve ANBP member's ability to deliver biological control products with expected quality parameters, the ANBP board of directors recently met with representatives of the American Society for Testing and Materials (ASTM). The ASTM is one of the largest standard development systems in the world and provides a forum for producers, users, consumers and those having a general interest (government and academia) to meet as stakeholders on common ground to write standards for materials, products, systems and services. The ASTM is a non-profit organization where volunteer members and stakeholders serve on technical committees to develop standards. Most of ASTM income comes from the sale of standards publications and the individual memberships are low ($65/year). Possible standards that could be developed with the ANBP include: counting methods, organism identification, fitness, labeling, packaging and purity. Research funding will be needed to pursue the more complex issues such as product fitness. It has been proposed that the first standards be developed on well-known biological control agents where there is reliable information and existing guidelines such as ANBP product profiles and the international IOBC Working Group on Quality Control of Mass Reared Arthropods. The ASTM is applying for National Biological Control Institute (NBCI) funding for its next organizational meeting, which will be held with ANBP board members and other interested parties on June 12, 1998 in Berkeley, California. For more information on this or other ANBP topic contact Maclay Burt, Executive Director, Association of Natural Bio-control Producers, 10202 Cowan Heights Drive, Santa Ana, California 92705, e-mail: maclayb@aol.com

The Professional Group of Producers of Beneficial Arthropods of the International Biocontrol Manufacturers' Association (IBMA): A Status Report

W. J. RAVENSBERG and K. J. F. BOLCKMANS

International Biocontrol Manufacturers' Association
5, Quai Voltaire F
75007 Paris
France

Introduction

The International Biocontrol Manufacturers' Association, the IBMA, was founded in November 1995 to form a platform for biocontrol manufacturers to discuss issues common to their industry, and to establish an officially recognized organization of the biocontrol manufacturers towards national and international authorities. The headquarters and offices of the association are based in Paris, France.

Under the leadership of Mr. Bernard Blum, the association has been organized into professional groups and working groups. There are four professional groups within the IBMA: Macrobials, Microbials, Pheromones and Natural Products. The working groups deal with issues common to all professional groups such as quality control, registration, legislation, ethics (code of conduct), promotion, etc.

The members of the IBMA believe that the biocontrol industry has reached a stage where self-regulation is important for the further evolution of the biocontrol industry and for the use of beneficial insects for biological pest control in general. Currently, the Macrobials group focuses on 3 issues:

-implementation of Quality Control
-harmonization of labeling
-representation towards the authorities.

Quality Control Implementation by Certification of Producers

The production of quality natural enemies is an essential component of a successful future for commercial biological control. Historically, beneficial insects and mites have been tested by the producers themselves with their own internal protocols, with little involvement from outside agents, to meet their own requirements for quality products. In order to standardize criteria for quality control, researchers and industry representatives have been

collaborating for many years on the development of guidelines for product testing. The next logical step is the sound implementation of these criteria.

The principal goal of the EC CONCERTED ACTION PL 921076: **"Designing and implementing quality control of beneficial insects: towards more reliable biological pest control"** was to develop consistent and easy-to-use tests for commercially produced natural enemies. The next question is: who is going to check the results of these tests? Clearly, compliance with these guidelines by the biocontrol industry will need to be verified by an independent organization(s) if these guidelines are to have any meaning.

One option calls for the products to be tested according to the established criteria by a third party, either governmental or nongovernmental. This would ensure independent, random and credible results. Testing of beneficial insects and mites is an expensive proposition, however, for it requires large numbers of beneficial arthropods, living crop plants, food for natural enemies, and most importantly, experience with these tests.

Possible candidates for this task include: national plant protection services, universities, experimental stations or certifying agencies. In some countries such as the Netherlands, the products are already tested twice yearly by the Plant Protection Service for identity and purity. These services are highly specialized, however, and every country may not have the resources, skills or equipment necessary to conduct such tests. Lack of standardization due to small sample size, infrequence and inexperience may also hinder the collection of consistent data. A further disadvantage is the greater likelihood of error where several different organisations are involved.

A second option calls for the products to be checked by those who have the necessary ingredients for meaningful testing available: the producers themselves. Producers could test their products according to the accepted protocols, and carefully document the results (comparable to GLP guidelines). This would ensure continuity and consistency in the results of these tests. Another important point is that this option is much more affordable for both large and small operations, where cost might otherwise be an obstacle. An outside organization will then inspect the records once or twice a year, verifying compliance with the Quality Control Guidelines. A certification program could also be established at a later date. Certification of quality control could be applied either to a range of specific products, or to the producers themselves.

The disadvantages of this option include: the risk of selective sampling, diminished confidence in the results and lack of independence of the testers. Even so, given that the need for continuity and standardization is great, an autonomous organization may still provide the best solution for reliable evaluation of testing methods and results. Several international organizations, with experience in similar issues of quality control certification in agriculturally-based industries, provide these services on a worldwide basis. These companies are well versed in evaluating quality control norms. The information collected could also be useful at a later date in establishing a database on quality control standards.

With such a scheme, a self-regulating system could be implemented worldwide that is acceptable to regulatory authorities. This will be especially useful for simplifying registration procedures in countries where registration of natural enemies is currently necessary. Government organizations may still play an active role in key areas including: identification of species, assessment of harmful organisms, and resolution of phytosanitary issues. Other key organizations, such as the European Plant Protection Organization (EPPO), the International Organization for Biological Control (IOBC), will certainly have a role to play in related issues of concern to the industry.

In summary, stakeholders in biocontrol have developed workable guidelines for quality control. Evaluation of testing methodology and data by an outside agency represents a logical, consistent, and affordable means to ensure compliance with quality standards for the production of commercially produced beneficial arthropods. Similar quality control programs (ISO, GLP, NEN) exist upon which a scheme can be based. Certification of producers is an option for the future.

The Macrobials group of the IBMA will continue the efforts of the EC Concerted Action by organizing annual workshops on:

-development and criteria for the remaining beneficials
-validation of the existing guidelines and criteria
-implementation of Quality Control.

Harmonization of Labeling of Beneficial Insects

Labeling of beneficial arthropods is currently very diverse, without any standardization. Often there is no species name, product name, producer's name, numbers per package or batch number. Instructions for use are to be found in a leaflet, on the label, or are often simply absent. Some countries such as Switzerland, Sweden and Austria have decided to legislate labeling of beneficials. In other countries authorities are also starting to develop rules. Unfortunately, such regulations are often developed by drawing inappropriate analogies to chemical pesticides and the authorities fail to consult the industry. At the moment there is a total absence of international standardization of these regulations. Therefore, producers have to act to develop uniform labels and prevent unworkable labeling requirements.

The only similarity between chemical pesticides and beneficials is that the aim of both products is to control pests. On the other hand, there are large differences between chemicals and beneficials in the nature of the product or "active ingredient", the range of products, the packaging (requirements, size, number of packages per hectare), the risk of the products to the user, consumer and food products, the risk of the products to the environment, the methods of application and the liability. The logical consequence is that labeling should be designed entirely differently.

It is the view of the IBMA that a clear and uniform labeling of packages of beneficials is necessary. Minimal label requirements have been identified as:

-the identity of the beneficial (scientific name)

-the name and address of the producer and supplier
-the target (taxa, symbols, scientific names)
-the number of specimen per package
-the batch number
-the storage temperature.

Optionally the following information can be added:

-relevant information on dose, when, where and how to release, etc.
-“use by” date.

Packages of beneficial arthropods are often rather small and do not always allow for the use of labels which contain a lot of information as is customary for labels of chemical pesticides. Typically, several packages of the same beneficial will be released into a greenhouse or field at one time. In most cases multiple releases of the same beneficial are required. The same species of beneficial arthropod can be used in several different crops, each with different release strategies and doses. Recommendations do not only vary per crop but also per season, culture, region, etc. and may be changed often as new information and experience become available. Beneficial arthropods are generally sold together with technical advice by an experienced biocontrol advisor. Accordingly, it is more appropriate to minimize these instructions on the label but to provide them to the customer on a leaflet. A reference to the instruction leaflet should be present on the label.

In order to resolve the need to develop labels in many different languages, one company, Koppert Biological Systems B.V. has developed a range of pictogrammes. Developing and printing labels in many different languages is not only expensive but also logistically complicated when one has to dispatch products with a very limited shelf life. Production and packaging beneficials is a typical case of a just-in-time system. When pictogrammes are used, the products can be labeled immediately after packaging. When labels in the language of each country are used, packages can only be labeled just prior to dispatch, which may lead to a certain loss of quality, due to extra handling time. Koppert Biological Systems B.V. has made its pictogrammes available for use to all IBMA members.

It is the aim of the Macrobials group to develop a standardized short label and an instruction system for the use of the product (leaflet). An internationally harmonized system of labeling has to be developed by contacting producers outside Europe (ANBP in North America, ABC in Australia, JBCA in Japan, etc.) and by informing all authorities concerned.

Registration of Beneficial Arthropods

Beneficials are different from chemical pesticides. Legislation has been developed for chemical pesticides because of issues such as phytotoxicity, potential hazards for the user, consumer or animals, worker exposure, residues on food or in the environment, side-effects on non-target organisms, resistance development, etc. Biocontrol has been developed as an alternative to chemical pest control because of these very issues.

Currently, little regulation exists for the use and import of (non-indigenous) beneficial arthropods. Exceptions are the USA, Great Britain, Canada, Japan, Poland, Sweden, Austria, and Switzerland. Other countries are increasingly asking for more information before approving import permits, however. There is also no harmonization of the legislation for beneficials of all these countries. Several national and international authorities are currently working on registration legislation for beneficial arthropods.

Inappropriate regulation designed by legislators with no knowledge of the characteristics of biocontrol and without consulting the biocontrol industry is a serious potential threat for the future not only for the biocontrol producers but also for the use of beneficial arthropods for biological pest control. For example, import taxation and high registration fees have made the access to the Swedish market very difficult. Over-regulation of the introduction of non-indigenous beneficial insects may hinder the development of "new" beneficials and have a negative impact on the further development of biocontrol packages.

Conclusions

The goal of the IBMA is to effectively represent the producers of beneficial arthropods and provide a unified voice for the biocontrol industry worldwide. Recognition of the unique aspects of our industry will result in legislation that is appropriate and relevant. Contacts have been established with the EPPO, IOBC, OECD and EU, where IBMA members now regularly represent our industry.

While a certain level of regulation is inevitable, we hold the view that self-regulation will allow the industry to achieve industry-wide quality control standards. Further, we believe that the biocontrol producers should work together to design and implement international standards for product labeling. The IOBC has an important role to play in both these issues and their collaboration will allow for greater acceptance of our guidelines.

Communication with ourselves and with regulatory authorities is essential if we wish to attain uniform legislation, which stimulates the further development of a sound and prosperous biocontrol industry.

The Regulation of Invertebrate Biological Control Agents (Insects, Mites and Nematodes) in Canada

M. J. KELLEHER

Health Canada, Pest Management Regulatory Agency, CANADA

Abstract. Invertebrate Biological Control Agents (IBCAs) are regulated jointly by the Animal and Plant Health Directorate (APHD) of Agriculture and Agri-Food Canada and the Pest Management Regulatory Agency (PMRA) of Health Canada. The following provides an overview of current regulations and anticipated changes to them.

APHD is responsible for the regulation of importation and domestic movement of IBCAs. As a member of the North American Plant Protection Organization, Canada has adopted the organization's standards for the evaluation of the potential a species' potential to affect plants. Evaluations by APHD include entomophagous organisms (parasitoids and hyperparasitoids) because they can have indirect effects on plants or ecosystems through community disruption. When necessary, import permits can stipulate time limits for use and the number of organisms to be transported. These permits can also restrict release to certain geographical regions or quarantine the organism to secure holding facilities. To streamline the process, APHD plans to recognize organisms that have a history of favorable risk assessments by creating two lists of precedented species that will no longer need an import permit: one list of organisms requiring notification and another list of organisms no longer requiring notification or an import permit.

The PMRA is responsible for the regulation of pest control products in Canada, and is developing guidelines for the regulation of commercial Invertebrate Biological Pest Control Agents (IBPCAs). Each product is assessed for safety to the environment and human health, efficacy and quality control. The PMRA strictly controls the text of labels on pest control products and issues an approval number, printed on the product label, when it is satisfied that the product meets the established criteria. Thereafter, it is an offense to use a product or make claims about a product beyond the explicit text of the label.

In developing a regulatory approach for IBPCAs, the fundamental differences between these living organisms and conventional chemical pesticides have been taken into consideration. Advice was solicited from industry, research scientists, and various government agencies during the review of numerous drafts of the guidelines and two workshops held since 1993. A broad perception exists that most IBPCA products are potentially safer to human health and the environment than conventional chemical pesticides. Consequently, a multi-tiered strategy based on hazard/risk has been adopted and, in many cases, data requirements may be waived entirely provided that a scientific rationale is given. The PMRA can also access the expertise of a Technical Advisory Committee, composed of scientists from various biocontrol disciplines, when needed. We feel that the various stakeholders appreciate this commonsense approach to regulation.

QUALITY MANAGEMENT

TQM (Total Quality Management): Managing Quality, Not Just Controlling or Measuring It

M. BURT

Executive Director of the Association of Natural Bio-control Producers
California, USA

Abstract. Arguably, Quality Assurance is the most formidable and critical challenge facing producers of live biological organisms, whether produced for research or for commercial use. "Horror stories" abound of experiences with production contamination, host material problems, employee-caused contamination, transit survival, etc. The results are loss of production, cost over-runs, shipping delays to customers, and the effect on the producer's or researcher's reputation and credibility.

TQM is a proven and powerful management tool that can provide significant quality control over living organisms first demonstrated by W. Edwards Deming in Japan after World War II. TQM is a "way of life" for countless organizations throughout the world. TQM focuses on the customer's needs and expectations.

Instead of traditional quality inspection "after the fact" and just prior to delivery to the customer, TQM embraces (1) standardizing process conditions, (2) stabilizing the process, including ongoing statistical process control (SPC) during the process. The result "goodbye" to costly rejects and "hello" to quality improvement and lower costs!!!

TQM pushes down conventional "top-down" management to the people who know most about the product or service and the surrounding quality issues. TQM is a challenging "cultural change" and requires visionary leadership. Of note, there are demonstrated successes with living entities, along with dramatic results in small organizations, or even departments/functions.

This presentation reviews the TQM "process" and most importantly, how to get started.

The Speaker. Maclay Burt is former Director of Agricultural Operations of Hunt-Wesson Foods, the world's largest tomato processing organization. He successfully instituted and managed a TQM environment with perishable "living" crops. Mr. Burt is the Executive Director of the Association of natural Bio-control Producers, as well as a private agribusiness and management consultant.

Examples of biocontrol production control or quality disasters

- Production contamination, wrong specie
- Squash, potato rot
- Equipment or machinery failure
- Employee contamination of the Mother Culture
- Transit survival, counts, shipping delays

What is "Total Quality Management" (TQM) all about? TQM is a powerful tool that provides significant control over ***living organisms***.

- Origins/history; it all began with W. Edwards Deming in Japan after World War II
- Concept/philosophy
  1. Understands who the "customers" are: ***external*** (traditional outside sales customers) and ***internal*** customers (the next function or department in the "manufacturing" sequence).
  2. Recognizes what the "customer" needs or wants
  3. Provides the needed product or service at the highest possible quality and service level (not just "cost-efficient" - ***costs will decrease!***)
- ISO 9000 is a more recent and more sophisticated system for absolute quality standards and assurance. ISO 9000 offers a certification program to assure customers that standards will be met by vendors.

Why implement TQM?

- Quality improves dramatically
- Cost goes down, ***even in a price pressure market***
- Stabilizes the process from person-to-person, shift-to-shift, facility-to-facility
- No more "after-the-fact" inspection and rejects; "inspection" control is an ongoing process
- Traditional department and customer barriers go down; manage for the ***enterprise*** (the business as a whole, not just individual operating departments)
- Numerical goals and work standards will disappear

Who does TQM?

- Nearly all industries in Japan
- Federal Express
- America West Airlines
- Ford
- GM Cadillac
- Florida Power and Light
- Harley Davidson
- Oscar Meyer
- Campbell Soup

TQM: A "cultural" change. Ideally, TQM involves the entire organization or company. But in the case of small organizations with limited resources and staffing, "mini" TQM applications tailored to specific problems can yield impressive results.

- ***Responsibility*** is pushed down to "empower" ***the people who know the most about the job, process or service***. On-going teams identify their internal customers, the customers' needs and ***constantly*** comprehend and improve the process for their product or service.
- TQM's focus is on the ***process***; it's not an event or activity or a "thing." TQM separates the improvement process from people personalities and egos. "Top-down" management styles disappear.
- Quality improvement and cost reduction is a ***constant***, never-ending process; the improvement horizon is always moving.
- Reporting levels are reduced/streamlined.
- Management's role shifts to more emphasis on coaching.
- Jobs are not eliminated, but job content may change; personal careers are enhanced.

How in the world can <u>you</u> get started?

- There has to be a "visionary" in your organization, someone comfortable with ***change***, as well as a bit of a risk-taker.
- The CEO and top management must be committed to TQM ***throughout*** the organization; but if this isn't feasible, incredible progress can be made by individual groups.
- It is the ***leaders*** that make TQM happen, not just the people or the teams.
- Sweep in the vendors - plants (squash, lemons, beans), planter mix!

The TQM "Process" (Thanks to W. Edwards Deming!)

- Create a positive environment
- Develop the process model; identify the key steps
- What are the variables that impact the key steps?
- Standardize the process conditions
- Communicate process conditions
- Stabilize the process, apply STATISTICAL PROCESS CONTROL
- Improve the process

Implementing TQM - the hardest challenge. ***Don't be afraid of false starts; <u>do something</u>!!!***

- Typically, a consulting organization will help get the new philosophy off the ground. So, you don't have to invent the wheel! Consultant resources can range from "turn key" operations to short-term "jump start" assistance.
- There's no "cookbook" how to do it. But, there are books and videos that help explain the "process" steps. Pick out some "fat rabbit" problems that are easy to improve so you can show some ***immediate*** results. If the workforce is truly educated, they will accept some slippage along the way.
- The focus is on ***results***, not just activities or the process.
- ***Again, get started! You <u>can</u> manage living organisms!!!***

Getting started

- Flow-chart the steps in your process, hopefully your entire process from the very beginning and ending with receipt and use by your customers. If the larger scale approach isn't feasible, select a particularly troublesome part of your process
- Identify the critical steps in your process
- Identify the variables (i.e., temperature, humidity, host material, etc.) that impact these critical steps
- With your staff and the people closest to the variables, develop "standards" to control the variables
- Initiate Statistical Process Control techniques to stabilize the standardized steps
- Constantly improve the process; remember, the horizon is always moving!!

Executive Overview of ISO-9000 Combined with Strategic Planning

E. BORGES

InterQual
Santa Barbara, California, USA

Abstract. This presentation targets executive management. It takes the audience on a "tour" of ISO-9000 processes, while clarifying the requirements of the standard. This presentation also shows a systematic way to implement the requirements and achieve your business' strategic objectives at the same time.

The Speaker. Eliana Borges is a Certified Quality Systems Lead Auditor (RAB # Q04418), a UCSB Instructor, and a Consultant with experience working for one of the largest Registrars in the World (Det Norske Veritas - DNV). Ms. Borges has participated in numerous national and international Certification Audits. She facilitates the ISO-9000 Advocacy Group monthly meeting sponsored by the American Society for Quality Control. She is fluent in Spanish, Portuguese and English, and she is an Effective and Motivational Trainer, with expertise in ISO-9000 Systems Implementation and Assessments.

About InterQual…

Our Mission

InterQual is in the business of providing you with the Quality System services required to successfully and effectively compete in the Global Market during the 90's and beyond.

Why?

The competition is tough, pressuring businesses into cost reductions, internal systems improvement and better relationships with their customers and suppliers. A system to increase your sales, and productivity, is not a choice any longer - IT IS A NECESSITY. The economic turmoil of the 1990's has forced American Businesses to streamline operations, increase market share and expand into the global community.

The world market, however, is becoming very sophisticated and is emphasizing the demand for product quality and customer satisfaction.

What is ISO-9000?

ISO-9000 is a set of international standards to implement Quality Systems. ISO-9000 implementation provides you with the tools to integrate your company's objectives with a company-wide continuous process improvement system.

History

During the 70's & 80's the European Community (EU) was harmonizing standards to facilitate trade. In 1979 ISO formed Technical Committee to address growing confusion in international trade. The International Technical Committee 76 released its first version of the ISO-9000 standards in 1987. A revised version (94) is now in effect. Objective: to facilitate trade by providing a common terminology and requirements. Over 100 countries have adopted the ISO-9000 series as their Quality Standard - this number is expected to continue to grow.

Your organization is a reflection of YOU, so what are you projecting? What will your company be like in the year 2000 and beyond?

It is time you look into the ISO-9000 Certification Process!!!

Let's face it - things are picking up out there. It is now a big playing field (world market) and you have to prepare for the future. International customers require ISO standards for a multitude of products. The regulated industries (medical, construction, telecommunications, and others) have no choice but comply with these new requirements. Your employees need to be aware of the requirements and what they mean. Your suppliers need to be informed so they can prepare for the future. Don't wait to find out you lost a large share of the market to the competition - ACT NOW!

The Certification process is not the hardest hurdle. The real challenge is to get your system documented in a smart fashion and to get your people trained to achieve the objectives of the system.

Services

We are a group of highly qualified professionals, with a broad range of experience in the fields of Electronics, Computers and Peripherals, Aerospace, Telecommunications, Medical and Automotive. All of our professionals are trained ISO-9000 Lead Assessors who work under the supervision of a Certified Quality Systems Lead Auditor (RAB or IQA Certified). Our services include (but are not limited to):

* Multilingual support (English, Portuguese and Spanish).

* ISO-9000 (ISO-9001, 9002 and 9003) Pre-Assessments.

* ISO-9000 Training

* Executive Overview (including Strategic Planning relationship to ISO- 9000 - open to the public or on-site)

* Internal Quality Audit Workshops (open to the public or on- site, tailored to your business, including hands-on experience for the Internal Quality Auditors.)

* Management of the Implementation Effort - Preparation of milestone schedules and assistance in achieving implementation at minimum cost and on schedule.

* Preparation of Manuals, Procedures and Work Instructions - We fold your system description into our electronic media templates, and let you play with the drafts until you are happy.

* Support of Management Reviews of the Quality System, Corrective Action System and Internal Quality Audits.

What are the milestones?

* Train Executive Management - They set policy for everyone to follow. Find out what ISO-9000 is, and what it does for you. Focus on the benefits derived from its implementation, such as: adoption of TQM techniques, continuous process improvement & cycle time reduction. ISO-9000 implements a cultural change in the organization, as a direct result of quality training and product quality awareness.

* Train the Internal Quality Audit Task Force - if ISO-9000 implementation could be compared with a train, the Executive Management functions as the Engineer and the task force is the engine pulling it forward.

* Define your company's strategy - Define objectives and measurements. Clarify the quality policy and company priorities. Assign responsibilities for policy definition and implementation. Assign a corporate champion (ISO representative).

* Document your system - Flowchart, write your Quality Manual, Procedures and Work Instructions ... and remember to keep it simple!

* Scope the job - Assess your company's existing quality system. Use internal auditors and an independent outside ISO consultant or Registrar. Identify areas of non compliance to ISO-9000 and corrective action required.

* Plan the ISO-9000 Implementation - Plan the whole process. Define the resources and management support to achieve the milestones. Determine skills and availability of in-house resources. Obtain outside consultants or training providers as necessary. Assign work teams. Establish company standards for all elements of the total quality system, including policies, procedures, work instructions, documentation and records. Develop an ISO matrix to provide the overall framework and assure integration of all elements.

* Upgrade Operations to ISO-9000 requirements - Take the opportunity to re-engineer your processes to improve productivity and eliminate waste. Document the improved processes and procedures.

* Train all employees - Management and Workers - Train all employees, both management and workers, in the new system. Assure that all managers and employees understand and “buy in” to the company-wide quality system.

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Evaluation of Proposed Association of Natural Bio-control Producers (ANBP) Quality Assurance Standards

J. C. WHEATLEY

Crop Science Department, California Polytechnic State University, San Luis Obispo, CA.

Abstract. Twelve students in California Polytechnic State University's (San Luis Obispo, CA) Crop Science 47OX, Research Methods in Entomology class evaluated proposed Association of Natural BioControl Producers (ANBP) quality assurance standards for five beneficial organisms: (1) *Cryptolaemus montrouzieri* Mulsant, (2) *Phytoseiulus persimilis* Athias-Henriot, (3) *Trichogramma* spp., (4) *Aphytis melinus* (DeBach), and (5) *Chrysoperta rufilabris* (Burmeister). Evaluations were initially conducted by the class as a whole to test the feasibility of the proposed testing protocols. Later, small groups of 2 to 3 students repeated each test using refined techniques to gather and record data on each organism for analysis. In all but two instances organisms supplied by commercial insectaries met the minimum standards proposed by ANBP. The most common problems encountered in applying the proposed protocols to actual lots of beneficials were in ambiguity of sampling techniques: Sample size, method of obtaining sample, randomization, etc. Extensive time requirements and gender determination, especially with *P. persimilis*, were also problematic.

Introduction and Discussion

The winter quarter at Cal Poly was over on March 20th, and the Quality Control/Product Certification class completed. Most of the lab work is done and the student teams' posters will be on display at the ANBP-AAIE meeting in February. Four of the posters were displayed at the CAPCA meeting in Anaheim and one won the $250 student competition. Each 2-person team (there were 6 teams) was responsible for the testing and write up of one of the five organisms that we studied: *Trichogramma* sp., *Cryptolaemus montrouzieri* (mealybug destroyer), *Phytoseiulus persimilis* (predaceous mite), *Aphytis melinus* (California red scale parasitoid), and *Chysoperia rufilabris* (a green lacewing).

Contributing insectaries were Buena Biosystems (Santa Paula, CA); Oxnard Pest Control (Oxnard, CA); Fillmore Citrus Protective District (Fillmore. CA); Associates Insectary (Santa Paula, CA); Beneficial Insectary (Redlands, CA); Sespe Creek Insectary (Lindsay, CA); Bunting Biological (Ventura, CA); Rincon-Vitova (Riverside, CA), and Biotactics (Riverside, CA). In addition to furnishing us with beneficial insects and mites, many of the insectary owners, managers, and rearing personnel drove to San Luis Obispo and made presentations to the class. Each organism was tested twice - once by the entire class in a formal laboratory setting and again by the team assigned to do further lab work and documentation on that particular creature.

Each organism presented unique challenges. We started the class with *Trichogramma*, thinking that since the product is shipped and enters commerce in the form of parasitized host eggs, this might be a relatively easy one on which to begin. To some extent, that was true.

Jake Blehm brought up some of his product in two delivery systems - host eggs (*Sitatroga cereale*) glued onto paper that was scored into smaller "release units" and another system consisting of parasitized host eggs in small paper bags termed "Blopac". We elected to work with the eggs glued onto paper. The first dilemma revolved around a crucial point - sampling - specifically, where to sample (what portion(s) of the paper and what size sample, i.e., number of eggs, to pull. We eventually settled on using a punch to cut out equal size subsamples from the paper. After experimenting with round punches of several sizes, we adopted the oblong, oval-shaped punch frequently used by entomologists to make points for mounting small specimens. That shape more readily lent itself to accurate counting of eggs by the greatest number of students.

The next step was differentiating parasitized from non-parasitized host eggs. With the use of the microscope video camera set-up, Jake was able to point out the color change in the parasitized eggs to the class. The students then determined the percent parasitized eggs as called for on the ANBP product profile. Samples were then put into vials and stored in a temperature chamber at 24EC, 14:l0 L:D to await the emergence of the parasitoids. This was all pretty straight forward and only a few logistics problems surfaced, such as finding a material with which to cover the opening of the glass vials that would allow for moisture passage yet contain the small wasps. A black organdy fabric was found at a local fabric shop and a double layer of it provided sufficient ventilation while still securing the adult wasps.

Sexing of the tiny wasps was based on shape of antennae segments and pubescence. Although microscopic in size, this task proved to be rather simple and the students made the separations easily.

Since the ANBP profile and IOBC workshop guidelines were rather detailed for *Trichogramma* spp., we decided to go further with this organism, so a fecundity trial was set up by the student team of Derron Dike and Karen Christiansen. The students traveled to a contributing insectary (more than one source was used for the beneficials) for a source of fresh *Sitotroga* eggs and parasitoids the following week. The fecundity test was run according to the guidelines in the Proceedings of the IOBC Working Group on Beneficial Agents, 1991. All *Trichogramma* samples met ANBP quality standard.

As it turned out, the first organism was the most straight forward. Working with *Cryptolaemous* also proceeded smoothly, once we figured out that an ice bath slowed the small beetles enough for accurate counting and handling. Sexing the beetles took some practice but the students became proficient very quickly. All mealybug destroyer samples met ANBP standards for number in container (count), percent mortality, and sex ratio.

Lacewing testing was much more involved, since mortality through egg hatch and two larval molts was specified. The time to set up the test was considerable but the samples we worked with also met the ANBP guidelines. Some problems were encountered with having a sufficient supply of host eggs (*Sitotroga*) fresh enough for the tiny larva. Sexing of the small larvae was out of the question and the usefulness of this requirement may have to be reevaluated.

Working with *Phytoseiulus persimilis* and *Aphytis melinus* was saved for last, which was just as well, because if we had started with these the whole project might have floundered. Handling the active adult stage of *Aphytis* proved to be more interesting and challenging than anticipated, and after inhaling hundreds of the little creatures we had to admit that we have not found a really good sampling technique yet - at least not one that samples the entire lot. Sexing was simple and counting, although tedious, could be accomplished accurately. We did validate the volumetric measures used by one insectary - the old UC Riverside work was still valid.

The sampling of mites shipped in vermiculite turned out to be a lot of fun. We used several small seed dividers and found one that made very precise subsamples, enabling us to count the mites easily. Sexing was to have been done as an adjunct to the fecundity test, but the whole thing broke down when our spider mite infested bean plants crashed just prior to the test date. We tried to substitute the substrate with statis leaves, but those did not hold up for the duration of the test period. Basically, the mite test needs to be redone, as does the sampling portion of the *Aphytis* test. That work is planned for the Winter.

Refinement of the sample sizes for all the tests will also be done this winter. Funding from NBCI actually reached the campus in October 1994, so I will have some release time to work on the project again. Additionally, two graduate students are available and one, Launnie Ginn, is currently working on the mite test. The $2000 that ANBP gave us last spring for matching funds actually kept the project alive and my department head, George Gowgani, was trusting enough to advance me monies prior to that even getting to campus, so a lot of people worked pretty hard last winter and hopefully the full report will be done on the original five organisms by this spring. I hope to see many of you at the February meeting, although I will not be able to attend until Monday, after the ANSP meeting. This report is rather casual I realize, long on narrative and short on the sort of data we all love, but you'll just have to wait until spring for the boring part.

Sampling Techniques for Quality Evaluation of *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) and *Phytoseiulus persimilis* (Acari: Phytoseiidae)

S. M. THOMPSON and J. C. WHEATLEY

Crop Science Department, California Polytechnic State University, San Luis Obispo, CA.

Abstract. A sampling methodology was developed for two commericial, beneficial organisms, *Trichogramma* spp. and *Phytoseiulus persimilis* Athias Henriot. Sampling techniques were needed as an adjunct to evaluation of producers' compliance with product profiles. *Trichogramma* spp. was handled in two forms, eggs glued onto a sheet of perforated paper and loose eggs mixed with vermiculite and host eggs for food. The predaceous mite product, *P. persimilis* was shipped in 500 ml plastic bottles containing adult mites in vermiculite media. Samples of each product were tested to determine the accuracy of the count, the mortality rate, sex ratio, and percent parasitism as specified in the respective product profile. Cluster sampling was used for *Trichogramma* eggs on paper. Simple random sampling was used for loose *Trichogramma* eggs and for *P. persimilis.*

Chrysoperla rufilabris (Burmeister) (Neuroptera: Chrysopidae) Quality Assurance Techniques

R. FOSTER, L. COLLINS and J. C. WHEATLEY

Crop Science Department, California Polytechnic State University, San Luis Obispo, CA

Abstract. Quality assurance testing procedures proposed by the Association of Natural BioControl Producers and the International Organization for Biological Control were evaluated. Eggs and second instar larvae of *Chrysoperla rufilabris* were evaluated separately according to their individual standards. Eggs were evaluated for: (1) quantity, (2) longevity, (3) hatching rate and (4) predator quality. Larvae were evaluated for composition (development of second instar to third instar larvae within five days). The guidelines were reasonably straightforward to apply, and required 21.5 student hours over a five-day period. *C. rufilabris* furnished by a commercial insectary exceeded all minimum standards proposed by IOBC and by the ANBP for both life stages of the product.

Cryptolaemus montrouzieri Mulsant (Coleoptera: Coccinellidae) Quality Assurance Techniques

N. LaGRILLE, R. GOERTZEN and J. C. WHEATLEY

Crop Science Department, California Polytechnic State University, San Luis Obispo, CA

Abstract. Proposed Association of Natural Biocontrol Producers' quality standards for commercially reared *Cryptolaemus montrouzieri* were evaluated. Test criteria proposed and evaluated were: (1) counts, (2) shipping mortality, and (3) sex ratio. The entire testing procedure took twenty-two hours including set-up, counting, and recording data. Samples furnished by commercial insectaries met the ANBP quality minimums. Problems were encountered with the evaluation protocols in pulling random samples for sexing, training lab personnel to make accurate gender determination, and in keeping the very active beetles under control during testing.

Phytoseiulus persimilis Athias-Henriot (Acari: Phytoseiidae) Quality Assurance Techniques

T. MARCI, M. MASON, L. GINN and J. C. WHEATLEY

Crop Science Department, California Polytechnic State University, San Luis Obispo, CA

Abstract. The predatory mite *Phytoseiulus persimilis* was subjected to quality testing to assess the product as sold and shipped, for compliance with industry standards proposed by the Association of Natural Biocontrol Producers. Testing methods and techniques were evaluated for personnel and time requirements. Twenty-three hours were required to complete the test. Counts of commercial *P. persimilis* shipments were within guidelines for quantity. A five-day test was run to determine sex ratio, fecundity and longevity but was unsuccessful due to problems with the leaf substate. A new test parameter, "Reproductive Index" (RI), derived from sex ratio, fecundity and longevity data is proposed: RI = total mite forms produced in five days/total number of mites tested for fecundity. This measurement would be less time-consuming and be an accurate reflection of the mites' reproductive capacity.

The Population Genetics of Mass-rearing

L. NUNNEY

Dept. of Biology University of California
Riverside, CA 92521
909 787 5011
Nunney@Citrus.ucr.edu

Abstract. Research to improve the effectiveness of mass-rearing is of central importance to biological control. While most research has concentrated on the basic issue of how to maximize yield, it can be argued that quality and not quantity is crucial to a successful release. For example, the SIT release of Medfly requires the production of large numbers of high quality flies. Our ability to produce large numbers of flies is without question: the Hawaiian rearing facility can produce more than 400 million pupae per week. It is less clear that the flies are of a high enough quality to compete successfully with wild conspecifics.

The theory of population genetics applied to mass-rearing suggests some information that can be used to evaluate the likely success of a release program: 1. the genetic structure, size and geographical origins of the founding population; 2. the effective size of the population in the first few generations of captivity; 3. the degree of adaptation to the rearing facility, measured as a change in such factors as life history parameters and mating patterns.

In establishing a mass-rearing program, the size of the initial sample is crucial. Thousands of individuals are infinitely better than one or two. However, it is less clear whether the mixing of individuals from different geographical locations is advantageous - the answer is almost certainly species specific. But having brought a population into captivity, there is a real danger that over the first few generations the initial benefit of a large number of founders will be lost. This can occur if a few genotypes are by chance pre-adapted to the rearing conditions and swamp out the others. The problem can be minimized by dividing the sample into very small breeding units, so that the reproductive success of a larger number of individuals is ensured.

In general, it is to be expected that the quality of a mass-reared strain will decline over time. Thus, it is important to establish a protocol for either the establishment and mass-rearing of new populations or for successfully incorporating new genetic variation into the original population. This issue can be particularly problematic when genetically manipulated stocks are involved because of the technical problems associated with producing large numbers of genetically diverse individuals all carrying the same manipulated DNA. It is clear that the tendency of genetic manipulation to result in inbred, laboratory-adapted and/or low fitness individuals must be avoided.

Adaptation to the mass-rearing conditions is inevitable (for example, in captive Medfly stocks, there is typically a marked reduction in the pre-reproductive adult period from about 10 to 5 days). Some adaptation may, in fact, be necessary for the success of mass-rearing. Thus,

research is needed to determine (i) what traits are favored under mass-rearing; (ii) the extent to which such traits reduce field performance; and (iii) how to minimize the selection for traits that reduce field performance. The information can be used to determine when to discard or augment a captive population.

Finally, no guidelines can guarantee success. The final test must be in field trials, comparing, when possible, the performance of mass-reared individuals to the true "wild type". It has been shown time and again that genotypes that function perfectly adequately in the laboratory often fall far short of adequacy when compared to their wild conspecifics.

The Goal of Mass-Rearing

Effective mass-rearing is of central importance to successful biological control, particularly now that augmentative biological control is being recognized as an important alternative to chemical control (see Parella et al., 1992). The goal of mass-rearing is to facilitate the release of large numbers of high quality biological control agents, and research to improve the effectiveness of mass-rearing should have high priority.

A mass-rearing program is suboptimal if it results in low numbers and/or low quality. Of these two criteria, numbers are easily assessed but, in general, quality is not. As a result, it is not surprising that most research has concentrated on the basic issue of how to maximize the yield from a rearing facility; however, it can be argued that quality may often be crucial to a successful release. For example, the SIT release of medfly requires the production of large numbers of high quality flies. Our ability to produce large numbers of flies is without question: the Hawaiian rearing facility can produce more than 400 million pupae per week. It is less clear that the flies are of a high enough quality to compete successfully with wild conspecifics (see Shelly et al., 1994).

The Problem

In any captive breeding program, the goal of maximizing the number of individuals produced is sound. From this it would appear that under most circumstances the problem of captive breeding is to produce high numbers while simply maintaining quality. The problem is that "simply" maintaining quality may not be easy and, in many cases, may not be possible.

When captive breeding is linked to biological control, the organisms are implicitly being required to perform efficiently in two very different environments, the rearing facility and field. This presents a dilemma that is at the very core of evolutionary biology: a generalist is usually "a jack-of-all-trades and master of none", whereas specialists are presumed to trade-off some of their generalist abilities. In the natural environment, both strategies can be highly successful, but in the context of captive breeding, there is a danger of trading some of the specialized abilities that make the species a successful control agent for equally specialized abilities that allow it to maximize reproductive output in a rearing facility.

This problem of "trade-off" can be viewed graphically (Fig. 1). When control is achieved by augmentative release, it is usually the case that a decline in quality can be offset

by releasing more individuals. This relationship creates a series of curves, with each curve defining combinations of quality and quantity that will achieve the same level of control. Superimposed on this background is the control agent itself. A population fresh from the natural environment, if founded appropriately, will have high quality, but may breed poorly under artificial conditions. Usually, this breeding success can be enhanced over time as a result of selection: those genotypes that breed successfully will become more abundant and the genetic composition of the breeding stock will change. Unfortunately, it is very unlikely that such a change will enhance field performance; it is much more likely that field performance (i.e., quality) will decline as the effectiveness of the rearing (i.e., quantity) increases (the solid curve in Fig. 1).

After a few generations (about 10-20), it is likely that the rapid adaptation to the rearing facility will slow and the resulting "domesticated" stock may be quite stable. There is, however, a danger that over the longer term, inbreeding may become a problem and both quantity and quality will begin to decline (the lower portion of the solid curve, Fig 1).

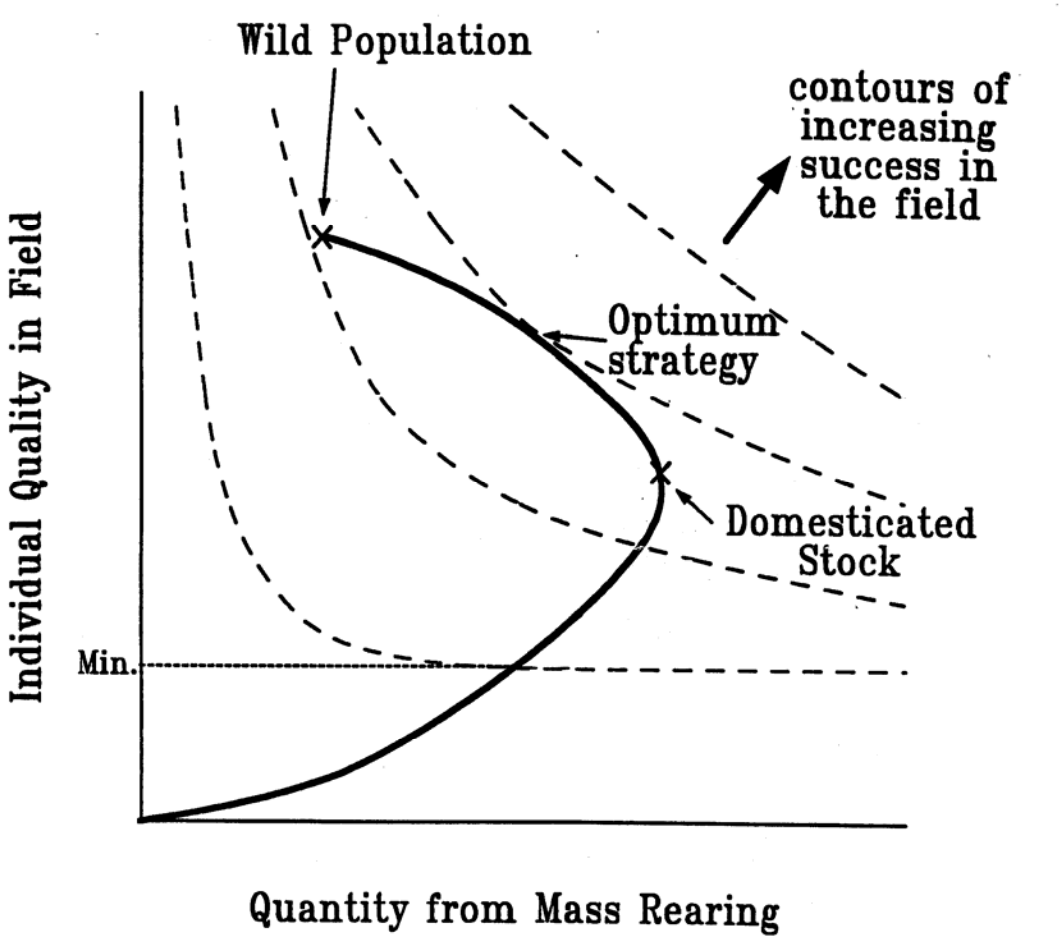

Figure 1. A graphical representation of expected trade-off between the numbers produced in mass-rearing and quality of performance in the field. The solid curve defines the trade-off and each dashed curve defines the combinations of quality and quantity that could achieve a given goal.

The precise shape of the curve linking the wild population and the domesticated stock will vary, but it is probable that in many circumstances the optimum stock, yielding the highest level of success, will be defined by some point between these two. The solution is either to change the rearing conditions so that the trade-off is minimized or to prevent the stock reaching the "domesticated" equilibrium.

Evidence for a Trade-Off - The Case of the Melon Fly Males

The melon fly (*Bactrocera cucurbitae*) was successfully eliminated from the island of Kume-zima in Japan in 1977 using SIT (sterile insect technique). This technique relies for its success on the mating ability of the captive-reared sterile males. Ito (1988) reviewed the lessons of this eradication, focusing particularly on the problems raised by the "domestication" of the male flies due to their continued mass-rearing. The field releases of sterile males began after about 10 generations of captivity, and estimated field success of the released males compared to wild type males was quite high, at about 75%. However, after gen. 10 a steady decline was apparent, so that by gen. 15 the success was below 50% and by gen. 18 it was only 20%. These field effects were completely hidden in laboratory experiments that still showed a very high relative success (around 100%) for the sterilized flies.

It was shown that two factors affected mating success. First, the flight ability of the mass-reared flies decreased (by about 50%), limiting their dispersal ability; and second, after courtship, the mass-reared males were rejected by wild females with a high probability perhaps because of differences from normal in their courtship wing vibrations.

A Strategy for Mass-Rearing

The theory of population genetics applied to mass-rearing suggests some information that can be used to evaluate the likely success of a release program: 1. the genetic structure, size and geographical origins of founding population; 2. the effective (or genetic) size of the population in the first few generations of captivity; 3. the degree of adaptation to the rearing facility, measured as a change in such factors as life history parameters and mating patterns.

In establishing a mass-rearing program, the size of the initial sample is crucial. Thousands of individuals are infinitely better than one or two, and a minimum of several hundred is certainly advisable. The source populations should, where possible, be from regions that are climatically similar to release sites. However, it is less clear whether the mixing of individuals from different geographical locations is advantageous - the answer is almost certainly species-specific. In some cases such crosses could lead to the breakdown of geographically co-adapted gene complexes, leading to an unpredictable shift in some traits. This can be a major problem if, for example, in a SIT program the shift is in mating behavior.

Having brought a population into captivity, there is a real danger that over the first few generations the initial benefit of a large number of founders will be lost. This can occur if there is the all too typical "crash-recovery" cycle (see Leppla et al., 1983), whereby a few genotypes are by chance pre-adapted to the rearing conditions and swamp out the others. The

problem can be minimized by dividing the sample into very small breeding units, so that the reproductive success of a larger number of individuals is ensured.

Adaptation to the mass-rearing conditions is inevitable (for example, in captive stocks of the medfly, *Ceratitis capitata*, there is typically a marked reduction in the pre-reproductive adult period from about 10 to 5 days: see Wong and Nakahara, 1978). As noted earlier, some adaptation may, in fact, be necessary for the success of mass-rearing. However, a strategy is needed to avoid too much domestication. One approach is to favor specific traits through the design of the rearing facility. Boller (1972) has suggested incorporating "luxury" stimuli that have no effect on yield but might serve to maintain essential behavioral traits and to build in some suboptimal conditions such as remote food sources and fluctuating temperature to maintain other types of essential traits. A related strategy suggested by Mackauer (1976) is to rear the stocks intermittently under semi-natural conditions, using field cages and, where feasible, field longevity can be promoted by recapturing released individuals.

A more direct way of preventing the captive stock from reaching its "domestication" equilibrium is to supplement stocks with wild caught individuals. Catching and adding individuals from the wild may present little problem; however, this does not ensure that these new individuals contribute to the strain. Maladapted to the rearing environment, they may reproduce poorly or not at all. It may be necessary to hybridize the wild genotypes with the laboratory strain under semi-natural conditions, before the new genes can be successfully introduced. And finally, the introduction should be monitored in terms of some phenotypic measure that differs between the wild and captive strain. Following supplementation with wild-caught genotypes, there should be a measurable shift in the monitored character to demonstrate success.

Supplementation with wild-caught individuals is intended to prevent the expected decline in quality of a mass-reared strain. It is important to establish a protocol either for continued supplementation, or, if this is impractical, for the periodic establishment of new strains that will eventually become the mass-rearing strain, replacing the current declining stock.

The Problems of Genetic Engineering

Genetic engineering opens up a wealth of new possibilities for biological control agents. However, it also presents particular problems for mass-rearing. The creation of genetically manipulated stocks inevitably involves the loss of background genetic variability. The problem is how to produce large numbers of genetically diverse individuals all carrying the same manipulated DNA. In particular, it is clear that the tendency of genetic manipulation to result in inbred, laboratory-adapted and/or low fitness individuals must be avoided.

In genetically well-defined organisms, such as *Drosophila melanogaster*, it is quite easy to outbreed an engineered stock, while retaining its novel characteristics; however, this is not generally the case. Thus part of the research effort dedicated to creating new engineered stocks should address the problem of placing the beneficial traits on a diverse genetic background that will perform successfully in the field, and not just under protected laboratory conditions.

Conclusions

No guidelines can guarantee success. The final test must be in field trials, comparing, when possible, the performance of mass reared individuals to the true "wild type". For practical reasons, standardized laboratory tests are an attractive alternative; however, such test must be distinguishing something far more subtle than pathological inadequacies. In particular, tests must distinguish the small performance differences that define a drop in field quality. For example, a criterion of flight ability used for evaluating medfly involved successfully flying out of a 20cm tall cylinder (Boller et al., 1981). This distinguishes the totally inadequate flies from others, but consider the more realistic evaluation discussed by Ito (1988). He noted how the flight ability of a mass-reared strain of melon fly was severely compromised - although they could still fly for over an hour, this was half the flight time of wild-type strain. The medfly approach could never hope to detect these kinds of important differences.

Field testing mass-rearing stocks under realistic conditions is time consuming and difficult. But the limited data available suggest that it is crucial. Boller (1972) made an important philosophical distinction between the prevailing view of production efficiency (cost per individual), which emphasizes quantity and has little regard for quality, and the alternative view of the cost to achieve a goal, which weights quantity and quality in the ways diagrammed in Fig 1. His insight is equally relevant almost 25 years later.

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Quality Control of Natural Enemies: Mass Rearing Systems

N. C. LEPPLA

University of Florida, Institute of Food and Agricultural Sciences, Central Florida Research and Education Center, Apopka, Florida 32703-8504, U. S. A.

Abstract. The current challenge to mass rearing biological control agents is to greatly reduce the cost of their production and application. Quality control procedures can be used to optimize rearing operations, minimize the deterioration of agents during shipment and release, and maximize their efficacy in the field. The objective is to use few, relatively inexpensive, and carefully administered natural enemies to control pest populations with precision. It is necessary to periodically assess the competitiveness of colonized strains, monitor them for genetic and behavioral changes, enforce production control (monitor all rearing operations in terms of personnel, materials, equipment, schedules, environments, etc.), establish process control (sample immature insect stages to predict quality and determine sources of efficiency), perform product control at the rearing facility and at critical points in the field, measure field performance, and provide feedback to optimize production and application. Total quality control in the mass rearing of biological control agents is composed of eight generic elements: management, research, methods development, material, production, utilization, personnel, and quality control. Although often not individually identified, all of these elements are present in pest management systems based on mass-reared natural enemies and each has internal control points. Coordination across the interdependent elements of these systems and feedback to management provides a means of eliminating unnecessary costs and delivering the most efficacious biological control agents.

NEMATODE QUALITY CONTROL

Entomopathogenic Nematode Total Quality Management

R. MILLER

Business Manager, Nematode Products
biosys
Columbia, MD

Definitions of "Quality"

"Quality Control" (QC) and related terms have many different connotations. An adequate definition for Quality Control may be found in the dictionary: e.g., "A system for verifying and maintaining a desired level of quality in a product or process by careful planning, continued inspection and corrective action where required." (Stein, 1980). Many other definitions would also suffice.

The definition of "Quality" is even more elusive, but may be defined by end user behavior. Evolution of a manufacturer's QC system involves conscious or unconscious reactions to customer behavior. If a product is of acceptable quality, and has a perceived value (relative to alternatives) equal to or greater than its price, end users will purchase and consume that product. This is especially valid for repeat purchases of a product. If the end user perception of quality is inadequate relative to price, he or she will not buy the product. This forces the manufacturer to respond in some way to create a better or different product to win sales and prosper in the marketplace. Total Quality Management is a philosophy that looks at improving any aspect of the product that may influence product success with the end user.

Entomopathogenic Nematode Quality

Entomopathogenic nematode "quality", when applied to steinernematid and heterorhabditid nematodes and their associated bacteria from a technical perspective, is usually defined as a set of linked parameters to be monitored and evaluated, such as:

a) minimum nematode viability (percent viable)
b) minimum total viable nematodes (per unit of product)
c) minimum nematode virulence (as indicated via bioassay)
d) maximum nematode age (after harvest, formulation, shelf life, etc.)
e) physiological, biochemical or morphological measurements
f) demonstrated performance on labeled targets

Viability/Total Viable Nematodes

The activities of viability assessment and quantification of total viable nematodes are at the core of all nematode research and development activities. They are also central functions of manufacturing and "QC", in that each harvested batch, each minimum unit of packaging and subsequent product application, is dependent upon accurate viability assessment and quantification. These two functions contribute greatly to product consistency. Over packing

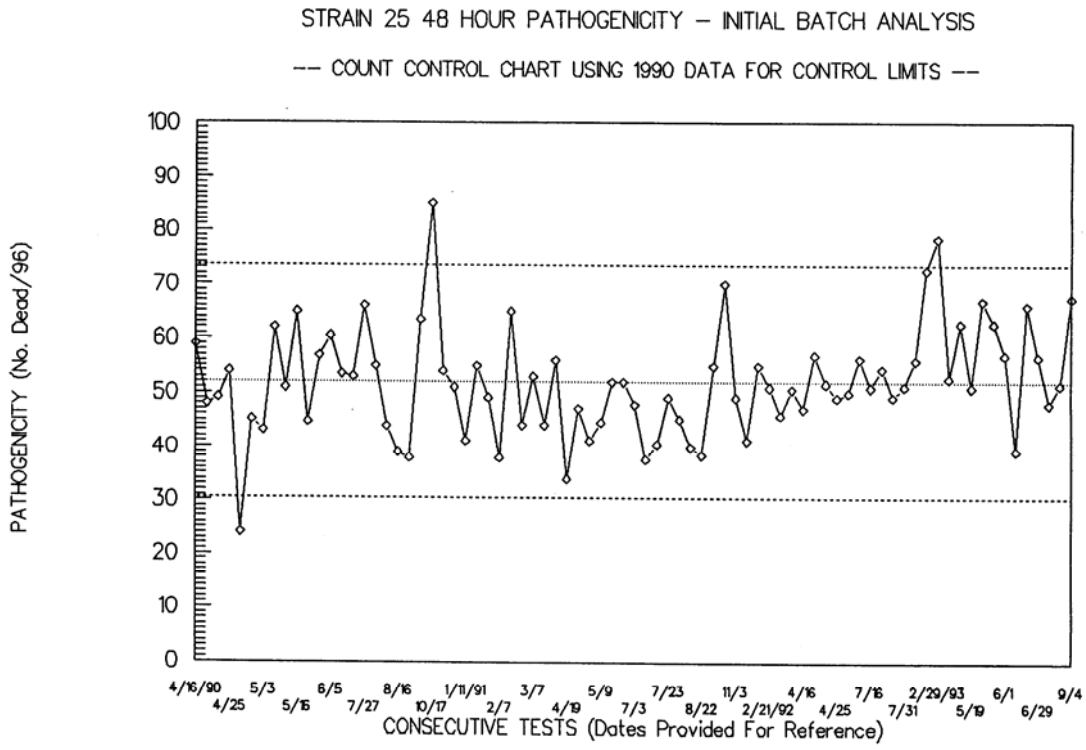
is a method of ensuring minimum total viable nematodes over a period of storage. It may be used to extend the shelf life of a product in certain situations.

Nematode Virulence

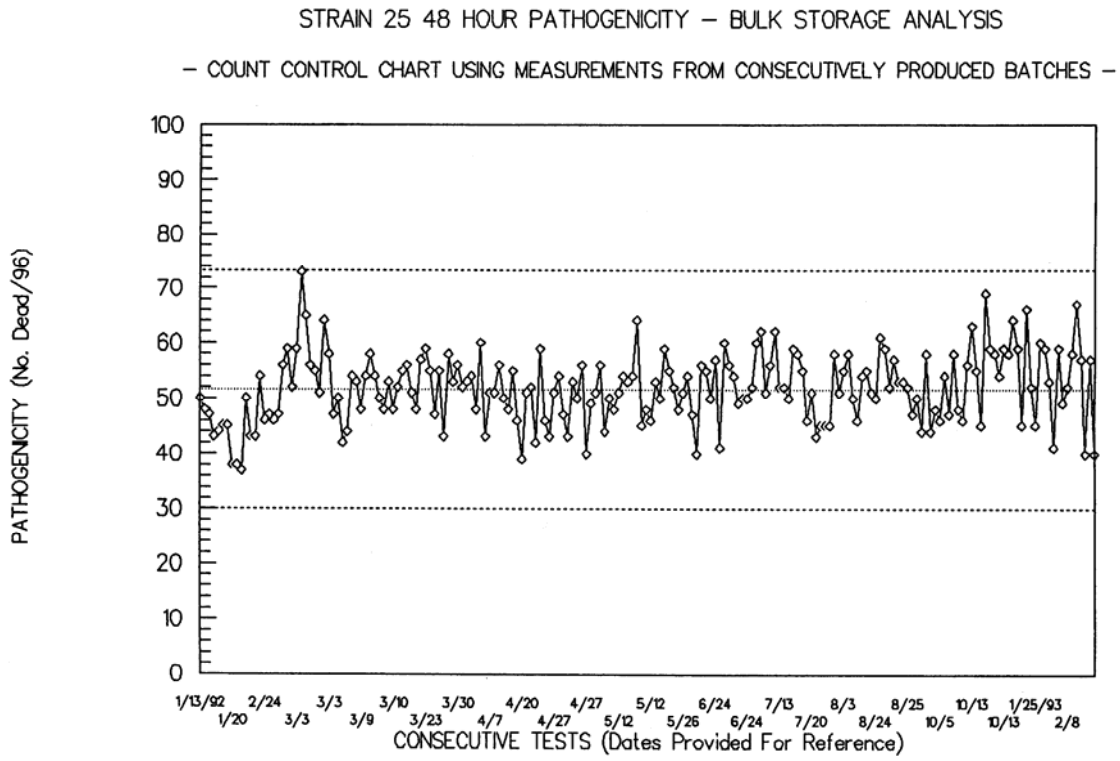
The importance of virulence assessment cannot be underestimated. Virulent nematodes enable consistent field development and performance for the end user, provided the product is used correctly. Several approaches have been reported, but bioassays utilizing indicator species are almost universally used (Molyneux et al., 1983; Georgis, 1990). Some aspect of time to kill (LT_{50}) or rate to kill (LC_{50}) is commonly used. A number of published techniques use high ratios (5:1 or greater) of infective juvenile (IJ) nematodes to insect hosts per arena (usually Petri dishes) (Georgis, 1990). Multiple IJ bioassays will provide a gross indication of virulence, but a small percentage of virulent IJs will mask the presence of a large percentage of nonvirulent IJs.

Another bioassay (Miller, 1989) has been developed utilizing a single IJ per insect (1:1) confined in a sealed cell well. By measuring virulence of individual IJs, weak or nonvirulent individuals are exposed within the population, providing a relative virulence indicator with greater resolution. This bioassay is used for product control and process control within a nematode species/strain, not for comparisons between species/strains. Timed minimum mortality standards are routinely assessed (e.g., 40% kill after 48 hrs) (see examples below for initial and bulk storage analysis). With this bioassay, batch-to-batch virulence may be monitored over time.

PROCESS CONTROL CHARTS



PROCESS CONTROL CHARTS



Nematode Age

Nematode age also contributes to end user satisfaction. Relative to nematode species/strain, culture conditions, storage conditions, formulation and labeled targets, age of the nematode plays an important role in performance. Use of batch codes, expiration dating and similar techniques is necessary to track and control the inventory life (refrigerated storage time prior to sale) and shelf life (refrigerated or room temperature storage time prior to application). **biosys** monitors and controls: time from production to formulation; from formulation to packaging; packaging to receipt by end user and likely end user storage time before use. Working closely with distribution channel partners is important to controlling this variable.

Morphological, Biochemical or Physiological Measurements

Other measurements taken on either the nematodes or their mutualistic bacteria, whether morphological, biochemical or physiological, need to be routinely performed to ensure the

consistency of the product from batch to batch. The use of such process control and product control practices, whether adapted from published research or developed independently, are largely proprietary. They are, however, integrally linked to the effort to produce consistent, quality products.

Product Validation: Field Efficacy

All of the above measurements are relatively meaningless unless field efficacy data are developed for labeled targets at labeled rates. The most efficient, and perhaps believable, means of developing such data are with third party researchers, preferably governmental (i.e., USDA), university and industry consultants). Reaching a consensus on the efficacy of a given nematode product at a given rate on a specific target by third parties will give the end user a clear message about the product. Lacking such, the "quality" of a given product will be ambiguous.

Statistical Design/Analysis

The techniques developed for these activities need to be efficient, accurate and economic. Most importantly, they have to be consistently deployed by the various personnel within the organization to be meaningful. Coping with inherent variance is an important part of statistical design and analysis of the various measurements. For instance, viability assessments by a single person can vary 10% or more; variance between two similarly trained technicians can be as high as 20%. Dealing with the variance in these data requires that procedures have adequate statistical design (sufficient replication and sample size).

Product Quality Perceptions by the End User

From an end user perspective, important perceptions of quality relative to entomopathogenic nematode products (or most any product) are:

a) consistency (reasonably consistent performance as advertised)
b) value (value = benefits÷price)
c) ease of use (relative to competitive products or alternatives - a combination of storage time requirements and convenient usage procedure)
d) availability (relative to alternatives)

Consistency

To achieve consistency, Standard Operating Procedures (SOPs) are often implemented and used for procedures in production, formulation, quality control/assurance, field development and even marketing/sales. These SOPs are usually living documents, used to guide workers actions. Changes and improvements are agreed upon by managers affecting or imposing control over the process/product. SOPs may include specifications and acceptable variances for time, temperature or nematode characteristics at critical points during the procedure being controlled. At **biosys**, we have found that senior management support of SOPs and any changes in them is critical to maintain consistency.

Value

Although value issues may not often be dealt with in terms of a technical discussion of "Quality" issues, it is a critically important parameter of marketing success (or failure), and end users constantly make purchasing decisions after judging value relative to price (where value = benefits÷price). The highest quality product, if not priced reasonably, will fail to sell--because end users judge its value ("quality") to be unacceptably low. This is important when comparing competitive products (even when dissimilar in nature) because a low-priced, medium-quality product may be judged a better value than a high-priced, high-quality product by consumers.

Ease of use

Great expenditures of effort have been made to make entomopathogenic nematodes easier to use (Georgis, 1990). A number of formulations have been tested and developed, only to be replaced by significantly better formulations. Some factors that are important when considering ease of use include:

1) Is the product easy to mix and apply in a wide range of applicators, including conventional sprayers?
2) Does use of the product result in substantial packaging waste?
3) Label Restrictions -
    a) Is irrigation needed? Do alternative products require irrigation?
    b) Is the product compatible with other common inputs such as fertilizer, pesticides, soil amendments, etc?
    c) Is the product "labeled" for the end user's intended use?
    d) Does the product have advantages over chemical alternatives?
4) Does the product have storage time after purchase but before use?
    a) Can the product be partially used, stored and finished at a later date?
5) Regulatory Paperwork and other "hassles" of application
    a) Is the product exempt from posting application notice requirements, etc?
    b) Is the product exempt from Worker Protection Safety regulations?
    c) Does the product have reentry restrictions?
6) Personal Protection Equipment.Is the applicator required to wear:
    a) Long pants/long shirt sleeves/gloves?
    b) A respirator?
    c) Goggles or protective eyewear?
7) Product Safety
    a) Is the product inherently safe?
    b) Are nontargets at risk?
    c) Is the product phytotoxic to any species of plant?

Product Availability

Availability of a product may also be considered a "non-technical" factor in any discussion on quality. However, product availability issues for dated nematode products have to be dealt with and have a significant effect on both the technical aspects of quality and the

end user's perception of "quality". If the product is difficult for the end user to locate and purchase when needed, sales will be slow or insignificant. If the product is marketed in distribution channels that make it readily available, but compromise other aspects of "quality", sales will be slow to develop. A reasonable compromise between widespread availability and distribution/shipping strategies that minimally preserve quality must be reached if significant sales are to develop over the long term. Issues of distribution stewardship policies, shipping and end user availability are some of the most challenging issues to solve in the effort to successfully market "quality" nematode products.

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Entomopathogenic Nematode Products and Quality Evaluations

K. MASON[1], R. L. RIDGWAY[2] and L. A. MERRILL[3]

[1]MicroBio Ltd., Church Street, Thriplow, Herts SG8 7RE UK
[2]Insect Biocontrol Laboratory, U.S. Department of Agriculture, Room 101, Building 402, BARC-East, Beltsville, MD 20705, USA
[3]M&R Durango Inc., 6565 Hwy. 172, Ignacio, CO 81122, USA

Abstract - An increasing number of nematode products are entering the market place and increased communications among producers and users on quality control procedures are desired to facilitate the production and use of viable and effective nematode products. Six nematode producers from the U.S. and Europe participated in a workshop with a number of users and researchers agreed that reliable virulence and other quality control testing was an essential component of nematode production. A range of different quality control tests were discussed and it was acknowledged that the tests to be used would vary considerably depending on the process and procedures used for the production of nematodes. Workshop participants agreed that all concerned should cooperate to provide users with as much practical information as is necessary to ensure the correct use of nematodes. Development of standard testing protocols for use in conducting independent quality control tests are desirable.

Introduction

As advancements in science and technology lead to the development of additional biologically based pest control products, there is a need for increased understanding and cooperation among all those involved with different groups of products. Such a need related to entomopathogenic nematode became increasingly evident as additional nematode based products enter the market place. Increased communications among producers and users relative to quality evaluation was identified as an important need as a result of a special conference and workshop on mass-reared natural enemies held in association with the annual meeting of the Entomological Society of America in the fall of 1993. In view of the long standing efforts of the International Organization of Biological Control (IOBC) on quality of mass-reared arthropods, a meeting of the global IOBC working group on quality control of mass-reared arthropods appeared to provide an excellent setting for a workshop on quality control of nematodes. Therefore, the 8th meeting of the global IOBC working group was selected as an appropriate forum within which to conduct such a workshop.

Participants

The nematode workshop was structured around commercial producers of nematodes. Representatives from four companies located in the U.S. and two companies located in Europe participated. The companies and their representatives were Biologic Co., Willow Hill, PA, USA, N. Pye; biosys, Columbia, MD, USA, R. W. Miller; Ecogen, Inc., Longhorne, PA, USA, S. Selvan; Koppert B.V., Veilingweg, The Netherlands, W. Ravensberg; MicroBio Ltd., Thriplow, US, K. Mason; and M&R Durango Inc., Ignacio, CO, USA, L. A. Merrill. R. L.

Ridgway served as moderator for the workshop. Companies represented ranged from moderately large companies operating internationally to small family owned concerns. Other participants included producers of other biological organisms, users, regulators, and researchers from throughout the world.

The nematode workshop was initiated by an overview presentation on entomopathogenic nematode total quality management by R. W. Miller, which appears elsewhere in these proceedings. This presentation was followed by specific presentations by company representatives that indicated that the nematodes were produced on live insects or solid media, or by liquid fermentation. The various presentations also detailed the ways in which nematode quality is achieved and maintains during production, storage, and distribution. Although there had not been previous collaboration on quality control procedures among producers, there was general agreement that sharing information, at lease in a general way, about quality criteria and ways of assessing them was desirable. Also, it was agreed that since entomopathogenic nematodes are now used routinely as part of integrated pest management (IPM) programs, quality standards should be established for these products and simple quality methods and guidelines that can be implemented by advisors and users were needed in addition to the more sophisticated quality control procedures used by nematode producers.

Commercially Available Species

The six companies represented agreed that the following species of nematodes are being marketed:

Heterorhabditis bacteriophora (Poinar)
Heterorhabditis megidis (Poinar, Jackson, and Klein)
Phasmarhabditis hermaphrodita (Schneider)
Steinernema carpocapsae (Weiser)
Steinernema feltiae (Filipjev)
Steinernema riobravis (Cabanillas, Poinar, and Raulston)
Steinernema scapterisci (Nguyen and Smart)

Quality Control During Production

Quality control during production varies considerably depending on which production system is used. Therefore, the details are likely to be proprietary; however, a number of general guidelines were presented. For example, in all systems it is important that an optimum number of infective nematodes is introduced and that they are free from contaminants that would negatively impact production. Also, in the case of *Photohabdus* spp. the bacteria should be in the primary phase. With in-vitro systems, checks are usually carried out periodically during production to ensure correct nematode development and sterility of the system, and also determine the optimum timing of harvest. With in-vivo systems, various effects of the nematodes on the host can be measured or observed to evaluate infectivity and nematode development throughout the entire process.

Quality Control Procedures at Harvest

All producers recognize that a test or virulence or pathogenicity is fundamental to a nematode quality control program. However, simple microscopic examination of a suspension of nematodes will provide a great deal of information and almost certainly will be a part of the quality control protocols used by a producer. The kinds of information available from a microscopic examination include (1) total nematode numbers, (2) percentage live, viable nematodes, (3) percentage infective juveniles, (4) measurements of length and width, and (5) relatively dark color indicative of good lipid reserves.

The virulence test of choice will vary considerably depending on the nematode species, the process and the procedures used for production of the nematodes, but whatever test is used should measure the different factors involved in the pathogenic process. To work effectively the nematodes must locate, penetrate, and release sufficient symbiotic bacteria to kill the target insect. It is possible to examine the factors involved in virulence separately. Various researchers have looked at the ability of nematodes to move through different media towards a target insect. One company is currently evaluating a simple test of motility that involves the time taken for 50% of a population to move out of a test arena. Tests are also available to measure the number of bacteria that are carried by the nematodes. Such tests are important since the nematodes that do not retain any bacteria are not effective.

Most producers rely on some kind of bioassay procedure using insects as a primary measure of virulence. The target insect is obviously the best insect to use, but in many cases it is not available in sufficient quantities. The most common test insects are larvae of *Galleria mellonella* (Lepidoptera) and *Tenebrio molitor* (Coleoptera). Test insects in the orders Diptera and Orthoptera are also used by some. When a non-target host is used in the bioassay, it is important to compare it periodically with a test against the target insects.

A one-on-one procedure developed by one company provides a measure of infectivity of individual nematodes and exposes the ineffective proportion of a population. Other producers use a different assay that tests the nematodes under conditions that mimic those in the field. Test insect larvae are mixed with moist peat moss or coconut fiber. The nematodes are introduced as a suspension, and so must detect, move towards, penetrate and kill the larvae to provide a positive response. Routine bioassay results are usually expressed in terms of LC50, LT50, or minimum percentage killed after a specific time. It is crucial that a minimum performance standard is set, and that product failing to meet the standard is rejected.

Quality Control Procedures During and After Storage

Some of the smaller nematode producers ship only freshly produced material. Other companies formulate and store nematodes and it is important for these companies that quality can easily be tested when produce is taken from storage. As well as visual checks on survival, motility, etc., lipid content of the nematodes is useful quality control indicator for nematodes that have been in storage. Lipid content can be determined by chemical analysis but this is expensive and time consuming. There is a close correlation between nematode dry weight and lipid content; therefore, dry weight is a simple measure that can be used to evaluate a product

following storage. Experience with different formulations and different strains allows producers to provide an expiration date for each batch of product. Producers and users should be confident that at the time of expiration the product will still perform as expected.

Customer Satisfaction

Several participants made the point that the end-user makes the ultimate decision about product quality. If he/she perceives a product to be cost-effective and makes repeat purchases then product quality is obviously satisfactory. It was also emphasized that producers can do a great deal to improve customer satisfaction. Batch coding material makes investigation of any complaint easier. Clearly stated storage and handling requirements together with a realistic expiration date ensure the nematodes do not deteriorate in the hands of the end-user. Producers also noted that some users need a great deal of technical support and encouragement when using nematodes for the first time. It may be useful if the end-user or the advisory worker can make a simple qualitative visual check on nematode numbers, survival and movement before the product is applied.

Discussion

Following the presentations by producers, a lively discussion occurred among producers, users, and researchers in response to a range of questions. Of broad interest were the markets in which nematodes are being sold and used. Producers indicate they were selling in one or more of the following markets: (1) glasshouse crops - control of fungus gnats and vine weevil, (2) mushrooms - control of fungus gnats, (3) turf - control of pests such as mole crickets, white grubs, and surface feeding caterpillars, (4) ornamentals - control of vine weevil, (5) citrus - control of citrus weevils, (6) cranberries - control of weevils/girdlers, (7) artichoke - control of plume moth, (8)outdoor flea control, and (9) other uses such as on mint and in the home and garden markets.

A number of other questions dealt with quality control testing procedures. Researchers and some producers were particularly interested in a motility test as an indicator of likely field performance. It was restated that many other factors influence efficacy - host finding, movement, penetration, bacterial release, etc., and that these in turn could be influenced by environmental factors such as soil type and irrigation. Therefore, the exclusive use of a single, simple test was discouraged. Although it was recognized that quality control tests conducted by producers must measure several traits to ensure desired quality, the need for a simple test that can be carried out by users or their advisors was also recognized. It was suggested that microscopic examination might be used by some users but the precise procedure would vary from product to product. Therefore, each producer should consider providing the user with a description of a simple test for their products.

Finally, the question was raised concerning the percentage of the production cost that was devoted to quality control by producers. Several producers concurred that whatever the cost it was insignificant compared to the losses likely to occur, if an adequate quality control program was not carried out.

Conclusions

Entomopathogenic nematodes are now used as an integral part of many IPM programs; seven species of nematodes are now being sold in a wide range of products.

Established quality control procedures are necessary for the production of viable and effective nematode products. A range of test procedures is available for the evaluation of nematode quality, many have limitations, and no one technique is sufficient to test all of the factors that influence nematode efficacy. It is incumbent upon the manufacturers of these products, as well as advisors, to provide users with as much practical information as is necessary to ensure their correct use (e.g., product use labels and technical information sheets).

Standard testing protocols developed by nematode producers, and possibly researchers, are desirable so that meaningful independent quality tests can be carried out.

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QUALITY CONTROL
of PREDATORS and PARASITOIDS

Effects of Inbreeding on the Quality of the Predatory Bug *Podisus maculiventris*

P. DeCLERCQ and D. DEGHEELE

Laboratory of Agrozoology, Department of Crop Protection, Faculty of Agricultural & Applied Biological Sciences, University of Gent, Coupure Links 653, B-9000 Gent, BELGIUM

Abstract. Inbreeding is often considered to be an important factor in the quality deterioration of laboratory-reared insects. The current paper discusses the effects of consanguinity in the laboratory rearing of the predatory bug *Podisus maculiventris* (SAY) (Heteroptera: Pentatomidae). This predator has been recognized for its potential to suppress various lepidopterous and coleopterous crop pests. Throughout 10 generations following introduction into the laboratory, several developmental and reproductive parameters were assessed to compare the quality of a reference population with that of two inbred populations. The latter were started from a small number of brother-sister founders and subsequently kept at low population size.

After up to 10 generations of laboratory rearing, developmental and reproductive traits did not vary dramatically between the reference and inbreeding treatments. Nevertheless, total fecundity, egg weight, egg batch, developmental rate of nymphs and nymphal survival were always higher in the reference population than in both inbred populations. However, weight and size, as expressed by humeral width, of adults in the reference population were intermediate between those of adults in the two inbred populations. Considering these findings, additional experiments were carried out to evaluate the correlation between weight, humeral width and fecundity of adult females in laboratory- reared *P. maculiventris*. There was a positive relationship between weight of 14-day-old females and humeral width ($r = 0.66$), but both of these parameters were only weakly associated with total fecundity, as expressed by the total number of eggs per female ($r = 0.18$ and 0.26, respectively). The results of this study suggest that high levels of consanguinity may affect development and reproduction of laboratory-reared beneficials to some extent but do not necessarily result in a dramatic reduction of their quality.

Quality Control Parameters of Mass-Reared *Phytoseiulus macropilis* (Banks) (Acari: Phytoseiidae) Using *Panonychus citri* McGregor (Acari: Tetranychidae) as Prey

M. RAMOS, J. ALEMAN and H. RODRIGUEZ

Plant Protection Division, National Center of Animal and Plant Health
Apdo 10, San Jose de las Lajas, La Havana, 32700, CUBA

Abstract. A series of biological assays on the characteristics of mass-reared *Phytoseiulus macropilis* were studied to estimate the parameters of quality control in this process. Prey (*Panonychus citri* Mc Gregor) and predator densities in rear units were determined. Futhermore, sexual rate, fecundity and predation during five days after copulation and longevity in starvation were tested to implement quality control protocols. Shewart's charts were used. The results suggest that all the parameters are useful to establish the quality control in mass-reared *P. macropilis* and they can be applied routinely to secure the quality. Nevertheless, it is possible to reduce the number of trials to prey and predator densities, considering the reproduction center conditions and the personal available.

Effect of Ovipositional Experience on Host Discrimination in an Egg Parasitoid, *Trichogramma chilonis* ISHII

K. MIURA[1], I. OHTA[1], S. MATSUDA[2] and M. KOBAYASHI[1]

[1]Laboratory of Insect Pest Control, Chugoku National Agricultural Experiment Station
Fukuyama 721, Japan
[2]Laboratory of Insect Management, Faculty of Agriculture, Shimane University
Matsue 690, Japan

Abstract. Females of *Trichogramma chilonis* that had never oviposited in any host eggs after emergence supposedly do not to distinguish between parasitized and unparasitized host eggs. However, experienced females distinctly discriminated parasitized host eggs from unparasitized eggs. Acceptability of unparasitized host eggs was 90% or greater irrespective of the host type that females had experienced. On the other hand, acceptance of parasitized host eggs decreased to 15% with females that had one prior experience with a parasitized host egg. Females that experienced oviposition on an unparasitized host egg began to accept parasitized host eggs more frequently 24 h after the experience. Inexperienced females inserted their ovipositors into hosts parasitized 3 days earlier, but they did not oviposit in hosts parasitized 5, 7 or 9 days earlier. These results are considered to be evidence that *T. chilonis* has the ability to discriminate host eggs parasitized by a conspecific female. The oviposition behavior of *T. chilonis* can be explained by the assumption that the wasp behaves to maximize her own inclusive fitness through her lifetime.

Introduction

The diamondback moth, *Plutella xylostella* (L.), is a serious pest of cruciferous crops in many countries. Development of alternative control methods, including biological control, is needed to cope with the recent development of insecticide resistance in *P. xylostella*. *Trichogramma chilonis* ISHII is an important solitary egg parasitoid of *P. xylostella* (Iga, 1985; Okada, 1989); thus, we have been investigating the possibility of a biological control program against *P. xylostella* using *T. chilonis* (Miura and Kobayashi, 1993; Miura and Kobayashi, 1995).

Superparasitism is known to occur frequently in both the laboratory and the field (e.g., Van Alphen and Nell, 1982; Hubbard et al., 1987). In mass culture of parasitoids the problem of superparasitism may lead to very expensive rearing procedures in order to prevent high parasitoid mortalities, development of small and weak adults, as well as strongly male-biased sex ratios (Waage, 1986; Van Lenteren, 1986). Understanding progeny and sex allocation may greatly improve methods for maximizing productivity of *Trichogramma* rearing units (Waage and Godfray, 1985).

Suzuki et al. (1984) reported the occurrence of superparasitism in *T. chilonis*, but it has been unknown whether *T. chilonis* has the ability to discriminate between parasitized and unparasitized host eggs. The present study deals with two aspects related to the host

discrimination: 1) Do females of *T. chilonis* discriminate parasitized host eggs from unparasitized ones? and 2) If they do, how does a previous ovipositional experience influence the host discrimination?

Materials and Methods

The population of *T. chilonis* used in the present study was part of stock cultures at the Shimane University and Chugoku National Agricultural Experiment Station, which were derived from the original colony introduced from Taiwan (Hirashima et al., 1990) and reared on Mediterranean flour moth, *Ephestia kuehniella* Zellar, eggs under 24^{o}C and 16L-8D conditions. The host, *P. xylostella*, was collected from cabbage fields near Matsue, Shimane Prefecture, Japan in 1990, and reared on cabbage leaves in a cabinet controlled at 24^{o}C. Moths were introduced into a rearing cage (34x34x26cm) containing a piece of Sealon film (ca. 10x10cm) (Fuji Photo Film Co., Ltd.) as a substrate for laying eggs, and fed a 20% honey solution.

Three experiments were carried out in the laboratory. The first was carried out using combinations of two host types and three parasitoid types as follows: Hosts - unparasitized eggs less than 24 h old (UE) of *P. xylostella* or same aged eggs parasitized 10 min before the test (PE); Parasitoids - females mated within 24 h after emergence which had never ovipositied before the test (IW), the same aged mated females which had experienced oviposition once within 10 min of the test on UE host (EWU), or the same aged mated females which had experienced oviposition once 10 min before the test on PE host (EWP). The wasps used in these experiments were reared at 24^{o}C with 16L-8D and fed on sugar and water absorbed in a small piece of filter paper. We carried out this experiment with each of the 6 combinations. One host egg on a piece of Sealon film and one female parasitoid were introduced into a Petri dish (9 cm in diameter). The area two cm around the egg were observed under a binocular microscope equipped with a video apparatus. The host egg was regarded as "rejected" if the female did not oviposit after contacting the egg three times. Twelve of each of the UE and PE eggs parasitized by IW parasitoids were dissected to count the number of parasitoid eggs laid. The rest of the parasitized eggs were reared at 24^{o}C and 16L-8D photophase for 30 days to determine the sex ratio and number of emerging parasitoids per host.

Next, we tested acceptability of PE hosts using EWU parasitoids. The EWU parasitoids were further divided into three types with respect to the interval between the first oviposition and the start of the test; i.e., less than 10 min (EWU-0), 24 h (EWU-1), or 48 h (EWU-2). The other procedures were the same as in the first experiment.

Lastly, we tested if the number of days after the host eggs had been parasitized had an effect on host discirmination using IW parasitoids. Host eggs that had been parasitized 10 min, 1-, 3-, 5-, 7- or 9-days after conspecific parasitism were presented to IW parasitoids. Other methods were the same as described above, but hosts used were *E. kuehniella* eggs less than 24 h old for only this experiment.

Results

Results of the first experiment are summarized in Table 1. There was no significant difference between the percentage acceptance of unparasitized and parasitized eggs by inexperienced females (IW). No difference was observed in their pattern of ovipositional behavior or the duration of each step in oviposition when using different host types (Table 2).

On the other hand, the percentage acceptance by experienced females (both EWU and EWP) was significantly higher for unparasitized hosts (UE). Acceptability of UE hosts was 90% or more irrespective of which host type females had experienced. However, acceptance of PE hosts decreased to a lower level by EWU (15.0%) and EWP (60.0%) females (Table 1).

The results of dissections of the UE and PE hosts parasitized by IW females showed that only one egg was laid in a host attacked once, while two eggs were laid in a host that was attacked twice. Despite the different number of parasitoid eggs laid in a single host egg between hosts attacked once and twice, only one progeny emerged from a given host egg (Table 3). Thus, superparasitism occurred when a host egg was attacked twice by different females. The female ratio was lower in parasitoids emerging from eggs that were attacked twice by different females than it was from hosts attacked once, but not significantly (Table 3).

Table 1. Acceptance of unparasitized eggs (UE) and parasitized eggs (PE) of *P. xylostella* by *T. chilonis* females. Females were divided into three types based on their oviposition experience: inexperienced (IW), experienced to oviposit once on UE hosts (EWU), and experience once on PE hosts (EWP).

Type of Parasitoids	Type of Host Eggs	No. of Females Tested	% Acceptance[1]
IW	UE	35	82.9 a
IW	PE	32	81.3 a
EWU	UE	24	91.7 a
EWU	PE	20	15.0 b
EWP	UE	20	90.0 a
EWP	PE	20	60.0 b

[1]Values followed by different letters in the same column differed significantly at the 5% level (FISHER'S exact probability test).

Table 2. Duration of each step of ovipositional sequence by inexperienced females (IW) to unparasitized (UE) and parasitized (PE) host eggs.

Type of Host Eggs	No. of Females Observed	Duration (Mean D.D., s)[1]			
		Drumming	Tapping & Drilling	Ovipositing	Total Time on Host
UE	12	14±11 a	75±40 a	53±41 a	235±112 a
PE	11	16± 7 a	50±40 a	47±47 a	156±111 a

[1]Means followed by the same letter are not significantly different at the 5% level (MANN-WHITNEY's U-test).

Table 3. Percentage of adult emergence and sex ratio of *T. chilonis* from *P. xylostella* eggs attacked once or twice.

No. of Times Host Eggs Attacked	No. of Host Eggs Tested	No. of Eggs from which either 0, 1, or 2 Progeny Emerged			Female Ratio of Emerging Progeny[1] (%)
		0	1	2	
1	13	3	10	0	100.0 a
2	22	5	17	0	76.5 a

[1]Means followed by the same letter are not significantly different at the 5% level (FISHER's exact probability test).

Most of the EWU-0 females (17 of 20 females) rejected parasitized host eggs, but half of the EWU-1 and more than half of the EWU-2 females accepted them (Table 4). The percentage acceptance was significantly different between EWU-0 females and EWU-1 or EWU-2 females ($P<0.05$, Fisher's exact probability test). This indicates that the response of experienced females to parasitized hosts changed after a period of 24 h of no exposure to host eggs.

Table 5 shows the effect of number of days after previous parasitism of hosts on host discrimination by IW parasitoids. All IW females accepted hosts parasitized within 10 min. More than 40% of IW females, however, rejected hosts parasitized 1-day earlier, and they did not oviposite in hosts parasitized 3-, 5-, 7- or 9-days earlier.

Table 4. Acceptance of parasitized eggs of *P. xylostella* (PE) by *T. chilonis* females once experienced to oviposit on an unparasitized egg (EWU). The EWU females were divided into three types with different ovipostional intervals.

Experience Condition of Females	Interval of Oviposition[1]	No. of Females Tested	% Acceptance[2]
EWU-0	<10min	20	15.0 a
EWU-1	24 h	12	50.0 b
EWU-2	48 h	12	58.3 b

[1]Between the time of the first oviposition on UE and the time of the initiation of the test.
[2]Values followed by different letters in the same column differed significantly at the 5% level (FISHER's exact probability test).

Table 5. Percentage oviposition of inexperienced famels of *T. chilonis* when they attacked *E. kuehniella* eggs at different tiemes after parasitism.

Days after First Parasitism	No. of Eggs Tested	Percentage of Oviposition
0(<10min)	12	100
1	12	58.3
3	11	0
5	12	0
7	12	0
9	12	0

Discussion

Many parasitoids have the ability to discriminate between unparasitized and parasitized hosts and show some degree of restraint in laying eggs in the latter (e.g., Rabb and Bradley, 1970; Van Lenteren, 1976, 1981; Bosque and Rabinovich, 1979; Noda, 1990). Van Lenteren (1976) and Klomp et al. (1980) stated that some parasitoid species lacked the ability to discriminate between parasitized and unparasitized hosts and the discrimination had to be learned by experience with unparasitized hosts. On the other hand, Van Alphen et al. (1987) and Noda (1990) showed, on the basis of a difference in parasitoid behavior, that inexperienced females of *Trichogramma evanescens*, *Leptopilina heterotoma* and *Gryon japonicum* have the ability to discriminate hosts, and thought that host discrimination does not

need to be learned in these species.

Suzuki et al. (1984) showed that inexperienced females of *T. chilonis* did not distinguish between parasitized and unparasitized *Papilio xuthus* host eggs. In our experiment, the difference in the oviposition behavior of inexperienced females with parasitized and unparasitized host eggs was also not obvious. However, more than 40 % of inexperienced females did not oviposit in hosts parasitized 1-day earlier and they rejected hosts parasitized 3-, 5-, 7- or 9-days earlier, although they could oviposit in 3-day old hosts (Miura, unpublished). These test results with inexperienced females show that they have ability to discriminate parasitized hosts eggs.

On the other hand, the percentage acceptance of parasitized host eggs varied according to previous oviposition experience. A female parasitoid that has had experience with an unparasitized host egg is known not to oviposit in a parasitized host egg (e.g., Van Lenteren, 1976; Noda, 1990). In the present study, even when a female had experienced a parasitized host egg, the percentage acceptance of a parasitized host egg by the female was significantly lower than that of an unparasitized host egg. Moreover, the response of females that had attacked an unparasitized host egg changed after 24 h without exposure to host eggs. Therefore, the explanation that a female acquires the ability to discriminate between parasitized and unparasitized host eggs by learning with unparasitized eggs cannot be given, because of the fact that the degree of host discrimination by a female varied with their oviposition experience. These results also are considered to be evidence that *T. chilonis* has the ability to discriminate host eggs parasitized by a conspecific female. The mechanism of discrimination needs to be further investigated.

Superparasitism is viewed as potentially beneficial under certain circumstance (Ikawa and Suzuki, 1982; Iwasa et al., 1984; Mangl, 1987; Van Alphen et al., 1987). Ikawa and Suzuki (1982) found that the degree of host discrimination varied according to the previous oviposition experiences for *Apanteles glomeratus*, and explained it by the assumption that the parasitoid female throughout her life would behave to maximize her reproductive success, based on the presumed density of the unparasitized hosts. They thought that when unparasitized hosts were few, parasitoids would be better off ovipositing in both iparasitized and unparasitized hosts in order to maximize their lifetime reproductive success, even though the individual reproductive success of an egg laid in a parasitized host would be smaller than in an unparasitized host.

The same explanation as Ikawa and Suzuki's (1982) statement can be given for the present results. In the present study, *T. chilonis* survival rate for progeny laid by a second female in a host egg within 10 min of first being parasitized was almost equal to that of progeny laid by the first female. *T. chilonis* females emerge with matured ovaries (Hirashima et al., 1990). The total number of eggs parasitized during their lifetime was highest when host eggs were offered to a female immediately after emergence and decreased toward the end of her lifetime, but the mean of their longevity was similar in each case (Hirashima et al., 1990). If parasitoid wasps are not able to encounter hosts for long intervals, reproductive success of the parasitoid could be smaller.

A female that did not encounter any host eggs after emergence would estimate the density of unparasitized host eggs to be low. Therefore, she would oviposit in a parasitized host egg as readily as she would in an unparasitized host egg. A female that had encountered an unparasitized host egg would estimate the density of unparasitized host eggs to be high, so she would not readily oviposit in a parasitized host egg. A female that encountered parasitized host eggs successively at short intervals would estimate the host density to be high, so she would not oviposit in a parasitized host egg more readily than in an unparasitized host egg. On the other hand, even if a female experienced an unparasitized host egg, she may oviposit in a parasitized host egg after a long-time of isolation from hosts. She would estimate the density of host eggs to be low. Thus, the ovipsition behavior of *T. chilonis* in the present study can be explained by the assumption that the wasp behaves to maximize her own inclusive fitness through her lifetime.

Superparasitism should affect the survival and sex ratio of parasitoids. We may predict from LMC and LRC theory that the sex ratio allocated to hosts changes when the ratio of parasitoid to host changes. Male-biased sex ratios in *T. chilonis* cultures may occur when the ratio of parasitoid to host increases. The level of superparasitism, however, should be greater as the rate of finding unparasitized hosts decreases. Low productivity of *T. chilonis* in mass culture may result from mortality in superparasitized hosts. Therefore, we should pay attention to the rate of encounter of unparasitized hosts by inexperienced females. This knowledge could serve as a guide for modifying rearing systems for *T. chilonis*.

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Changes in the Rearing Process to Improve the Quality of Mass Production of the Fruit Fly Parasitoid *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae)

J. CANCINO, P. VILLALOBOS and S. De La TORRE

Programa Moscamed SAGAR. 2da. Ave. Sur No. 5. Tapachula, Chis. México

Introduction

Diachasmimorpha longicaudata is a solitary endoparasitoid of fruit flies (Diptera: Tephritidae), which was introduced in to Mexico at the end of the 1950s (Jimenez, 1956; Clausen, 1956). Its ability to adapt to a wide range of enviromental conditions and its capacity to develop in different species of *Anastrepha* spp. were the main attributes used to select this parasitoid species for use in a program of biological control of tephritidits in Mexico. In addition, *D. longicaudata* has been reared under laboratory conditions with excellent results (Chong, 1962; Marucci and Clancy, 1950; Greany et al, 1976). The mass production of this parasitoid has made it possible to use it in augmentative releases, a tecnique that has been considered very efficient to the suppression of fruit fly populations in the field (Wong et al. 1991; Knipling, 1992; Sivinski et al. 1996; Montoya et al. 2000).

In 1994, the National Campaign Against the Fruit Flies (SAGAR-IICA) in Mexico established a colony of *D. longicaudata* with a capacity to produce 50 million pupae weekly. The production has been used to do mass releases of the parasitoid in fruit zones infested with fruit flies according to a national plan (SARH, 1995). The methods of mass rearing were modified and adapted from techniques described by Wong and Ramadan (1992) using larvae of *Anastrepha ludens* (Loew) as the host (Cancino, 2000). This processes of improving the mass rearing system for *D. longicaudata* represents an experience that we feel could be important in the establishment of other parasitoid rearing systems in the future.

In this document the most important problems encountered during the process of establishing a *D. longicaudata* colony in the Moscafrut Plant in Metapa de Domínguez, Chis, are reported. The analysis were designed by practical evaluations that gave direct solutions. These evaluations were designed in accordance with results from quality control measurements in the process of mass production. In agreement with this, the transcendent problems were located in specific steps or techniques of the production process.

The information presented should be of interest in the establishment of colonies of *D. longicaudata* in other laboratories.

Materials and Methods

The evaluations were done in the Moscafrut Plant (SAGAR-IICA) in 1994 from August to October, in which mass rearing of *D. longicaudata* was established with a production capacity of 50 million pupae per week. The basic problems encountered while increasing the

production of *D. longicaudata* were: a) a high percentage of mortality in host larvae and parasitoids during development and b) a decrease in the percentage of adult parasitoid emergence. In order to find the operative changes in the rearing procedures needed to correct these problems the following evaluations were done:

1. Effect of post-exposition period in host larvae. Eight day old host larvae of *Anastrepha ludens* were exposed to parasitization using "cassette type" oviposition units. This consists of two plastic squares (14 x 20 cm) with a hole in the middle united with hinges made from a piece of organza cloth piece. The organza cloth also covered the hole in the plastic squares on both sides. In the middle about 2000 larvae with 30 gr of diet were put. The larvae were then exposed to parasitoids in "Metapa type" modular cages. Each module is formed by 12 cages of aluminium structure integrated in two piles of 6 cages. In each cage (32 x 42 x 32 cm) 1500 parasitoids (2♀:1♂) fed with honey were maintained. The larvae were exposed for a period of 2 hrs.

The process established at the begining included that the exposed larvae were submited to a post-exposition period in which the development of larvae was completed in the diet. The mortality of exposed larvae on some ocassions was near 90%. This parameter was used as a main indicator to do the following evaluations that were included during the activities of production:

a). Relationship of size and weight of larvae to emergence. Three samples of 10 ml of larvae per lot from 45 lots were taken before exposure to parasitoids and weighed with an analytical balance. The correlation between the averages of weight and volume (larvae/10 ml) with the dates of corresponding emergence were then determined.

b). Effect of postexposition period on the mortality of host larvae. A given lot of parasitized host larvae were maintained in diet for two different postexposition times - 24 and 48 hrs. The host larvae were put in trays (77 x 40 x 4 cm) and then put in pils (30 trays/pil), at density of one litre of larvae (about 32,000 larvae) per 1.1 kg of diet. Afterwards, the larvae were separated from the diet by washing and put in vermiculite. In each tray 1 L of larvae per 1 L of vermiculite was put. After 72 h, three samples were taken at random from the pil and in the trays from the larvae maintained in vermiculite. Each sample consisted of 100 hosts, in which the number of larvae, pupae and died larvae were counted. With this date percent mortality per lot for the two periods of exposition was obtained. Before adult emergence, in the same manner, samples of 100 pupae were taken to obtain the number of adult parasitoids emergenced per lot. The average number of dead larvae and the percentage of emergence for each period of postexposition were analyzed statistically by an ANOVA test ($\alpha = 0.05$).

2. Effect of the relative humidity on the viability of pupae. Two lots of 1 million larvae each were exposed to either 50-70% relative humidity or 70-80% RH. The evaluations were made in normal larval development rooms at a constant temperature of 26°C. The insects were maintained in these rooms for a period of 14 days. At the end of the development period, three samples by pil and tray were taken. In each sample of pupae, the percentage of pupae that were damaged or dead and the percentage of emergence were counted. In the first case the measurements were made by dissections and in the second the pupae were maintained until the

adults emerged in cilindric plastic cointainers (8 x 4 cm). Thirty lots of production were evaluated. The average percent mortality and emergence were analyzed by an ANOVA test ($\alpha = 0.05$).

3. Effect of time of host larvae exposition to parasitoids. This test was made using two different exposure times to parasitoids - 2 and 3 hrs. The evaluation was made with two samples of 5 L of larvae (150,000 larvae) from the same lot of production. The larvae were in a tray (53 x 60 x 11 cm), from which portions of about 2,000 larvae with diet were taken. These portions were put in "cassette type" units of parasitation and exposed in cages containing mass reared parasitoids (Metapa cage with 1,500 parasitoids; 2♀:1♂). After the exposition, the larvae with diet were maintained in trays, and then separated from the diet by washing. After larvae had been collected they were put in vermiculite in trays and maintained at 26°C for 14 days. After pupation but before the adults emerged three samples of 100 pupae per treatment were taken. The samples were put in cilindrical plastic containers (8 x 4 cm) until adult emergence. The number of adults emerged and the sex-ratio were recorded. For each treatmeant 30 replicates were done, the average emergence and sex ratio were analyzed by ANOVA ($\alpha = 0.05$).

Results

1. Effect of postexposition period in host larvae. In Fig. 1 the positive relationship between the weight of host larvae and parasitoid emergence is shown. An r^2 value of 63.2 between these parameters was obtained. From this relationship it can be determined that using an average host weight of 24±2 mg it is possible to obtain 45 to 60% adult emergence. The results for the relationship between volume of larvae (No. larvae/10 ml) and emergence only resulted in an $r^2 = 20.81$.

The results from the evaluation of the postexposition period can be observed in Table 1. With a postexposition period of 48 hr larval mortality increased and adult emergence decreased. There was a significant difference in both parameters obtained in the two periods of post-exposition evaluated.

2. Effect of relative humidity on the viability of pupae. The mortality in the inmature stages of parasitoid development was reduced when a lower relative humidity was used (Table 2). At 50-70% RH the mortality of pupae decreased to only 25.29±0.72%. In adittion, the percentage of adult emergence increased to an average of 61.91±1.83%. These values were statisticly different when compared with the results at 70-80% RH.

3. Effect of time of host larvae exposition to parasitoids. A shorter exposure time (2 hrs vs. 3 hrs) increased the number of parasitoids produced. With an exposure time of 2 hr the percent emergence increased to 67.17±1.22% vs. only 33.76±1.33% obtained with an exposure time of 3 hrs. However, the sex-ratio was similar in both cases (Table 3).

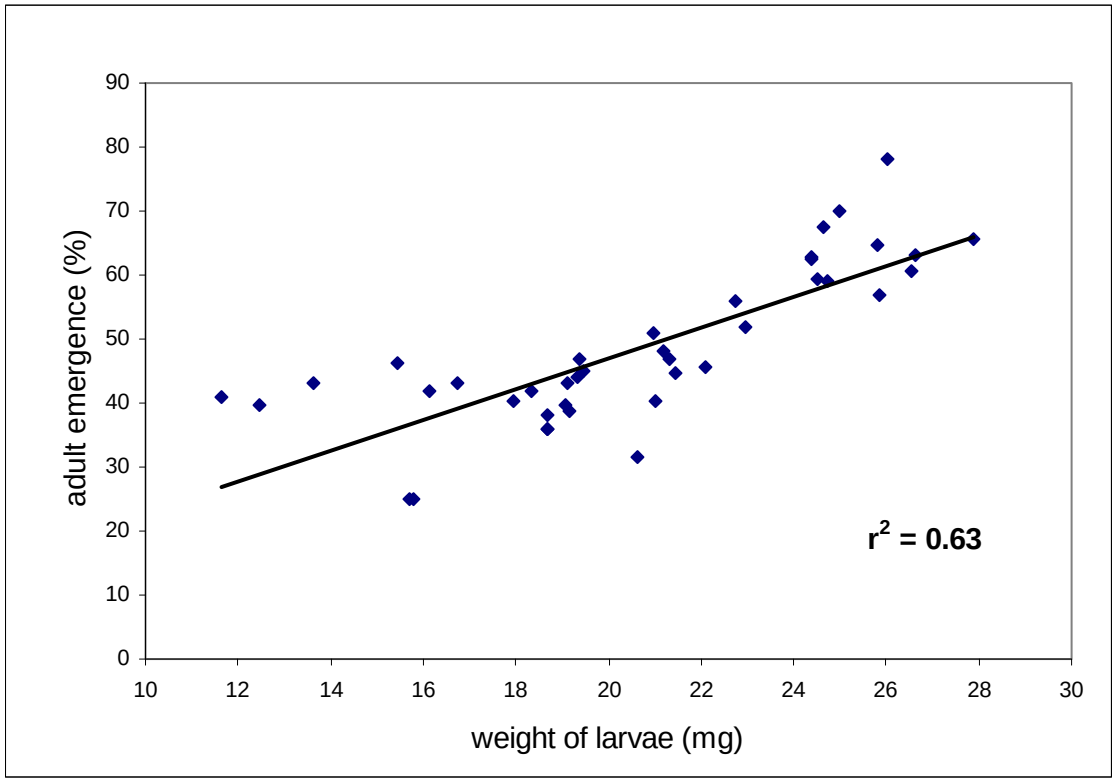

Figure 1. Relationship between adult emergence of *D. longicaudata* and the weight of host larvae obtained during the process of mass rearing establishment.

Table 1. Average results (± ES) of larval mortality and emergence using post-exposure periods of 24 and 48 hrs in the rearing of *Diachasmimorpha longicaudata*.

Post-exposure Period (hours)	Larval Mortality	Emergence (%)
24	10.01±0.35 a	63.85±0.86 a
48	26.61±1.90 b	43.93±1.33 b

Values in columns followed by different letters imply significant differences, ANOVA (α= 0.05).

Table 2. Average results (± ES) of percent damage and emergence obtained at 50-70% and 70-80% relative humidity in the rearing of *Diachasmimorpha longicaudata.*

Relative Humidity (%)	Pupal Mortality	Emergence (%)
50-70	25.29±0.72 a	61.91±1.83 a
70-80	40.24±1.74 b	39.87±1.48 b

Values in columns followed by different letters imply significant differences, ANOVA (α= 0.05).

Table 3. Average results (± ES) of percent emergence of parasitoids when host larvae were exposed for periods of 2 and 3 hours in the rearing of *Diachasmimorpha longicaudata*.

Exposure Time (hours)	Percent Emergence	Sex Ratio (F:M)
2	67.17±1.22 a	2.35:1
3	33.76±1.33 b	2.40:1

Values in columns followed by different letters imply significant differences, ANOVA (α= 0.05).

Discussion

In the quality control of *D. longicaudata* mass rearing, simple procedures can provide important information. For example, the measurement of host weight was found to be an important parameter and could help maintain the quality of the production process. A minimum average weight of 24.0±2 mg in *A. ludens* larvae could be used as an important indicator of this parameter.

However, the problem of high mortality of parasitized host larvae had a more immediate solution, i.e., the reduction of the postexposition period. This may have reduced metabolic heat, which often accompanies the use of high densities. This problem is very frequent in different mass rearing systems for fruit flies (Tanaka et al., 1972). The effect of an increase in larval mortality can be drastic. In experiences with *D. longicaudata* mass rearing it has been shown that the presence of a greater than 20% host mortality can provoke an exponential increase in parasitoid mortality, which is charasteristic of parasitized hosts being contaminated with microorganisms.

The high mortality could be the result of a fast virulance of pathogenic microorganism that affects the host development and the parasitoid. Conditions which result in levels of host mortality under 10% give better conditions to parasitoids to develop.

Although the problems of host larval mortality were solved, the mortality in inmature stages of parasitoid development was maintained. The application of 50-70% RH resulted in a decrease in mortality in the inmatures stages of the parasitoids and an increase in emergence rates. The use of high densities of puparíums with parasitoids in development at high humidity provoked problems. The residues of diet and organic material in the vermiculite could promote opportunistic organisms that increase with the favorable conditions. When the relative humidity was high (70-80%) in the parasitoid development room it was very common to find the proliferation of fungus, mites and larvae of *Drosophila* and *Foridae* flies. However, Ashley et al. (1976) found that humidity was an important factor in the development of inmature stages of *D. longicaudata* into the puparium and they reported that 80% humidity is very recommendable. Here, we found that a reduction in relative humidity improved parasitoid production and reduced problems with contaminants. It is suggested that to maintain the humidity in the trays, one should apply 200 ml of water per tray (with 1 l parasitaized larvae/ 1 l of vermiculite) at seven days after the parasitoids begin to develop.

Finally, we also showed that a reduction in the time of larval exposition increased the emergence adult parasitoids. This probably indicates that superparasitism in the *D. longicaudata* colony was a fundamental problem. Superparasitism has been reported previously in this parasitoid (Lawrence, 1998). During the process of establishing the colony it was observed that with increasing time of exposure the rate of parasitism increased as well. Unfortunately, this manifested itself in a reduction in the number of adults emerging. The high level of parasitism could have its origin in the loss of attributes to find adequate hosts to oviposit. This could be a result of maintaining the parasitoid colony using artificial units of oviposition for an extended period of time. When the evaluations were done, the *D. longicaudata* colony had been maintained for a period of about 100 generations under mass

rearing conditions. During the establishment phase a more dynamic process was used, which included basically the use of major quantities of larvae and parasitoids with more artificial techniques.

The emergence of parasitoids obtained with 2 hrs of larval exposure was an important indicator of technique adjustment in the mass rearing process. However, the origin of this behavior in the parasitoid colony suggests that an evaluation of exposure time should be done periodically.

The evaluations which were done gave very important information which allowed us to find solutions to urgent troubles in the process of establishing a parasitoid colony for mass rearing. With the application of these changes it was possible reach our production goal of 50 million pupae per week. The changes applied could be considered as corrections in the methods of production as a result of process quality analysis.

The methodologies used in production and process control are very important aspects in the quality control of insects. In the process of establishment an evaluation of indicators of quality should be indispensable. Changes in in the rearing process as sugggested by such an evaluationon on ocassion can give drastic results. In this work during the establishment of the rearing methodology, three problems were presented that independently affected production and had their solution in changes to the techniques found out in the evaluations based on dates of quality. Each one of these problems had the following estimation of loss: the larval mortality affected 70% of the production and the reduction of emergence affected 20%.

Both problems were detected by the daily measurements of quality control parameters. Unfortunately, with *D. longicaudata*, mass rearing quality control standards have not been established. The information given by Messing et al. (1993), Purcell et al. (1994) and Cancino and Yoc (1993) is only partial, because they do not integrate all of the information. It is very important to work to establish standards and design simple and effective evaluation techniques - two basic aspects of quality control (Chambers, 1977; Leppla, 1993). The increasing importance of *D. longicuadata* in Mexico and other countries as a method of biological control for a number of pest fruit flies obligates us to consider the inclusion of quality control standards into our mass rearing systems. This could refesult in a constant benefit and help avoid problems in mass rearing.

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Quality Control Criteria for Mass-Rearing *Lixophaga diatraeae*

J. ALEMAN[1], L. PLANA[1], M. VIDAL[2], G. LLANES[2] and M. DELGADO[3]

[1]Centro Nacional de Sanidad Agropecuaria (CENSA), Carretera Jamaica y Autopista Nacional, Apdo. 10 San Jose de las Lajas, La Habana, Cuba. Tel. 63014. Fax 53(64)63897, email: ccoonsa@conini.inf.cu

[2]Instituto Cubano de Investigaciones Azucareras (ICINAZ), Quivican, La Habana. Cuba.

[3]Facultad de Agronomia, Instituto Superior de Ciencias Agropecurias. San Jose de las Lajas, La Habana, Cuba.

Abstract. Sugar cane borer, *Diatraea saccharalis*, is the most harmful pest to the sugar cane crop in Cuba. *Lixophaga diatraeae* has been the main biological control agent in the Caribbean islands. This tachinid fly is mass reared in fifty laboratories. The aim of this work was the implementation of quality control parameters associated to biological stages of *L. diatraeae* and its substitution host *Galleria mellonella* to detect quality problems. The parameters were percentages of parasitized *G. mellonella* larvae, larval mortality, larval yield and percentages of pupae for the factitious host. Flight ability and mating propensity for the parasitoid also were evaluated. Larval and pupal cycles, length of pupal period, pupal and adult malformation, pupal emergence, sex ratio, fecundity and adult longevity were studied for both species. A method to evaluate parasitization capacity of *L. diatraeae* on *D. saccharalis* was assayed under insectary conditions for the first time in Cuba. Tolerance ranges and mean limits were calculated for each parameter and were displayed in Shewhart charts. The main results and their effects on mass rearing quality were analyzed.

Quality Control Parameters of Wild and Mass Reared *Diachasmimorpha longicaudata* (Ashmead), A Fruit Fly Parasitoid

J. CANCINO[1], J. L. CANCINO[1], M. MARTÍNEZ[1] and P. LIEDO[2]

[1]Programa Moscamed, SAGAR, 2da. Ave. Sur No. 5, Tapachula, Chis., Mexico
[2]El Colegio de la Frontera Sur, Carretera Antiguo Aeropuerto Km. 1.5, Tapachula, Chiapas, Mexico

Introduction

The parasitoid *Diachasmimorpha longicaudata* (Hymenopthera: Braconidae) has been mass reared at the Metapa facility for about 10 years. There is capacity at this facility to produce up to 50 million pupae per week (ca. 50% adult parasitoid emergence), and these parasitoids have been used for augmentative biological control of *Anastrepha* species in Mexico.

Because of this long period under artificial rearing conditions, it was decided to analyze the status of the colony regarding its quality. According to Chambers and Boller (1977), the starting point to develop quality control methods and establish required parameters for mass reared insects should be the attributes of the wild ones. Studies that compare mass reared and wild insects to develop quality control methods and parameters have been made for tephritid fruit flies (Rössler, 1975; Greany and Szentesi, 1979; Foote and Carey, 1987; Vargas and Carey, 1989; Haymer, 1995), *Musca domestica* L. parasitoids (Geden et al., 1992), and *Trichogramma* wasps (Wajnberg and Pizzol, 1989; Bigler, 1996).

In the case of *D. longicaudata*, there have been a number of studies regarding the quality of mass reared insects under field and laboratory conditions (Messing et al., 1993; Purcell et al., 1994; Cancino and Yoc, 1993; Bautista and Harris, 1997), but there have not been comparisons of mass reared and wild insects. Our goal in this study was to compare the Metapa mass reared colony with insects collected from naturally parasitized fruit flies in order to contribute to the development of quality control standards.

Materials and Methods

The mass reared insects used for this study were obtained from the regular production of the "Moscafrut" facility in Metapa, Chiapas, Mexico (SAGAR – IICA). The wild insects were obtained from mango and orange fruits collected in the Valley of Mazapa de Madero, an isolated fruit growing area where no mass reared parasitoids have been released during the last eight years. These fruits were naturally infested by fruit flies and the larvae of these flies were naturally parasitized by *D. longicaudata.* After collection, the fruits were placed in plastic trays in the laboratory to allow any insects infesting the fruit to complete their development. The pupae obtained in this fashion were sampled to do the evaluations with pupae and adult parasitoids. Four biological attributes were evaluated comparing the wild and colony strains: size, flight ability, longevity, and fecundity.

Size was compared as pupal weight and adult morphological measurements. Each individual pupa was weighed using an analytic balance. Sample size was 1000 individuals for the wild strain and 500 individuals from the mass reared strain collected at random from several production batches. After weighing, each individual pupa was placed in a 2x2 cm cylindrical cell in order to correlate pupal weight with adult parasitoid emergence and sex. A random sample of 30 males and 30 females was used to make morphological measurements. The measurements taken were whole body length from head to tip of the ovipositor; large wing length, ovipositor length, and length of the back tibia. These data were analyzed by simple ANOVA (α= 0.05).

Flight ability was expressed as the percentage of parasitoids that could fly (percent fliers). Each sample was composed of 100 pupae that were placed inside of a 10 cm diameter x 8 cm height PVC black cylinder. The inside walls of the cylinders were coated with neutral powder so the parasitoids could not leave the cylinders by walking. These cylinders were placed in a screened cage (65cm x 65 cm x 45 cm) with a source of light (75 W) at 50 cm over the top of the cage. Samples were exposed to a 12:12 L:D period. The number of non-emerged pupae, dead parasitoids inside the cylinder (no fliers), fruit flies and empty pupal cases was recorded. The percent fliers was estimated as follows:

No. adult parasitoids outside the cylinder / (No. empty pupal cases – No. adult fruit flies) x 100

A total of 20 samples from the wild strain and 15 samples from the mass reared strain were evaluated. Percent fliers was transformed using arcsine and analyzed by ANOVA (α= 0.05).

Longevity was determined under laboratory conditions and samples were exposed to four different conditions:

- With honey and without water
- With water and without honey
- With water and with honey
- Without honey and without water

Cohorts of 30 females and 15 males were introduced into “Hawaii” type cages (27 x 27 x 27 cm). The number of dead parasitoids and their sex was recorded daily until all of the parasitoids in a cage had died. Four replicates per treatment were carried out for each group of parasitoids. Survival curves were elaborated for each treatment and sex, using the sum of individuals from the four cages.

Fecundity was determined in the laboratory using three types of parasitism units: fruit fly larvae in artificial diet, fruit fly larvae in laboratory infested fruits, and naturally field infested fruits.

In the case of artificial diet, two cohorts of 30 females and 15 males per group were placed in “Hawaii” type cages with honey and water. The parasitism units were Petri dish bottoms (9 cm in diameter x 0.7 cm height) in which 400 *Anastrepha ludens* (Loew) larvae with larval diet were placed and covered with mesh. These units were exposed daily for two

hours starting from the 5th day of age. After exposure, the larvae were sorted out from the diet by washing them and placed in plastic cylinder containers (5 cm diam. x 9 cm height) with vermiculite for pupation. Pupae were maintained in these containers at 26°C and 60-80% RH for 15 days. After this period, the number of emerged adult parasitoids was recorded and the number of offspring, by sex, per female per day was calculated. Fecundity curves were elaborated for each group of parasitoids.

In the case of laboratory infested fruits, two cohorts of 30 females and 15 males of both strains were evaluated. From the fifth day of age, *A. ludens* infested guava fruits were exposed during four hours per day for a period of 10 days. After exposure, the fruits were maintained for two days at 26°C and 60-80% RH. The fruits were then opened and the larvae extracted and placed in plastic containers with vermiculite for pupation. The number of male and female parasitoids that emerged was used to estimate the number of offspring per female per day. Fecundity curves were used to compare the fecundity of both groups of parasitoids.

Large field cages (3 m diameter x 2 m height) were used when naturally field infested fruit were tested. In each cage a cohort of 100 females and 50 males, of a given group, was released. The parasitoids were 5 days old and were maintained in the cage for 5 days while provided with honey and water. Three small mango trees were placed in the middle of each cage and 10 infested guavas were put on a circular gridiron of 50 cm diameter. These guava fruits were exposed for six hours and then removed and maintained during two days in the laboratory for larval maturation. After two days the fruits were opened and the larvae were placed in plastic containers with vermiculite for pupation. The pupae were maintained in the laboratory for 14 days at 26°C and 60-80% RH, and then the number of emerged male and female parasitoids, as well as adult fruit flies, was recorded. Percent parasitism was estimated as follows:

$$\% \text{ parasitism} = (\text{No. parasitoids})\,(100) / (\text{No. adult fruit flies} + \text{adult parasitoids})$$

Four replicates per strain were performed and the data (percent parasitism) were transformed to arcsine and analyzed by ANOVA ($\alpha = 0.05$). Environmental conditions during field exposure were 17-33°C and 60-80% RH.

Results

There were significant differences in pupal weight and size measurements, with exception of the size of the tibia in females. Wild parasitoids were greater in size than the mass reared ones. Table 1 shows the average pupal weight and adult morphological measurements for laboratory and wild parasitoids.

The differences in flight ability (percent fliers) were also significant (Table 2). Wild parasitoids showed greater percent fliers than mass reared parasitoids.

Table 3 shows the mean longevity of males and females of both strains under the four different conditions. Survival of the parasitoids exposed to these conditions is shown in Fig. 1. Food had a significant effect on parasitoid survival for both groups of parasitoids. In those

conditions where parasitoids had access to honey, survival was much greater than those without honey. Water contributed to a slightly greater survival. Wild females showed greater survival than mass reared females when they were provided with honey, but not when they were food deprived. In general, wild parasitoids were more sensitive to the absence of food and water.

Table 1. Means (± S.E.) of adult size (mm) and pupal weight (mg) in laboratory and wild parasitoids of *D. longicaudata*.

	Females		Males	
	Laboratory	Wild	Laboratory	Wild
Body length	9.84 ± 0.15 a	10.58 ± 0.41 b	4.28 ± 0.10 a	4.72 ± 0.10 b
Wing length	4.17 ± 0.05 a	4.44 ± 0.04 b	3.75 ± 0.06 a	4.22 ± 0.03 b
Tibia length	1.59 ± 0.02 a	1.65 ± 0.03 a	1.31 ± 0.03 a	1.50 ± 0.02 b
Ovipositor length	4.87 ± 0.10 a	5.49 ± 0.07 b	--	--
Pupal weight	10.55 ± 0.13 a	14.21 ± 0.21 b	9.61 ± 0.28 a	14.15 ± 0.53 b

Values followed by different letters between columns are significantly different, ANOVA ($\alpha = 0.05$).

Table 2. Means (± S.E.) of percent fliers in laboratory and wild strains of the parasitoid *D. longicaudata*.

Parasitoid Strain	Percent Fliers
Laboratory	83.48 ± 1.90 b
Wild	96.28 ± 0.74 a

Values followed by different letters between columns are significantly different, ANOVA ($\alpha = 0.05$).

Table 3. Mean (± S.E.) fecundity of laboratory and wild parasitoids obtained using different oviposition methods

		OFFSPRING[3]		
		Females	Males	Sex ratio
Cage with artificial oviposition unit[1]	laboratory	6.10 ± 0.62 a	2.32 ± 0.22 a	2.65 : 1
	wild	7.46 ± 0.61 a	5.93 ± 0.58 b	3.89 : 1
Cage with laboratory infested fruit[1]	laboratory	0.33 ± 0.18 a	0.23 ± 0.14 a	1.43 : 1
	wild	0.66 ± 0.48 a	0.16 ± 0.09 a	2.92 : 1
Field cage with naturally infested fruit[2]	laboratory	41.1 ± 7.91 a	19.5 ± 3.65 a	2.05 : 1
	wild	63.6 ± 15.01 b	35.8 ± 9.28 b	1.97 : 1

[1]Mean number of offspring per female during 10 days.
[2]Mean number of total offspring during 5 days. Initially there were 100 females and 50 males per cage.
[3]Values for the number of offspring followed by the same letter in a column are not significantly different, ANOVA ($\alpha = 0.05$).

The mean fecundity obtained under the three different conditions tested, as well as the sex ratio, is shown in Table 3. With exception of the male offspring from the artificial diet unit of oviposition, under laboratory conditions, there were no significant differences in fecundity; however, under field cage conditions, wild females showed significantly greater fecundity than mass reared females. There were not significant differences in sex ratio (females: male), but numerically, there was a greater proportion of females for the wild parasitoids under laboratory conditions, but smaller under field cage conditions.

The pattern of daily fecundity for the first ten days of adult life, obtained with artificial diet parasitism units, is shown in Fig. 2. The mass reared strain showed equal or greater fecundity throughout the ten-day period. The difference was greater in the case of male offspring.

Percent parasitism under field cage conditions was greater for the wild parasitoids during the first 4 days of exposure, and was identical with the mass reared at the fifth day of exposure, which was also the lowest rate for both groups of parasitoids (Fig. 3). During the first four days, parasitism by the wild parasitoids was always greater than 50%. In the case of the mass reared strain, parasitism was equal of greater than 40% during the first four days and it was greater than 50% only in the second day.

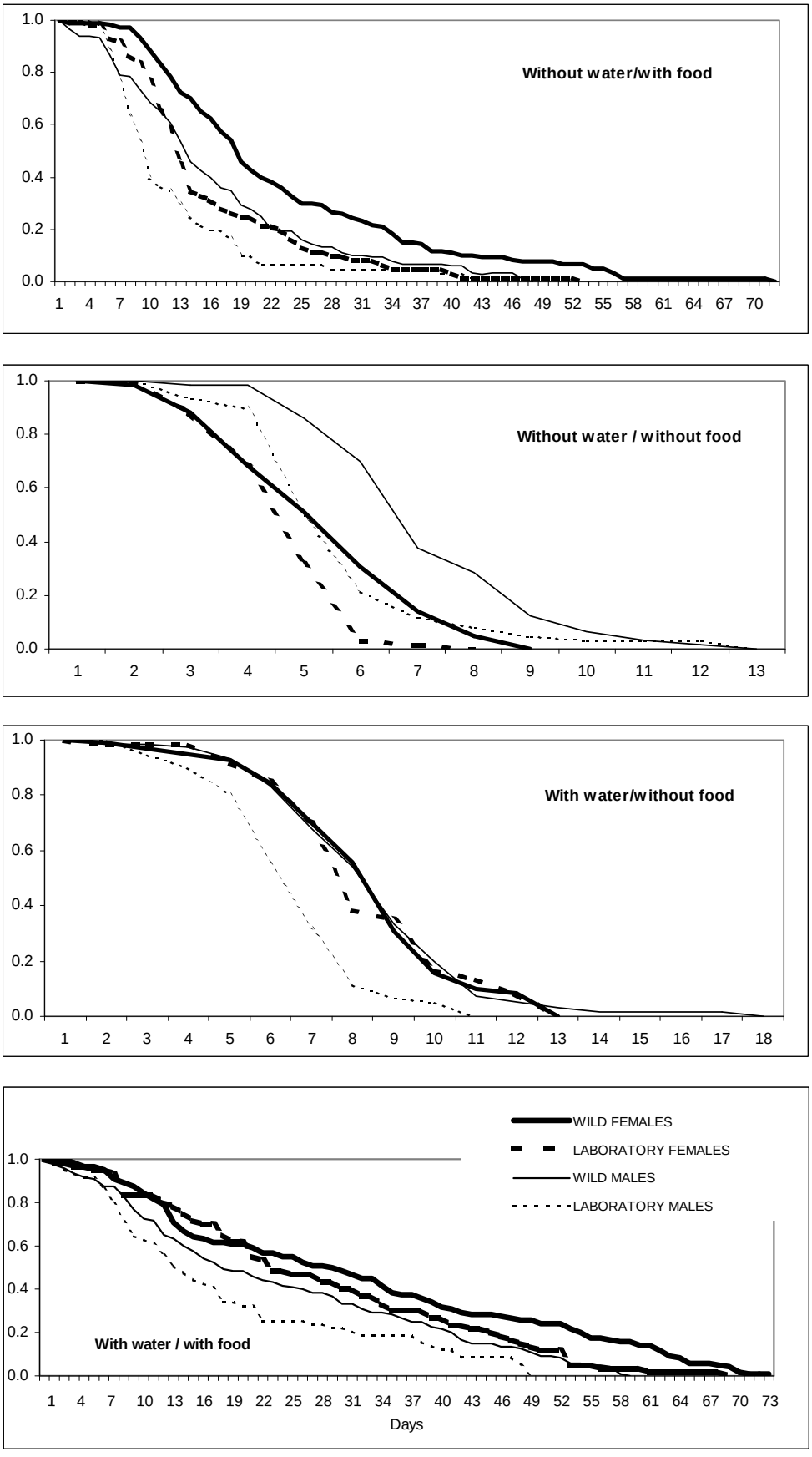
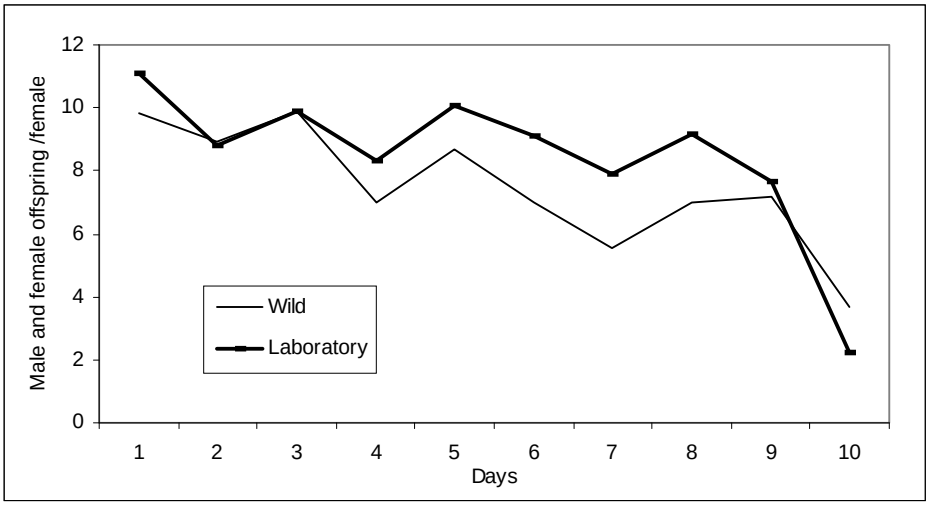
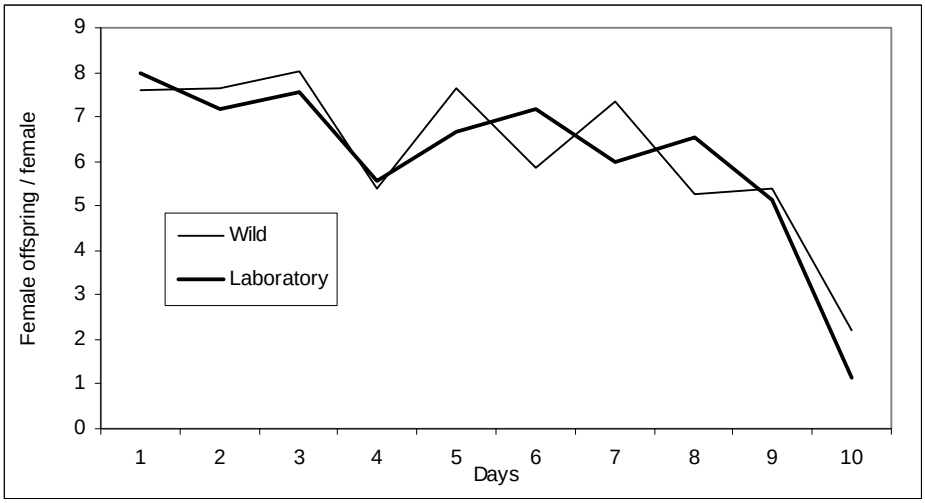


Fig. 1. Survival of laboratory and wild parasitoids with different conditions of water and food availability.





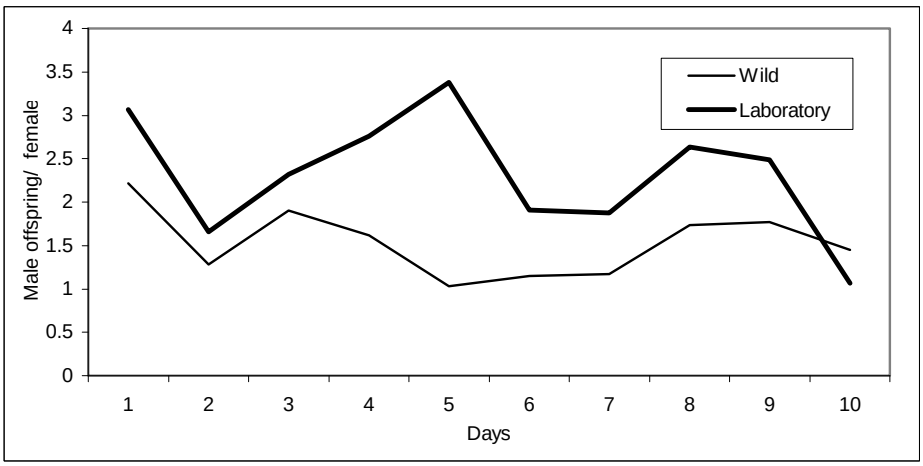


Fig. 2. Fecundity of laboratory and wild parasitoid strains using artificial parasitization units for oviposition

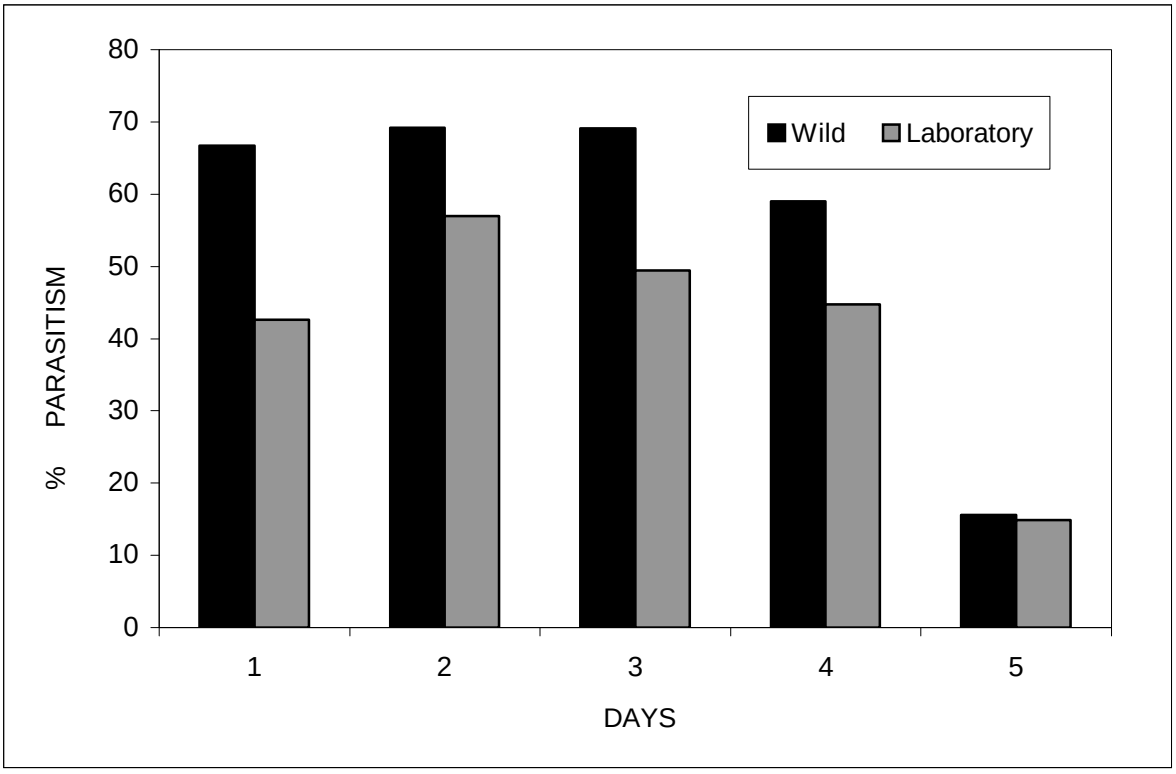


Fig. 3. Percentage larval parasitism in field cages with infested fruit using laboratory and wild parasitoids.

Discussion

The results obtained in pupal weight and adult size showed that the wild parasitoids were larger. This could have an effect on other parameters, such as the sex ratio, longevity and fecundity. These differences in size could be attributed to the host of origin. The mass reared parasitoids came from mass reared *A. ludens,* which according to quality control data from the Moscafrut facility have been reported to be smaller than wild larvae (Liedo and Carey, 1994).

The smaller size can represent a disadvantage to mass reared individuals. According to Lawrence (1981), small *D. longicaudata* individuals are displaced under competitive conditions and show shorter longevity and reduced fecundity. Host size could also affect the sex ratio of the offspring. Small hosts tend to produce more males in fruit fly parasitoids of the Opinae subfamily (Wong and Ramadan, 1992) and in parasitoids of the house fly (Mandeville and Mullens, 1990).

Longevity was greater for the wild strain. However, these parasitoids were very sensitive to the absence of food, whereas the mass reared strain was slightly more resistant to these stress conditions. This could be attributed to mass rearing selection, since under these conditions water is not provided.

Mass rearing conditions could also explain the lower flight ability of the mass reared strain, since these parasitoids are maintained in small space cages. The effect of small rearing cages in the flight ability of parasitoids has been demonstrated in a number of cases (e.g., Bush et al., 1976). The greater flight ability of the wild strain could also be attributed to the larger size of these parasitoids.

The fecundity rates observed in this study can also be explained by the effects of mass rearing. The greater fecundity of the mass reared strain, when artificial parasitism units or laboratory infested fruits were used, probably represents the adaptation of these individuals to laboratory conditions. This greater fecundity occurred despite the fact that the wild strain was greater in size and that size has normally been positively correlated with fecundity rates. However, under field cage conditions the wild strain showed much greater fecundity, which could be interpreted as a loss in the capacity of the mass reared strain to find hosts under more natural conditions.

Another interesting aspect in fecundity was the offspring sex-ratio. There are two possible explanations for the greater proportion of males in the mass reared strain: first, the low mating activity on laboratory conditions and second, the decrease in the capacity to select and discriminate hosts. It could be that the wild parasitoids had more opportunities to search and find adequate hosts, using large hosts to lay fecundated eggs and therefore, producing more females.

The results under field cage conditions are perhaps the best indicators of a loss in field performance, and therefore, in the quality of the mass reared parasitoids. These results might be an indication of a reduction in the searching ability of mass reared parasitoids under more close to natural field conditions.

The host searching capacity is an important attribute that should be maintained in parasitoids to be used as pest biological control agents. Modifications to mass rearing methods could help to maintain a good searching capacity. Bautista and Harris (1997) have reported that the materials and procedures used for the mass rearing of *D. longicaudata* can modify the behavior of adult parasitoids. According to Greany et al. (1997), *D. longicaudata* finds its host using volatiles emanated during the process of fruit infestation. Sivinski (1991) showed that in addition to olfactory cues, visual stimuli, such as the shape and size of the fruit, are important factors. Under artificial mass rearing conditions, both chemical and visual cues are lost and parasitoids have to adapt to the new laboratory conditions, so they can reproduce. In the long term, this could adversely affect their field performance.

Our results suggest that the long time under mass rearing conditions (> 100 generations) has gradually affected some important characteristics or biological attributes that might be important for an effective biological control program. Some corrective measures can be taken. For example, the size of the larval host could be an important factor to improve product quality and therefore, the optimal size must be determined and a quality control procedure should be developed to assure the correct host size (process quality control). This could contribute to overcome some disadvantages of mass reared parasitoids, such as longevity, fecundity, sex ratio, and flight ability. Other alternatives could be related to mass rearing conditions. For

example, reduction in cage densities and sex ratios, as long as is feasible. Larger cages to encourage a better flight ability. The introduction of wild parasitoids into the colony (refreshment) or the replacement of the whole colony, are always alternatives to solve quality problems, but the cost of these options must be considered as part of an integral analysis.

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Telenomus alsophilae Rearing Under Laboratory Conditions

C. A. RODAS

Smurfit Carton de Colombia
Department of Forest Research
Forest Tree Health Program
Fax (57 - 2) 442-5800
Apartado Aereo 219
Cali, Colombia

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Background

Telenomus alsophilae Viereck (Hym.: Scelionidae) was successfully introduced from Virginia (USA) to Colombia in order to control *Oxydia trychiata* outbreaks. The original host of *T. alsophilae* was *Alsophila pometaria* (Harris), but it also parasitizes *Phigalia titea* Cramer and *Abbotana clemataria* (J.E. Smith) eggs in the United States.

In Colombia, *T. alsophilae* has been efficiently mass released to control the following measuring worm species: *O. trychiata, O. platypterata, O. geminata, O. vesulia, O. olivata, Cargolia arana, C. pruna, Neuromelia ablinearia, Bassania schreiteri* and *Chrysomima semilutearia.*

O. trychiata and *C. semilutearia* are the most useful hosts for *T. alsophilae* mass rearing in Colombia; however, *C. semilutearia* is the most efficient because of its adaptability to laboratory conditions.

Rearing the Host

Pupae that are ready to emerge are placed in plastic trays with sterile sawdust and introduced into wood framed screen cages that have paper strips hanging from the ceiling. Adults begin to mate within one day of emergence and egg laying begins one day later.

Egg-masses are collected every day by cutting them from the paper strips and then gluing them to cardboard squares (about 9 x 6 cm). Both *O. trychiata* and *C. semilutearia* larvae are fed with *Pinus patula* foliage for 8-10 days in glass bottles (4000 cc capacity) and then transferred to wood framed screen cages (1.20 x 0.60 x 0.50 m) until the prepupal stage. Pupae with sawdust are caged in 14 x 18 cm plastic trays.

Rearing the Egg Parasitoid

Parasitization. Cards with the host egg-masses are exposed to about 2.000 adult *T. alsophilae* wasps for 24 hours in a 13.3 x 7 cm glass bottle. Every cohort of wasps parasitizes 20,000 - 25,000 *C. semilutearia* eggs. A female wasp is able to parasitize 73 to 230 *C. semilutearia* eggs (average 126 eggs) or 56 to 190 *O. trychiata* eggs (average 98 eggs). Parasitoids are fed every other day with a 33% honey solution.

Parasitoid Development. Cards with parasitized eggs are put in 13.3 x 7 cm glass bottles closed with a piece of cloth fixed with a rubber band. Parasitoid development takes 23 to 30 days (average 27.17) at 21^{o}C and 80% RH, and 45-50 days (average 48) at 17^{o}C and 75% RH.

Adult Parasitoid Management. Emerging adult wasps from different cohorts are mixed every day; food is supplied every other day using a piece of cotton soaked with a 33% honey solution. Depending on necessity, the adult parasitoids may be released, used to for colony maintenance, or stored in the dark with food at 14-16^{o}C and 75% relative humidity.

FIELD PERFORMANCE
of NATURAL ENEMIES

Genetic Fingerprinting for Quality Control and Field Evaluation of Mass-Reared Organisms

D. C. VACEK and R. A. RUIZ

USDA, APHIS, PPQ
Mission Biological Control Center
Mission, Texas 78573

Abstract. Genetic fingerprinting with randomly amplified polymorphic DNA (RAPD) provides a rapid and reliable method for insuring quality control of mass-reared biological control agents and identifying undescribed populations of potential biological control agents. The integration of molecular genetic techniques into quarantine importation and culture of exotic natural enemies has enhanced the implementation of biological control projects at the Mission Biological Control Center (MBCC). Voucher specimens of the natural enemies imported and cultured in the quarantine laboratory are provided to both systematists and the MBCC Genetics Diagnostics Laboratory. Specimens are rapidly identified using genetic techniques while systematic determinations are in progress. Additionally, genetic techniques are useful in recognition of biotypes, populations, and cryptic species that might otherwise go undetected in the quarantine importation process. We have successfully used these methods to document field establishment of mass-reared exotic natural enemies that are practically indistinguishable from natives and other released exotics. The advantages and limitations of methods used by the Genetics Diagnostics Laboratory and the application of this technology to quality assessment of laboratory colonies and to field evaluation of biological control agents are described.

The Relationship Between Laboratory and Field Tests on the Aphid Parasite *Aphidius colemani*

J. VAN SCHELT[1] and K. HORA[2]

[1] Koppert B.V., Berkel en Rodenrijs, The Netherlands
[2] Univ. of Amsterdam, Populationbiology/ISP, The Netherlands

Introduction

The IOBC-report by van Lenteren (1994) describes preliminary guidelines and methods for quality control on a number of commercially available beneficial insects. These tests are designed to be as realistic, simple, reliable and uniform as possible. This allows product control to be performed by the producers, after the product has passed through all handling procedures. The product should not be sold if any quality parameter falls below a certain threshold. These thresholds are to be determined within the IOBC-quality control working group, in which people from research institutes and producers of biological control products are represented. It was decided that for some insects it was necessary to demonstrate a relationship between low performance in the laboratory and a poor performance in the field. In this paper quality control tests with the aphid parasite *Aphidius colemani* are described. Good and poor quality wasps were tested in the laboratory for fecundity and flight capacity and in the field for dispersal and fecundity.

Material and Methods

All experiments were conducted at a temperature of 22°C, 70% RH and a light regime of 16L:8D, unless otherwise stated. *A. colemani* was reared on cucumber with *Aphis gossypii* as a host. Loose mummies were put in plastic containers where honey was provided as food for newly emerged wasps. The evening before an experiment newly emerged wasps were removed, this ensured wasps were no older than 16 hours. Earlier experiments demonstrated that mating takes place in the container. Wasps from the same batch were used in all the experiments. Poor quality wasps were produced by storing mummies from part of the batch at 8°C for two weeks.

Fecundity

Fecundity was tested using plastic trays with a layer of agar and a punched leaf disc of cucumber. (see van Schelt, 1993) 25 female wasps were each offered individually cotton aphid (*Aphis gossypii*) ad libitum for 24 hours. After 9 days the number of mummies in the tray was counted. Both fresh and stored wasps were tested.

Short range flight test

The short range flight test was based on the principle of the test described for *Encarsia formosa* by van Lenteren (1994). The openings of plastic PVC tubes (∅ 25 cm, height 60 cm.) were covered with a yellow sticky trap. The upper side of the trap was covered with plastic foil. A 75 ml. vial with 0.2 gram of mummies (100-150 wasps) close to emergence was placed in the bottom of the tube. Three days later the percentage of flying wasps was calculated as: number of

wasps on the trap/total number of emerged wasps x 100%. For each treatment (fresh/stored) 10 flight tubes were used.

Long range flight test

This test was conducted in a glasshouse measuring 300 meters long by 50 metres wide. The glasshouse contained a substrate grown cucumber crop of 2.5 meters in height in which no aphids had been spotted and no *Aphidius* had been introduced. Small pots containing winter wheat seedlings (var. ritmo) infested with wheat aphids (*Rhopalosiphum padi*) were used as an indirect measurement to determine the dispersal.

Three pots were placed every 2 meters with 2 meters between each pot. In total 96 plants were placed over a distance of 62 meters. At one end of the glasshouse 750 female wasps were released. Two days later the plants were collected from the glasshouse and placed individually into buckets. After nine days the presence/absence of mummies and the number of mummies per plant was assessed.

Two tests were carried out: one with fresh mummies and two weeks later with mummies from the same batch, which had been cold stored. Just before the two tests took place 20 plants were placed at random in the greenhouse to check for the presence of *Aphidius colemani*.

Results

In Fig. 1 the number of *A. gossypii* mummies produced is given for the fresh and stored wasps. A significant reduction of 70% in fecundity was found (Mann-Whitney, 5%).

Fig. 2 illustrates the percentage of flying wasps recorded in the tube. Though a significant difference was found, the relative decrease in flying capacity was only 20%. The wasps were quite evenly distributed over the sticky trap. This means that they actually flew rather than walked into the glue.

The preliminary tests to check for the presence of *A. colemani* were both negative. This means that apart from the released wasps there were no other aphid parasites present. Fig. 3 and 4 show the results from the glasshouse test. Though there was a trend for stored wasps to fly further than the fresh, the percentage of plants found was the same for both treatments (Kolmogorov-Smirnov,5%). In contrast if the number of mummies per plant is examined, fresh wasps produced significantly more mummies (KS,5%).

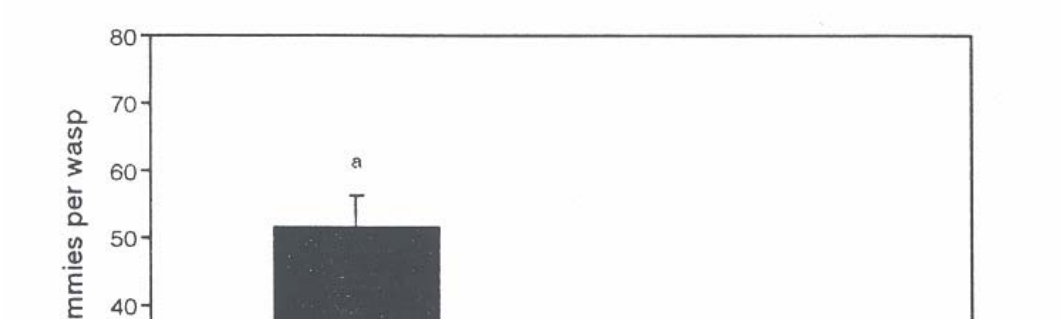

Fig. 1 The number of *A. gossypii* mummies produced per female in 24 hours (+s.e.), for wasps from fresh mummies and from mummies stored 2 weeks.

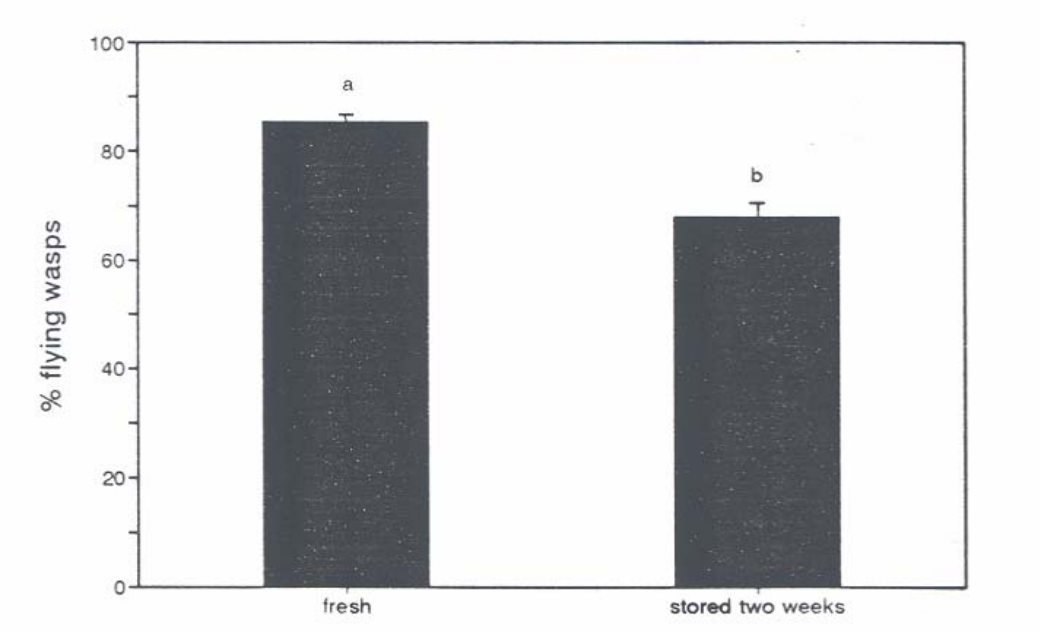

Fig. 2 The percentage flying wasps (+s.e.), determined on wasps emerged from fresh mummies and mummies stored for 2 weeks.

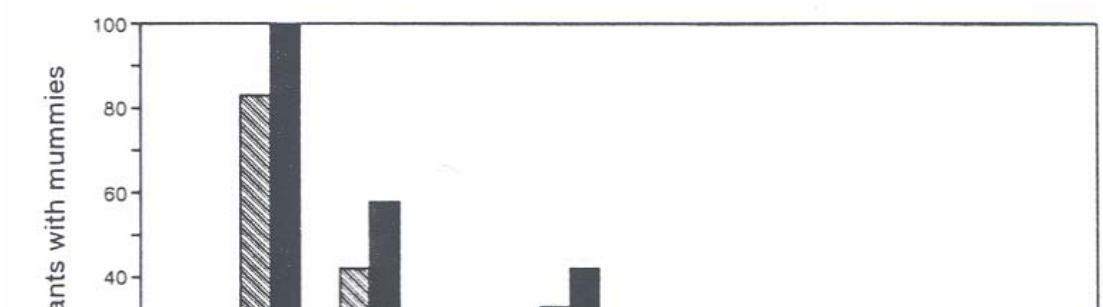

Fig. 3 The percentage of plants reached by *A. colemani* at different distances from their release point.

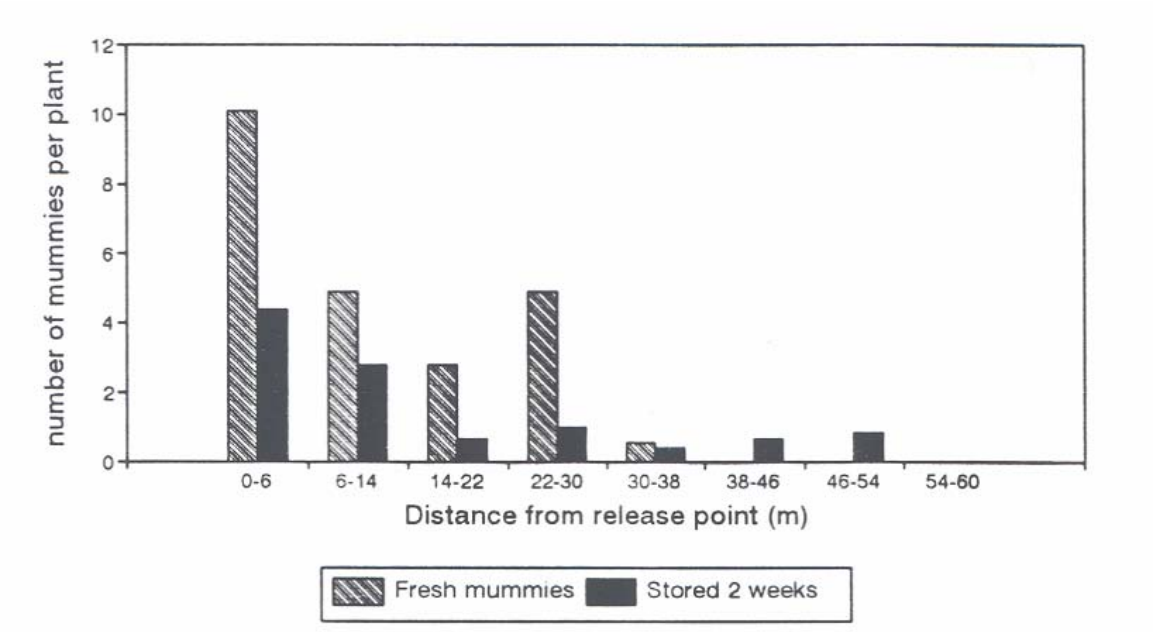

Fig. 4 The average number of mummies per plant at different distances from their release point.

Discussion

These tests partly demonstrate that a poor performance in the laboratory was reflected in a

poor performance in the field. The low fecundity in the lab was also found in the field, the reduction in flight capacity was not. Three explanations can be given for this. First, the difference in flight capacity between the two batches was rather small. It may be unrealistic to expect this subtle difference to be determined in the field. Secondly, assessment of the percentage of flying wasps may not be representative of actual dispersal in the field. In theory a small number of wasps can visit many pots and produce a small number of mummies per pot. It also possible to speculate that wasps stored for a long time loose weight and because of this can fly further than heavier fresh ones.

These field tests clarify the ability of *Aphidius colemani* to disperse and allow more precise experiments to be devised.

The relationship between the short range flight test and subsequent dispersal in the glasshouse has to be investigated further.

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Establishment and Dispersal of *Orgilus obscurator* (Ness) Measured After Implants of Parasitized *Rhyacionia buoliana* (Denis & Schiffermüller) Larvae in Plantations of *Pinus radiata* D. in Chile

J. L. SMITH, E. BRAVO N. and M. KRAUSKOFPT

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Abstract. The objective of the study was to evaluate the establishment and dispersal of *Orgilus obscurator* Ness, the larval parasite of *Rhyacionia buoliana* Denis & Schiffermhller, and the effect of the parasite on apical attack, after implanting two distinct rates of parasitzed larva. *R. buotiana* populations varied between 0 and 9,120 larva/hectare. Implantation efficiency was between 67.6% and 81.2%. On the average, 42% of the implants produced adult parasites. Parasitism in the implantation plot after one year fluctuated between 2,0% and 19,6%. Parasitization rates outside of the implantation plots, an area of approximately 13 hectares, varied between 0.3% and 4.0% after one year. Sex ratios, the number of males per female, were between 0 and 1.5. Dispersal of the parasite after one year was at least 180 meters from the implantation plot. Apical attack in the implantation plots increased from 1993-1994.

The conclusions of the study were the following: 1.) Methods to increase the percentage of successful implants should be explored in order to optimize the quantity of implanted material; 2.) The development of parasitized larva should be concentrated in the field where adult production, specifically females, is equal to or higher than present laboratories methods. If the implants are located in places where *O. obscurator* is to be established, there is no need to recover the adults that are produced; 3.) It is possible to establish and ensure mating of adult parasites with the implantation of 128 parasited larvae per point. If these implants are placed close together, parasite dispersal, in one year, can occur over at least a 13 hectare area; 4.) Performing parasitized larval implants is not enough to oppose apical attack produced by *R. buoliana*, therefore, additional control methods should be integrated.

Introduction

The laboratory work, training, production and use in the field realized by the Instituto de Investigaciones Agropecuaria (Agriculture and Cattle Research Institute), INIA, since 1986, demonstrate that it will be possible to establish in Chile the specific parasite of *Rhyacionia buoliana* Denis and Schiffermüller, *Orgilus obscurator* Ness (Cisternas et al. 1993; Espinoza, 1993). The technology of introducing in the field *R. buoliana* larvae that are parasitized by *O. obscurator* is one of the methods used to establish the parasite. The work performed by INIA showed that 600 parasitized larvae were enough to establish the parasite. It is important to know the minimum number of parasitized larval implants needed, in *Pinus radiata* Don plantations, to establish the parasite. Reducing the quantity of material at each implant point will facilitate

parasite establishment in more locations in less time. It is important to obtain this information to insure the rapid establishment of this biological control agent.

It is important, as well, to determine the range of *O. obscurator* dispersion in order to define the distance between implantation points. Juliett (1960) indicated that the average dispersion rate for *O. obscurator* males and females was 275.6 and 541.3 meters per day, respectively. However, this has not been confirmed in *P. radiata* plantations. Also, Juliet (1960) indicated that *O. obscurator* was not affected by wind and that its dispersion pattern depends on vegetation density. He also pointed out that adults disperse widely when large shoot moth infestations exist. The objective of our research was to evaluate *O. obscurator* establishment and dispersal under two parasitized larval implantation rates, and determine the effect on apical attack by *R. buoliana*.

Materials and Methods

The study took place in two year old *P. radiata* plantations with supposedly 2,000 larva per hectare.

Determination of pest levels, period 1993.

The population level present in each farm was confirmed before releasing parasitized larvae in the field. The number of sound and attacked shoots and buds, and the number of larvae were determined from a census of a line of 50 trees. After determining the quantity of larvae in the 50 trees, the population per hectare was calculated on the basis of 1,600 trees per hectare.

Efficiency of implantation of parasited larva, period 1993.

Parasited larval implants were conducted during the second half of June, 1993, until the end of October, 1993. In each plantation, an area of 256 trees (16 lines of 16 trees) or 64 trees (8 lines of 8 trees) was selected in which 512 or 128 parasitized larvae were implanted, respectively. The parasitized larvae were released two per tree in succulent shoots. They were covered for protection with a transparent plastic bag that was tied to the tree with wire. The bag had a gauze window for ventilation and water drainage. The implants were checked two or three times a week to confirm effective implantation. Larval deaths and losses were reported. When the implant proved effective, the bag was removed and the shoot remarked with wire. The bags were left for a maximum of ten days and then removed.

Adult emergence within implantation plots, period 1993.

In 1993, *O. obscurator* recovery in implantation plots was evaluated to determine the number of male and female parasites that emerged from the parasitized larvae. Samples consisting of 20% of the shoots where parasitized larval implants had proved effective were covered with a gauze net. Evaluation took place between November 1993 and January 1994. Two or three times a week nets were checked, looking for the presence of shoot moths and the parasite. If any agent was present, the kind and sex were recorded. After this, it was released and the gauze net and wire were removed from the shoot. By the end of January, all the remaining gauze nets were checked to determine and record the kind and sex of the agents that were present. The percentage of parasite emergence and the number of males and females were calculated.

Evaluation of establishment and parasitism within implantation plots, period 1994.

During the first half of December 1994, establishment and parasitism were evaluated in the implantation plots. A random sample of 30% of attacked shoots were collected in each plot. Selected shoots were cut and immediately analyzed in the field. The presence of *R. buoliana* larvae and *O. obscurator* agents were recorded. Live *R. buoliana* larvae were brought to the laboratory to determine the kind of agent that emerged.

Evaluation of parasitism and dispersal of *O. obscurator* outside of implantation plots, period 1994.

This evaluation was conducted between the second half of November and the first half of December, 1994. The evaluation consisted of an area outside the implantation plot with a radius of 180 meters in eight directions (N, NE, E, SE, S, SW, W, NW). Beginning at the border of each implantaion plot, six evaluation plots were established that were 20 meters apart along each directional axes. Each evaluation plot consisted of four lines of ten trees, oriented perpendicular to the directional axes. Each tree was evaluated independently. Up to ten attacked shoots were cut from each tree and analyzed in the field. The number of live and dead shoot moth larvae, pupae, and exuvia, and *O. obscurator* larvae and pupae were recorded, as well as the direction, evaluation plot, line and tree number. The evaluation started from the last plot (Nº6), 180 meters from the edge of the implantation plot, and advanced toward the implantation plot. When parasites were found, the evaluation at that distance from the implantation plot was completed in each direction. No further evaluations outside of that implantation plot were made. Live shoot moth larvae were put in capsules and brought to the laboratory for rearing in order to determine the kind of agent that would emerge. The rearing was conducted at 20-22^{0}C on artificial diet, and evaluated every seven days.

Apical Attack, periods 1993 and 1994.

During 1993 and 1994 the apical attack category of trees in each implantation plot was determined. This was achieved by viewing the apical area in order to determine attack levels according to the following classification (Bioforest Ltda., 1991):

0 = Sound apex, apical area does not present attacked buds.
1 = Attacked apex, apical area presents attacked buds.
2 = Apex with apical pruning, the main apex is twisted, broken or absent.

Results and Discussion

Determination of pest levels, period 1993.

Populations present in the surveyed plantations varied from 0 to 9,120 larva/hectare. It was requested that plantations have generally similar population levels (about 2,000 larva/hectare), but after the evaluation it was determined that plantations owned by Forestal Tornagaleones showed higher population levels, which varied from 6,112 to 9,120 larvae/hectare, while populations in

plantations owned by Forestal Valdivia S.A. varied from 0 to 2,464 larva/hectare (Table 1). The plantation where no moth larvae were found was at a higher elevation (ca. 500 meters) with strong winds, cold winters and summers, and had extensive weed populations, which opposed pine plants and limited growth. However, most of the farms had between 1,408 to 2,464 larva/hectare. Evaluations performed by the forestry companies showed different population estimates. This variation might be caused by the different evaluation systems used, by evaluations poorly conducted, or by the fact that *R. buoliana* population distribution is not homogenous (Ahumada and Smith, 1995). Work continued in these farms without considering population levels.

Table 1. 1993 population estimates for *R. buoliana*.

Plantation	Number of shoots from the evaluation of 50 trees			Shoots per Tree	Total Number of Larva	Larva per Hectare
	Healthy	Attacked	Total			
El Trebol	1655	265	1920	38	285	9120
Pichipaillaco	2625	195	2820	56	191	6112
San Ambrosio	931	100	1031	21	66	2112
Chanco	1478	120	1598	32	57	1824
El Salto P38	702	4	706	14	0	0
Santa Gisela	1113	144	1257	25	44	1408
La Junta	1027	113	1140	23	48	1536
Las Vegas	951	193	1144	23	77	2464

Efficiency of implantation of parasited larva, period 1993.

Implantation efficiency varied from 67.6% to 81.2%, with an average of 74.5%. Regarding larvae that did not implant, 15.1% were found dead, while 10.4% were lost (Table 2). It is possible that lost larvae implanted without showing implantation signs (resin formation), or more probably these agents escaped from the implantation bags. The best efficiency values were obtained from June implants. There were no parasitized larvae lost in these two plantations. It is possible that severe weather conditions in winter minimized parasitized larval movements (migration) and stimulated rapid implantation, or parasitized larvae died without leaving the bag. There is also the possibility that resin production in winter was more noticeable than in the spring, and helped to locate successful implants. It is important to consider implantation efficiency in order to improve the use of implants for establishing *O. obscurator* in the field.

Table 2. 1993 efficiency of implantation[a].

Plantation	Number of implants	Percentage of implants that died	Percentage of implants that were unrecovered	Percentage of effective implants
El Trebol	512	19,1	0	80,9
Pichipaillaco	512	18,7	0	81,2
San Ambrosio	512	9,4	9,3	81,3
Chanco	512	13,1	19,3	67,6
El Salto P38	128	10,9	14,1	75,0
Santa Gisela	128	17,2	11,7	71,1
La Junta	128	15,6	15,6	68,8
Las Vegas	128	16,4	12,5	71,1
Total	**2560**	**15,1**	**10,4**	**74,5**

[a] Implants = parasitized larva

Adult emergence within implantation plots, period 1993.

Signs (cocoons) or agents (females and males) of *O. obscurator* were collected in implantation plots, with parasite recovery that varied from 28% to 58% (42% average). Regarding parasite signs or agents collected, 45% were cocoons, 25% were females and 30% were males (Table 3). This efficiency level, related to the production of *O. obscurator* adults, was equal to or higher than efficiency rates presently found in production laboratories (personal information). This result indicated that *O. obscurator* development in the field inside *R. buoliana* larvae is equally or more efficient than in the laboratory. Therefore, it is valuable to implant parasitized larvae in the field in order to improve adult production. Furthermore, if the implants are placed in locations selected for the establishment of the parasite, it is not necessary to collect the emerging adult parasites. Material can be left to develop and emerge in the field. If the implanted parasitized larvae are at the same developmental stage as the larvae naturally present, adult parasite emergence should occur when there are larvae ready to be parasitized.

Table 3. Recovery of *O. obscurator* (1993) within the implantation plots (sample of 20% of the shoots with implants). [a b]

Plantation	Shoots evaluated	*O. obscurator* recovered	% Recovered	% Cocoons	% Females	% Males
El Trebol	83	25	30	36	40	24
San Ambrosio	83	44	53	27	38	34
El Salto P38	19	11	58	54	18	27
Santa Gisela	18	7	39	71	14	14
La Junta	18	5	28	60	0	40
Las Vegas	18	8	44	25	37	37
Total	**239**	**100**	**42**	**45**	**25**	**30**

[a] Plantations Pichipaillaco y Chanco were not evaluated.
[b] Percent cocoons, females, and males are related to the total recovered.

Evaluation of establishment and parasitism within implantation plots, period 1994.

O. obscurator specimens were recovered in four of the implantation plots. Parasitism varied from 2.0% to 19.6% (Table 4). The plantation San Ambrosio presented the highest percentage of parasitism. Also, in 1993 this plantation had the highest total number of *O. obscurator* adults recovered from shoots (44). However, not recovering parasites in the evaluation does not indicate that the parasite was not present. Its absence might be due to a variety of factors, such as: 1) the size of the sample could have been small related to the degree of attack present in the plantation; or 2) females could have flown during their ovipositional period to other trees not included in the plot. These females may have been attracted by the odor of plants damaged by *R. buoliana* attack and/or by their feces. It is probable that the long distance attraction (over one meter) is caused by chemical odors produced by insect damaged plants, rather than by feces or host stench. Various studies with Braconidae hymenopterans have shown that long distance parasite attraction to areas where hosts are present is favored by the odors of plant damage produced by the host (Turlings et al., 1990; Steinberg et al., 1993; Geervliet et al., 1994). Stench produced by larvae or their feces is less significant in long distance attraction. These smells help the parasite locate their host after arriving at the plant (Sato, 1979; Vinson, 1985; Nealis, 1986; Takabayashi and Takahashi, 1989; Turlings et al., 1991; McCall et al., 1993). Nevertheless, *O. obscurator* presence indicates that it was able to survive and reproduce, sometimes with significant parasitism levels after one year, as a result of parasitized larval implants.

Table 4. Evaluation of establishment and parasitism within implantation plots

(sample of 30% attacked shoots), period 1994.

Plantation	Attacked Shoots		Total Agents	*O. obscurator* Total	Percent Parasitism
	Total	Empty			
El Trebol	490	439	51	1	2,0
Pichipaillaco	299	288	11	0	0
San Ambrosio	165	99	56	11	19,6
Chanco	184	125	59	7	11,9
El Salto P38	10	9	1	0	0
Santa Gisela	36	17	19	2	10.5
La Junta	42	20	22	0	0
Las Vegas	75	68	7	0	0

Evaluation of parasitism and dispersal of *O. obscurator* outside of implantation plots, period 1994.

These evaluations, as well as the evaluations made inside implantation plots, showed a high level of empty attacked shoots (Tables 4 & 5). This could have been influenced by shoots that were previously attacked by shoot moth larvae that left the shoot during the larval migratory season. It could also be the result of predation or other unknown factors.

Regarding *O. obscurator* parasitism determined in the different plantations, between 4 and 30 parasites were recovered per plantation. Parasitism rates outside of implantation plots, an area of about 13 hectares, varied from 0.3 to 4.0% after one year. Sex ratios, in relation to males per female, varied from 0 to 1.5. It is important to note that the presence of females, and in general a sex ratio of one to one, meant that there were encounters and mating between males and females (Table 5). It appears parasitism levels were not influenced by the number of implants in each plantation. However, population levels should have some effect on parasitism levels. Unexpected population differences did not permit us to determine the effect of implant rate over parasitism.

Regarding movement or dispersal, it is still difficult to define a dispersal tendency related to directional axes (Table 6). *O. obscurator* was found present in all eight directional axes (less on the North axes). It appears that dispersal was not influenced by implant density. In both cases (128 and 512 parasited larva) parasites were encountered up to the last evaluation plot (approximate distances of 170-180 meters) (Table 6). Shoot moth populations in these plantations varied from 0 to 9,120 larva/ha. (1993 sampling). Dispersal could have been affected by the difficulty of finding larvae apt to be parasitized. Other factors that could influence dispersal are predominant winds during flight periods, odors emanating from attacked trees that stimulate parasite dispersal towards areas with higher larval concentrations, host produced stench, or other factors that have not been evaluated (topographic features, exposition, etc.). It is important to note that implants performed with both 128 and 512 parasitized larvae showed an effective establishment and dispersal of *O. obscurator* in *R. buoliana* populations that varied from 0 to

9,120 larva/hectare. This indicates that future implants with 128 larvae per point could establish the parasite, within one year, in an area of approximately 13 hectares (Table 6). It should also be noted that both implantation rates achieved the mating of males and females in the next parasite generation. This was demonstrated by the almost one to one sex ratio encountered in the study (Table 5).

Table 5. Evaluation of parasitism and dispersal of *O. obscurator* outside of the implantation plots, period 1994. [a]

Plantation	Attacked Shoots		Total	*O. obscurator* recovered				Percent
	Total	%Empty	Agents	Total	Dead	Males	Females	Parasitism
El Trebol	5355	89.0	587	8	2	3	3	1,4
Pichipaillaco	7953	91,6	667	3	0	0	3	0,4
San Ambrosio	14433	86,1	2003	24	3	8	13	1,2
Chanco	9675	85,7	1386	30	3	16	11	2,2
El Salto P38	2182	87,4	276	4	0	2	2	1,4
Santa Gisela	13684	83,6	2247	6	0	5	1	0,3
Las Juntas	9182	79,5	1879	5	0	3	2	0,3
Las Vegas	8150	93,9	498	20	7	6	7	4,0
Total	**70614**	**86,5**	**9543**	**100**	**15**	**43**	**42**	**1,05**

[a] Total agents correspond to *R.buoliana* and *O.obscurator*.

Apical attack, 1993 and 1994.

In 1993, an average of 79.5% of the trees in the implantation plot had unattacked apical areas, 13.8% of trees exhibited apical attack, and 2.3% of the trees exhibited apical pruning (Table 7). In 1994, the number of trees with sound apical areas decreased to 22.5%. Unattacked apical areas decreased 3.6 times in relation to 1993. The percentage of trees with attacked apical areas increased to 23.4%, which implies an increase of 1.6 times in relation to 1993. The number of trees with apical pruning increased to 23.3%, an increase of ten times in relation to 1993 (Table 7). The effect of the parasite on a decrease in apical attack was not observed. Therefore, control of apical attack should not be expected during the first year of parasite establishment. This indicates the need to integrate other activities, together with the establishment of *O. obscurator*, in order to control damage produced by *R. buoliana*.

Table 6. Dispersal of *O. obscurator* in plantations of radiata pine, December 1994.

Number of implants	Distance (meters)	Directional axes and the number of plantations were *O. obscurator* was encountered							
		N	NE	E	SE	S	SW	W	NW
512	20-30	2	0	1	1	1	1	2	2
	50-60	0	1	1	2	1	2	1	1
	80-90	1	1	1	0	3	1	2	0
	110-120	0	2	1	1	0	1	2	1
	140-150	0	0	1	1	0	0	1	1
	170-180	0	0	1	0	0	0	1	0
128	20-30	0	2	1	1	0	1	0	1
	50-60	1	0	1	0	0	0	0	1
	80-90	0	1	0	2	1	0	0	1
	110-120	0	1	1	2	0	1	0	2
	140-150	0	0	0	0	0	0	2	1
	170-180	0	0	0	0	0	1	0	1

Table 7. Category of apical attack, periods 1993 y 1994

Plantation	Categories 1993 (%)			Categories 1994 (%)		
	0	1	2	0	1	2
El Trebol	88,3	0	5,9	26,8	18,0	21,2
Pichipaillaco	92,3	0	3,1	20,2	23,3	44,4
San Ambrosio	67,6	28,2	1,1	7,5	29,0	23,0
Chanco	78,2	17,1	1,6	31,6	13,2	7,2
El Salto P38	100	0	0	46,9	48,4	4,7
Santa Gisela	57,8	34,4	0	17,2	31,2	25,0
La Junta	67,2	32,8	0	12,5	26,6	40,6
Las Vegas	67,2	28,1	0	28,1	29,7	9,4
% General	**80,0**	**15,1**	**2,3**	**22,5**	**23,4**	**23,3**

Category of attack:

0 = Sound apex
1 = Attacked apex
2 = Apex with apical pruning

Conclusions

1. Methods to increase the percentage of successful implants should be explored in order to optimize the quantity of implanted material.

2. The development of parasitized larva should be concentrated in the field where adult parasite production, specifically females, is equal to or higher than present laboratories methods. If the implants are located in places where *O. obscurator* is to be established, there is no need to recover the adults that are produced.

3. It is possible to establish and ensure mating of adult parasites with the implantation of 128 parasited larvae per point. If these implants are placed close together, parasite dispersal, in one year, can occur over at least a 13 hectare area.

4. Performing parasitized larval implants is not enough to oppose apical attack produced by *R. buoliana*, therfore, additional control methods should be integrated.

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Trichogramma spp. Control Damage Produced by *Rhyacionia buoliana* Denis & Schiffermüller in Plantations of *Pinus radiata* D. in Chile

S. OJEDA A., J. L. SMITH and M. KRAUSKOFPT

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Abstract. *Trichogramma pretiosum, T. exiguum, and T. embryophagum* were released in 2 year old radiata pine plantations to determine their capacities to parasitize sentinel host eggs and reduce attack produced by the european pine shoot moth, *Rhyacionia buoliana.* All species were released four times during the ovipositional period of the shoot moth. Each release was made at a rate of 5,000,000 parasitized eggs per hectare. Parasitization rates of sentinel host eggs were greater than 50% for each species. The reduction of attacked shoots, in relation to control plots, ranged between 76% and 25%. *T. pretiosum* and *T. exiguum,* two species collected from radiata pine plantations in Chile, provided significantly greater reduction in attacked shoots than *T. embryophagum* imported from Russia. It was speculated that these species were better adapted to searching for host eggs in radiata pine than their Russian counterpart. The future use of these species, for the control of *R. buoliana* in Chile, will depend greatly on the capacity to produce large quantities of parasites during the ovipositional period of the pest species. It is important to determine control levels in relation to release rates. This information will enable us to decide which species can be produced and released in quantities sufficient enough to provide adecuate control.

Introduction

The genera of *Trichogramma* include more than 100 species that mainly attack Lepidoptera eggs, and are found in a wide range of crops and hosts. Research on the use of these parasitoids for biological control started at the beginning of this century. By 1930, a method of producing *Trichogramma* on alternative hosts was developed (Hassan, 1994). At present, advanced techniques for insect mass production and release are available.

Efforts to control insect pests with *Trichogramma spp.* have achieved various results (Hassan, 1993). In agriculture, this parasitoid has been responsible for the decrease of different pest populations. In forestry, *Trichogramma* releases have been considered effective against *Choristoneura muriana, Dioryctria ebeli, Rhyacionia buoliana, Choristoneura fumiferana* and other forest defoliators (Belmont and Habeck, 1983; Tsankov et al., 1980). *Trichogramma* has traditionally been considered a polyphagous insect, but there is evidence that species differ in their searching capability, host preferences and adaptability to environmental conditions (Hassan, 1994). For this reason it is important to select appropriate species for use in biological control programs. Considering this point, *T. embryophagum, T. exiguum* and *T. pretiosum* were tested in the field against *R. buoliana*.

Trichogramma embryophagum was specifically isolated in Russia from *R. buoliana* eggs. *Trichogramma exiguum* and *T. pretiosum* are two native species that were detected in *Pinus*

radiata plantations located in the tenth region of Chile, and were considered to be adapted to environmental conditions of this region. The objective of this study was to determine each species capacity to parasitize sentinel host eggs and reduce attack produced by the European pine shoot moth, *Rhyacionia buoliana* Denis & Schiffermüller, after inundative releases in the field.

Materials and Methods

Production of *Trichogramma spp.* and the alternate host *Ephestia kuehniella* Zeller was accomplished according to the methodology detailed by Ojeda and Smith (1995). The *Trichogramma spp.* released in this study were *T. embryophagum* Moscow (M) and St. Petersburg (SP), *T. exiguum* and *T. pretiosum*. *Trichogramma embryophagum* was isolated from *R. buoliana* eggs and introduced to Chile from Russia. *Trichogramma exiguum* and *T. pretiosum* are Chilean native species that were found in pine plantations in the province of Valdivia. They were caught in traps baited with *E. kuehniella* eggs. Taxonomic identification of the native species was done by Dr. John Pinto, University of California, USA.

Establishment of test plots and release of parasitized eggs.

The study was conducted in a 1992 *Pinus radiata* plantation located in the province of Valdivia, X region, Chile. A completely randomized design was used with four treatments (*Trichogramma spp*) and one control, each with four repetitions. Each repetition consisted of a test plot made up of 25 trees (5 lines of five trees) planted at distances of 2.0 meters x 3.0 meters within and between lines, respectively. The plots were 150 m^2 and were located 50 m apart. The trees were on an average 1.5 meters high. Pheromone traps were set (Delta type with Biolure, Consep, Bend, Oregon bait) between plots to detect the emergence of the first *R. buoliana* males, and to calculate the beginning of the female ovipositional season. Traps were removed after the first male catch.

Four lines of 10 trees, inside the study area but outside of the test plots, were evaluated in order to determine *R. buoliana* population levels. A census of each tree was conducted to calculate the number of *R. buoliana* agents (larvae and pupae) present. The average number of agents per tree was multiplied by 1,600 to determine the population level per hectare of plantation.

Four releases were made of each *Trichogramma* species. These releases were distributed in such a way as to cover most of the *R. buoliana* egg-laying period. The first release occurred ten days after first male catch, and the other three at 10-day intervals. The parasitoid was release in the pupal stage, so that wasp emergence occurred in the field. *Trichogramma spp.* were released in every tree of each test plot at a rate of 5,0 x 10^6 parasitized eggs/hectare (Table 1). Adhesive cards with parasited eggs were stuck to the bottom of plastic petri dishes and hung in the middle third of each tree one day before scheduled emergence.

Table 1. Release of *Trichogramma spp.* per treatment.

Treatment	Parasitized eggs released[a]		
	Eggs per tree	Eggs per plot	Eggs per treatment
T. pretiosum	3.000	75.000	300.000
T. exiguum	3.000	75.000	300.000
T. embryophagum (Moscú)	3.000	75.000	300.000
T. embryophagum (SP)	3.000	75.000	300.000
Control	0	0	0
Total			1.200.000

[a] These values correspond to each release date. The total number of parasitized eggs released during the study was 4.800.000.

Emergence of *Trichogramma spp.* in the field.

Parasitized eggs were recovered 10 days after their release and brought to the laboratory. An area that corresponded to 20% of each adhesive card was selected at random and evaluated. The percentage of broken eggs was calculated for this area to determine the emergence level of each released species.

Installation and evaluation of sentinel host eggs.

Rhyacionia buoliana sentinel eggs, which were used to determine *Trichogramma spp.* parasitism, were produced according to methods used by Ojeda and Smith (1995). Two days after each release, sentinel eggs were placed in the five center trees of each plot. The sentinel eggs produced on pine shoots were distributed in the middle and upper third of each tree, and left in the field until 5 days after parasitoid emergence. A total of 5,560 sentinel eggs were installed, including control plots. After 5 days sentinal eggs were brought to the laboratory and reared at 25 ± 2 ^{0}C and 75 ± 10% H.R., for at least 7 days, until the *R. buoliana* eggs turned black. The sentinel eggs for each treatment were evaluated under a microscope to determine the percentage of parasitized eggs, and *Trichogramma spp.* were allowed to emerge. Statistic differences were determined by an analysis of variance (ANOVA) and means were separated using a least significant differences test (LSD) (MSTAT, Microcomputer Statistical Program, Michigan State University).

Effect of *Trichogramma spp.* on *R. buoliana* attack.

The effect of each treatment on the attack produced by *R. buoliana* was determined by evaluating the percentage of attacked shoots before and after the four releases. At each evaluation nine trees from the center of each test plot (repetition) were evaluated. The number of sound and attacked shoots was recorded. The effect of *Trichogramma* on attack was analyzed by applying the Henderson and Tilton formula (1955):

% Control = (1-(ALTa x ALCb) / (ALTb x ALCa)) x 100

ALTb = Attack level in the treatment (before)
ALTa = Attack level in the treatment (after)
ALCb = Attack level in the control (before)
ALCa = Attack level in the control (after)

$$\text{Attack level} = \frac{\text{Attacked Shoots}}{\text{Total Shoots}} \times 100$$

An ANOVA was conducted to determine statistical differences between treatments and repetitions. Means were separated using LSD.

Results

There existed an average of two *R. buoliana* agents (larva or pupa) per tree in the test plantation. This figure was multiplied by 1,600 trees per hectare to estimate the presence of 3,200 larvae per hectare.

Emergence of *Trichogramma spp.* in the field.

The emergence of *Trichogramma spp.* in the field after each release was considered optimal, just as in the study conducted by Ojeda and Smith (1995), and reached values similar to those routinely obtained in the laboratory (90.7%). The mean emergence value per treatment varied from 85.6% to 87.7% (Table 2).

Table 2. Field emergence of *Trichogramma spp.* after each release.

Treatment	Percent emergence per release date				
	Dec. 23	Jan. 2	Jan. 12	Jan. 22	Average
T. pretiosum	74,8	90,4	89,9	90,4	86,4
T. exiguum	80,2	89,3	90,3	91,0	87,7
T. embryophagum (M)	74,4	89,9	90,2	90,0	85,6
T. embryophagum (SP)	73,7	88,9	91,2	91,5	86,3

***Trichogramma spp.* parasitism in *R. buoliana* sentinel eggs.**

Trichogramma spp. parasitism in *R. buoliana* sentinel eggs reached levels in each release period that varied from 36.6% to 76.5%. The average per species for the four releases was higher than 50%. Nevertheless, statistical analysis did not demonstrate significant differences between treatments. *Trichogramma exiguum* and *T. embryophagum* (SP) showed the highest total parasitism rates, 62.6% and 61.8%, respectively. There was never parasitism of sentinel eggs in control plots, indicating that the 50 meter spacing was sufficient (Table 3).

Table 3. Percent parasitism of *Trichogramma spp.* in sentinel eggs of *R. buoliana.*

Treatment	Percent parasitism per release date				
	Dec. 23	Jan. 2	Jan. 12	Jan. 22	Average
T. pretiosum	49,9	76,5	48,6	36,6	52,9 a
T. exiguum	59,1	62,0	62,9	66,5	62,6 a
T. embryophagum (M)	48,5	57,6	48,4	60,4	53,5 a
T. embryophagum (SP)	48,3	73,9	62,3	62,7	61,8 a
Control	0	0	0	0	0 b

Values with the same letters are not statistically different (LSD, P < 0,05)

The emergence of *Trichogramma spp.* from parasitized sentinel eggs was between 81% and 83% (Table 4). Statistical analysis showed no significant differences between treatments. This result indicated that pine shoots were good substrates for the production of sentinel eggs. Similarly, it can be noted that all *Trichogramma spp.* developed to the adult stage, demonstrating it is an appropriate host. This confirms the same conclusion made by Ojeda y Smith (1995).

Table 4. Emergence in the laboratory of *Trichogramma spp.* from parasitized sentinel eggs of *R. buoliana*.

Treatment	Percent emergence
T. pretiosum	81,7 a
T. exiguum	81,0 a
T. embryophagum (M)	83,0 a
T. embryophagum (SP)	82,3 a

Values with the same letter are not statistically different (LSD, P< 0,05)

Effect of *Trichogramma spp.* on *R. buoliana* attack.

Attack levels before and after parasitoid release are shown in table 5. The rate of attacked shoots in each treatment was compared to the control by applying the Henderson and Tilton formula (1955). Attack levels in plots treated with *T. pretiosum, T. exiguum, T. embryophagum* (M), and *T. embryophagum* (SP) decreased by 75.4%, 50.7% 25.5 and 34.3%, respectively, in comparison to the control. Significant statistical differences were found between treatments but not between repetitions. The strongest effect on *R. buoliana* attack was achieved with *T. pretiosum* and *T. exiguum*, the two native species found in pine plantations.

Table 5. Determinationn of percent shoot attack [a] before and after the four releases of *Trichogramma spp.*

Treatment	% Attacked Shoots Nov. 1994	% Attacked Shoots Apr. 1995	% Reduction of Attacked Shoots [b]
T. pretiosum	27.3 (48)	3.9 (135)	75.4 a
T. exiguum	24.5 (50)	7.9 (124)	50.7 b
T. embryophagum (M)	24.8 (34)	14.5 (117)	25.5 d
T. embryophagum (SP)	18.5 (31)	8.9 (105)	34.3 c

[a] Numbers in parenthesis correspond to the average number of shoots per tree.
[b] Values with the same letter are not statistically different (LSD, $p < 0.05$).

Discussion

Trichogramma spp. parasitism on *R. buoliana* sentinel eggs reached important levels (over 50%), but no significant differences were observed. The real control effect of treatments on *R. buoliana* was determined through the analysis of the attack level in each treatment plot in relation to the control. The decrease in attack rate produced by the release of each *Trichogramma spp.* varied significantly. The highest control rates were achieved with *T. pretiosum* and *T. exiguum*, 75.4% and 50.7%, respectively. Both are native species found in *P. radiata* plantations. They are adapted to environmental conditions in their native range, which could explain their higher efficiency. However, the same tendency was not observed in sentinel eggs. The distribution and exposure of sentinel eggs allowed for easy access to all species, and possibly lead to similar parasitization levels. The effectiveness of *Trichogramma spp.* in the field depends on the behavior of the released species, and on various biological features that contribute to higher parasitization levels. The most relevant features include flight capability, searching ability in the host plant, preference for a certain host, multiplication rate, tolerance to extreme environmental conditions, emergence rates, fecundity, and life span (Cerutti & Bigler 1991, Hassan 1994). The higher effectiveness of native species could be credited to their ability to search for *R. buoliana* eggs in *P. radiata* plantations, and a better recognition and acceptance of the pest. Shoot moth eggs are part of the natural habitat for these species, which exist in native areas associated with *P. radiata* plantations. *Trichogramma embryophagum* (M) and (SP) reduced the attack level with respect to

the control at levels of 25.5% and 34.3%, respectively. These species were specifically isolated from *R. buoliana* eggs in Russia, so locating the naturally occurring host eggs in a foreign environment could restrict their performance in Chile. It is important to note that *T. embryophagum* (M) and (SP) have a longer developmental cycle than the native species (unpublished information, Bioforest S.A.), and therefore multiply slower in the field. This would lead to a lower natural reproduction rate during the *R. buoliana* egg-laying season.

In spite of these conclusions, the use of *T. embryophagum* for *R. buoliana* control should not be dismissed, and calls for further evaluation. Efficacy of this species in the field can be optimized by developing suitable release strategies. This implies determining the number of releases, the rate of parasitoid release, and timing and planning of parasitoid emergence in the field. Another important consideration is the effect of host density and distribution related to parasitoid functional response.

A native *Trichogramma* species is often chosen for biological control because it is supposedly better adapted to local environmental conditions. However, it might not be the best suited for a certain purpose, in spite of its effectiveness. *Trichogramma embryophagum* shows a comparative advantage over *T. pretiosum* and *T. exiguum* as it is able to be put into diapause. The control of diapause in insects is of great importance because it enables mass production and storage of large amounts of live material. This material can then be utilized during release periods (Zaslavski and Umarova, 1990). Using *Trichogramma* as a bioinsecticide for the control of *R. buoliana* requires not only determining the most effective species, but also the feasibility of its operational production.

According to our results, the use of *Trichogramma spp.* to control *R. buoliana* is biologically feasible, and could be an important element in integrated management programs. However, the operational use of *Trichogramma* requires technology to facilitate the continuous production of large amounts of high quality parasitoids (Smith et al., 1990). For this reason, it is necessary to investigate three main areas in the future: 1) Development of an efficient and cost effective mass production system; 2) Characterization and improvement of parasitoid quality; and 3) Development of a release strategy to optimize field efficiency. Successful research in these areas will enable the use and commercialization of *Trichogramma* for use in the Chilean forestry sector.

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Biological Control of Pests Attacking Ornamental Foliage in Florida

L. S. OSBORNE

University of Florida
CFREC-Apopka
2807 Binion Road
Apopka, FL 32703-8504
lso@icon.apk.ufl.edu
(407) 884-2034
(352) 392-9359 FAX

Abstract. The tropical ornamental foliage industry in Florida is extremely diverse. Plants are grown in greenhouses, slat sheds and full sun. The acreage each company has in production can be as little as a half acre to more that 50. The plant materials range from crops grown for as little as six weeks in 3-inch pots to 50-foot trees grown in the ground for years. Some growers specialize in a few types of plants and others are generalists with over one hundred plant varieties.

Because of this diversity, implementing biological-control programs has many unique problems as well as opportunities. The impediments to developing these programs were discussed in Osborne et al. 1994. The factors that relate directly to this meeting would be the lack of control options for the diverse pest complex associated with this industry, the need for positive feedback when beneficials are released, and quality control for natural enemies.

Aphids, mealybugs, scales and thrips are disruptive to spider mite biological-control programs on ornamental foliage plants in many regions of Florida. Although there are references that document efficacy of both parasitoids and predators on these pests, effective natural enemies are either not available, available in limited quantities or prohibitively expensive. Mealybug control will be discussed to illustrate this issue.

Quality control of commercial natural enemies is a major concern to growers. They have lost confidence in biological control because they have experienced many failures. The failure of purchased natural enemies to establish and control target pest species is usually placed on pesticide residues. It is my belief that this is only a partial answer and in certain cases not the case. The failure of *Phytoseiulus persimilis* to establish any level of control a few years back was due to something innately wrong with the predator. A system is desperately needed that would allow growers to verify the quality of natural enemies. Ideas on how this might be accomplished will be presented.

Quality of the delivery system and technical help are other issues that need to be addressed in this industry. One major producer has hired a technical representative that works with the Florida industry. This program has done more to further implementation of this technology than anything else to date. Secondly, the producers must have information and products to deliver which are useful to the ornamental industry. The successful transfer of

technology developed to manage pests of vegetables in northern greenhouses to the diverse ornamental industry in Florida is filled with unique challenges and will require special attention by the natural-enemy producers.

Osborne L. S., F. L. Petitt, Z. Landa and K. A. Hoelmer (1994). Biological control of pests attacking crops grown in protected cultivation: the Florida experience. In: D. Rosen, F.D. Bennett and J.L. Capinera, [eds.] Pest Management in the Subtropics: Biological Control - A Florida Perspective. Intercept, Andover, England, U.K.

HOST REARING and ARTIFICIAL DIETS

Production of Cerambycid Eggs for Mass-Rearing of Parasitoids

M. SERRÃO[1]; L. BONIFÁCIO[2] and J. CAMPOS[2]

[1]SOPORCEL S.A. Forestry Research Center, Apartado 15,
2065 ALCOENTRE, PORTUGAL
[2]CELPA Pulp & Paper Assoc., Rua Marquês Sá da Bandeira n 74, 1 Esq,
1000 LISBOA, PORTUGAL

Abstract. The egg parasitoid *Avetianella longoi* (Hymenoptera: Encyrtidae) is believed to be specific to *Phoracantha semipunctata* (Cerambycidae: Phoracanthini) in Portugal, where no other species of Phoracanthini are known. With the aim of reducing damages caused by the borer (a severe pest in Ecalyptus plantations) programs of I&D have been implemented by the Association of Pulp and Paper Companies (CELPA). Augmentative release programs of *A. longoi* were initiated at the end of 1993. Mass-rearing *A. longoi* is complicated by its host specificity, and requires mass-production of *P. semipunctata* eggs. The authors present and discuss methods of producing *P. semipunctata* eggs the research that is being carried out. Also considered are artificial rearing methods, including diets for *P. semipunctata* adult production and the use of artificial eggs.

Introduction

The egg parasitoid *Avetianella longoi* (Hymenoptera: Encyrtidae), first found in Portugal in 1991 by Paiva et al. (1991) is believed to be specific to *Phoracantha semipunctata* (Cerambycidae; Phoracanthini). In Portugal there are no other species of Phoracanthini known.

With the goal of reducing damages caused by the borer (a severe pest in Eucalyptus plantations) we implemented an IPM program starting in 1992. The main component of this program is the augmentative release of *A. longoi*, in order to improve natural parasitism and reduce tree mortality. Preliminary mass-rearing studies began at the end of 1993.

Mass-rearing of *A. longoi* is complicated by its host specificity, and requires mass production of *P. semipunctata* eggs. Behavior and feeding requirements of *P. semipunctata* appear to be the main constraint of egg mass production (quantity and quality). Therefore, the objective of this work was to optimize methods of producing *P. semipunctata* eggs. This work was conducted in a small scale rearing facility and was implemented in two experimental field releases of the egg parasitoid during 1995 (manuscript in prep.).

Materials and Methods

The insects were kept in cylindrical 12 x 12 cm aluminum mesh cages with glass Petri dishes (15 cm) on top and bottom, using a sex-ratio of 5 females to 3 males. The bottom dish was lined with filter paper. An 8 cm glass Petri dish wrapped with filter paper held an inverted plastic feeding tube (10 ml) stopped with a cotton roll. Beetles readily oviposited

under the wrapped dish. Eggs were collected and dead adults were removed three times a week to reduce insect handling. Stress caused by handling has a negative influence on oviposition rhythm.

All of these experiments were conducted under similar environmental conditions:

photoperiod - 14L:10D
temperature - 25 ± 2 ºC
relative humidity - 60 ± 10%

Behavior Study

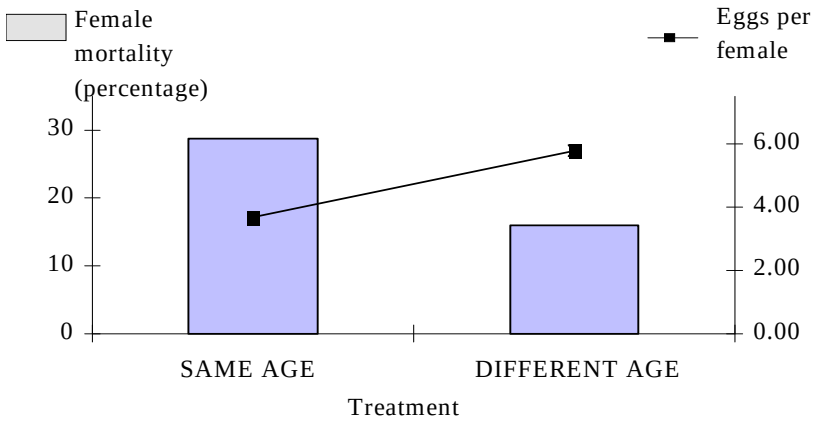
We have previously found that *P. semipunctata* oviposition can be increased by regularly recombining beetles, so that both sexes have novel mates. In this study, we examined the effect of adult age on oviposition rate. In the first treatment, beetles were recombined weekly within the same *date* of emergence ("SAME AGE" treatment); emergences occurring within a week period were considered to be of the same *date*. Dead beetles were not replaced, but the sex ratio was kept constant by reducing the number of cages. In the second treatment, different aged insects were recombined regardless of emergence date ("DIFFERENT AGE" treatment). In this treatment dead beetles were replaced with recently emerged insects. Insects were fed with a water solution of 5% sucrose. The treatment effect was examined by comparing weekly averages of the number of viable eggs (excluding dried or damaged eggs) and percentage of female mortality (Arcsine transformed; Zar, 1984). One Way Analysis of Variance (Minitab Inc.) showed significant differences between treatments for both variables:

Viable eggs

Source	DF	SS	MS	*F*	*P*
Treatment	1	12.77	12.77	8.14	**0.015****
Error	12	18.82	1.57		

Female mortality

Source	DF	SS	MS	*F*	*P*
Treatment	1	0.06791	0.06791	17.98	**0.001*****
Error	12	0.04531	0.00378		



Treatment	Female mortality (weekly percentage)		Eggs per female (weekly)	
	Average	SEMean	Average	SEMean
SAME AGE	28.733	0.013	3.67	0.67
DIFFERENT AGE	16.004	0.049	5.79	0.39

Feeding Study

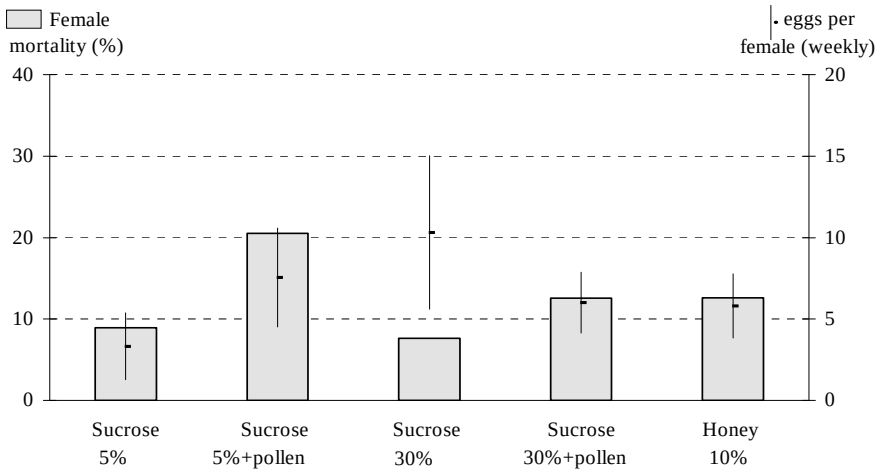
The purpose of the feeding study was to examine the influence of diet on the weekly production of viable eggs (excluding dried or damaged eggs) and percentage of female mortality. Five diets were tested: 5 % sucrose; 5 % sucrose with honey bee pollen; 30 % sucrose; 30 % sucrose with honey bee pollen; 10 % honey.

Honeybee pollen was applied to the cotton roll in the feeding tube, after filling it with diet. Insects from the laboratory colony were arbitrarily assigned to the different treatments. Dead beetles were replaced with recently emerged adults to maintain the original sex-ratio (5 f :3 m). Insects within the same treatment were recombined weekly. The treatment effect was examined by comparing weekly averages of the number of viable eggs and percentage of female mortality. Transformation failed to normalize data for viable eggs, so a non-parametric test was used (Mood Median Test; Minitab Inc.). The analysis showed that diet affected female mortality, but had no effect on viable egg production.

	Female mortality (percentage)				Viable eggs			
Diet	N<=	N>	Median	Q3-Q1	N<=	N>	Median	Q3-Q1
Suc. 5	10	2	0.050	0.050	9	3	0.00	1.88
Suc. 5+pollen	4	8	0.085	0.127	5	7	1.84	5.44
Suc. 30	10	2	0.000	0.050	6	6	1.13	6.45
Suc. 30+pollen	7	5	0.025	0.093	5	7	1.39	3.05
Honey 10	5	7	0.060	0.068	5	7	1.37	3.34

Chi-square = 10.69 df = 4 *P* = **0.031***

Chi-square = 4.00 df = 4 *P* = **0.407**



Treatment		Female mortality (weekly percentage)		Eggs per female (weekly)	
	N	Average	SEMean	Average	SEMean
Sucrose 5%	4	8.92	1.31	3.32	2.05
Sucrose 5% + pollen	4	20.50	2.01	7.54	3.04
Sucrose 30%	4	7.63	2.63	10.32	4.71
Sucrose 30% + pollen	4	12.56	5.21	6.00	1.87
Honey 10%	4	12.58	4.83	5.80	1.97

Discussion and Conclusions

Periodic mixing of different aged adults appeared to stimulate oviposition and reduce mortality.

The feeding study showed no significant diet effect on production of viable eggs, however the data indicate that increasing the concentration of sugar solution may improve oviposition. The significant diet effect on mortality was due to the 5% sucrose with pollen diet (mortality in the other treatments appeared to be similar).

We have applied the results of this study to improve the efficiency of *P. semipunctata* egg production by caging beetles of different ages and using a 30% sucrose diet. Unfortunately, the egg production rate was still insufficient for an economically viable mass production program for *A. longoi*. Currently we are investigating the possibility of developing artificial eggs for mass rearing *A. longoi*. However, this method may prove difficult due to the specificity of the parasitoid. Future research will also focus on improving the design of rearing cages and other aspects related mainly to oviposition stimulants.

Acknowledgements

We thank Drs. L. HANKS, J. MILLAR and T. PAINE, Department of Entomology, University of California, Riverside, for their valuable suggestions and discussion of rearing methodology. We also appreciate the helpful advice provided by Prof. CAROLA MEIERROSE, Department of Biology - University of Évora. This work would not have been possible without the technical assistance of ANA FERRO, and all technical staff of Forestry Protections Team of SOPORCEL - Forestry Research Centre. This work was supported by CELPA - Pulp and Paper Association.

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Procedures for Mass-Rearing the West Indian Fruit Fly *Anastrepha obliqua* (Macq.)

M. P. MORENO E., Y. GOMEZ S. and J. L. ZAVALA

Mexico Fruit Flies Complex. Apartado Postal 368, Tapachula 30700, Chiapas, Mexico.

Abstract. In order to maintain high levels of production of *Anastrepha obliqua* (Macquart), it was necessary to find an oviposition substrate that held up to mass rearing conditions and that kept the eggs in optimal conditions of humidity so as not to affect egg viability. After trying a variety of substrates, a cotton fabric commercially known as "tuzor" with 18 holes per lineal centimeter was decided on. This fabric is coated on both sides with a thin layer of transparent silicon. In the oviposition panel, the hydration of the eggs was maintained by applying 2% fusselleron over the silicon layer. A second bottle neck that needed to be addressed was the larval diet. The initial diet included texturized beets, but it was too granular and poorly assimilated by the larvae. Research on this part of the rearing process reslted in the development of a standard diet based on powdered cob, texturizer and Vanderzant^{MR} vitamins, in addition to the normal requirements for sugar, yeast, microbial inhibitors and water. Once the oviposition and diet problems were solved the current standard production process was established. This process has allowed us to keep the colony as a promising stock for massive release.

Introduction

With the goal of eliminating export restrictions to mango, citrus, caducipholious fruits, guava and other non traditional tropical commodities, as well as reducing the damage caused to these fruits by fruit flies, the Mexican National Campaign against Fruit Flies was undertaken. This campaign implied the building of a mass rearing facility in order to apply the Sterile Insect Technique (SIT), which would then be combined with integrated control strategies to reach control of the fruit flies of economic importance. The Campaign also involved active participation of fruit growers and State governments.

Among the species of economic importance that the Fruit Fly Mass Rearing Complex will produce at massive levels is *Anastrepha obliqua* (Macquart). At present a promising stock is available for large scale production. This paper describes the procedures used to, and advances that were made to, rear this species.

Colony

At present the reproductive colony is handled in Mission type cages with dimensions of 2.15 x 1.8 x 0.30 cm. The front and rear of the cage are covered with a mesh made of glass fiber having 49 holes per square centimeter. The center of the cage is covered with an oviposition sustratum, which is called the oviposition panel. Each cage houses 55,000 pupae, and with 90% emergence we obtain an average of 0.5 adults per square centimeter. This density has allowed us to increase egg production in spite of the recommendation by Moreno

(1993) that the maximum density per cage must not exceed 0.2 adults per square centimeter in cages of 30 x 30 x 30 cm^2 (5,400 cm^2).

In searching for an oviposition substrate that satisfied handling and production needs for mass rearing, and at the same time allowed the eggs to be kept well hydrated until the collection time, several bioessays were made. The first of the assessed substrates was parafilm paper (American Company^{MR}). Although at the beginning it appeared reasonable for egg production, it had the disadvantage that in order to keep the eggs hydrated it was necessary to constantly use an ultrasonic humidifier. This electronic device was not manufactured for this specific purpose and it frequently failed, which had a direct negative impact on egg quality. In addition, parafilm was a very fragile sustrate to work with and had to be replaced after only 5 days of use (the average number of days eggs are collected from an oviposition cage is 12 to 15 days), which was too costly for mass rearing use.

Several types of synthetic fabrics were later assessed (commercially known as organza, shiffon, etc.) that had 50 to 60 holes per linear centimeter. To these fabrics a thin layer of silicon was applied on both sides. Once the oviposition panel was placed on the cage several applications of 25 fusselleron were added to keep the eggs hydrated. Results of these experiments were not encouraging because the fusselleron did not adhere well to the fabrics and because the flies did not oviposit well through the small holes.

Later, the research laboratory in Weslaco, Texas, USA, reported that they found that a fabric with 16 to 18 holes per linear centimeter gave better results than oviposition into domes with natural red color paraffin (Moreno, 1994). As a result, a cotton fabric with 18 holes per linear centimeter was adapted to the vertical panel in the Mission type cages. This set up produced encouraging results and surpassed those obtained with other fabrics.

The current fabric is called "tuzor" and it is coated with a thin layer of silicon on both sides. This allowes for a uniform application of fusseleron, which in turn keeps the eggs hydrated. Finally, considering that colors yellow and green are the best attractants for *A. ludens* (Robacker et al., 1990), we assessed the response of *A. obliqua* to green, yellow, white and clear green silicon panels. The best results in egg production were obtained using clear green panels. With this color panel the egg production levels shown in in Figure 1 were obtained.

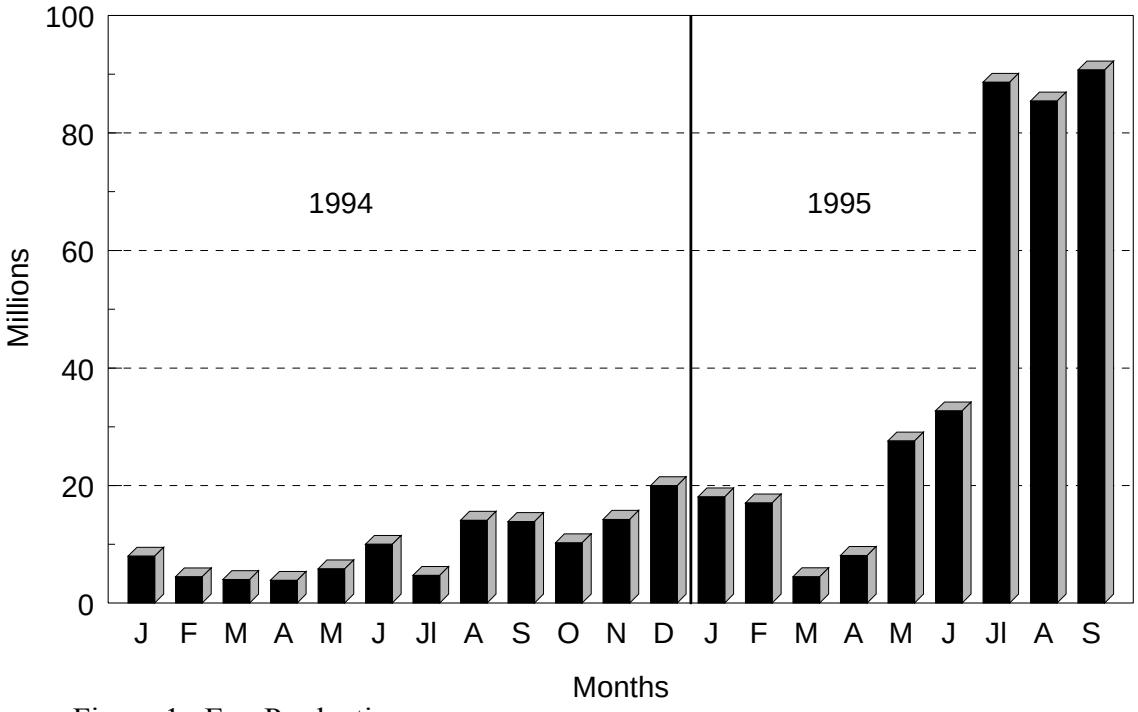

Figure 1. Egg Production.

Egg Incubation

To determine the adequate process for egg incubation several methods were evaluated. First, the eggs were placed on a moist piece of fabric within Petri dishes that were placed inside containers made of styrofoam and kept at 26-27ºC to favor hatching at the seeding time. However, since these containers were not hermetically sealed, gradual dehydration of the eggs over time was a problem. To solve this problem a light layer of fusseleron was placed on the fabric. Unfortunately, this set up was also a favorable medium for the development of microorganisms and contamination reduced egg hatch. In addition, as production increased the handling of these containers became inoperative. This generated the idea for developing a bubbling system by means of pumps for fish with a semi-industrial capacity. Eggs were collected in water and held in bottles bubbled with air at a constant temperature of 26-27ºC. Eggs were held like this for 48 hours before they were seeded onto diet. This system also made it feasible to disinfect the eggs prior to diet seeding, which improved egg-larval viability.

Diets

Knipling (1979), Guerra et al. (1984) and other authors state that for mass rearing purposes the insects have to be produced with the minimum of material resources, manpower and time that doesn't sacrafice the quality of the final product.

Originally, *A. obliqua* larvae were reared on a diet based on texturized beet, but as it was not operative for mass rearing the search for a better alternative was started. We started with a diet of gel texture that follows the formula of the Weslaco, Texas, USA, laboratory (Moreno et al., 1993). This diet is completely rich in nutrients for insects, although it has the disadvantage of a high cost and it requires more handling during its preparation. In order to lower costs a new diet was tested; it was based on cob powder as texturizer, sugar as a carbohydrate source, torula yeast as a protein source, microbial inhibitors, and Vanderzant^{MR} vitamins as insect fortifiers. After many trials a standard formula was developed that resulted in a good quality fly being produced.

Since this time, the *A. obliqua* colony has received special care since all the biological material is returned to the reproductive colony. Liedo and Carey (1994) recommend that special care must be taken in the rearing of flies that form the reproductive colony to minimize adverse effects on the quality of the flies used for release.

Larvae

The percentage of larval recuperation reflects the rearing efficiency from egg-larva in any insect mass rearing process. At the beginning of these studies there were problems in reaching high production levels that were related to egg collection and the non operativity of the larval diet; however, in mid 1994 larval recuperation started to become more consistent and the colony stock slowly became adapted to the mass rearing conditions. Figure 2 shows the increase in larval production obtained.

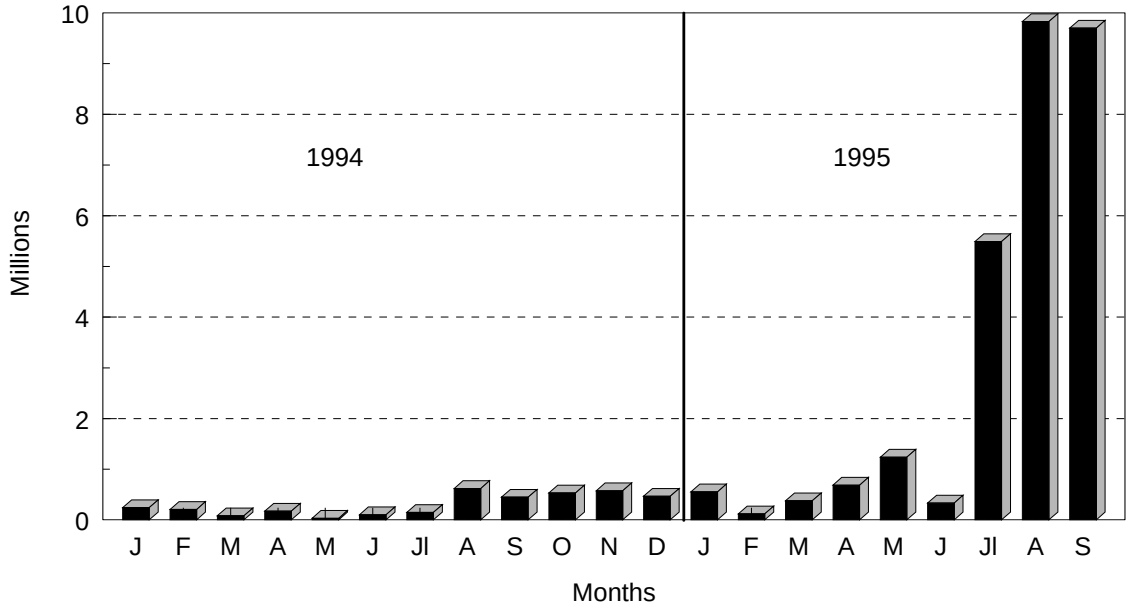

Figure 2. Larval Production.

Pupae

The transformation of larvae to pupae has always been 85-90%, which undoubtedly reflects a high larval quality. Figure 3 shows monthly pupal production levels, which have allowed us to keep a constant number of cages in the colony; this fact, together with the increase in the transformation from egg-larva will allow us to maintain mass production levels.

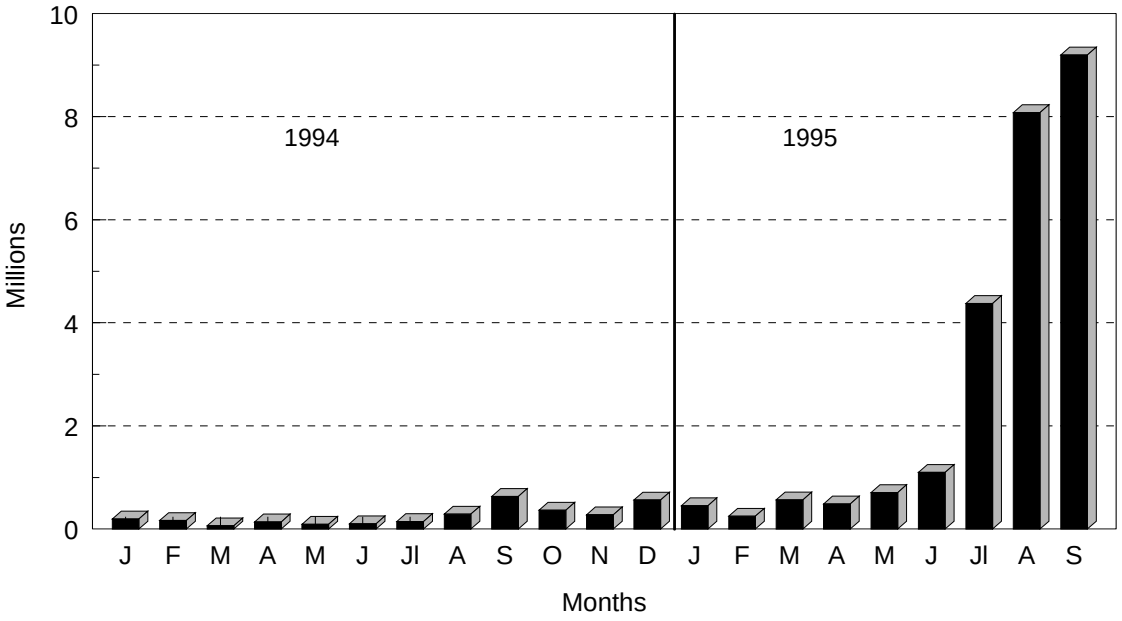

Figure 3. Pupal Production.

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Influence on Quality Related Characteristics of Rearing *Trichogramma spp.* in Hosts of Different Size or on an Artificial Diet

D. A. NORDLUND[1], S. M. GREENGERG[2], and Z. X. WU[3]

[1]USDA, ARS, Biological Control and Mass Rearing Research Unit, Mississippi State, Mississippi 39762, USA; [2]Texas Apicultural Experiment Station, Texas A&M University System, Weslaco, Texas 78596, USA; and [3]Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, Mississippi 39762, USA

Abstract. The quality of *Trichogramma* spp., in terms of effective performance in the field, is dependent on many factors including fecundity, longevity, and ability to locate and oviposit in the target host. Increased longevity and fecundity are influenced by the size of the adult female. With recent advances in the development of *in vitro* rearing techniques for these important parasitoids, there is a need to increase our understanding of quality, indicators of quality, and those rearing factors that contribute to improved quality. We found that the size of the host influences the size (body length) for *Trichogramma pretiosum* and *T. minutum* females and that body length in these species influenced the number of eggs produced by a *Trichogramma* female. We also found that the size of the rearing host can influence the percentage of stretched plastic artificial eggs that the females will oviposit in. *T. minutum* females reared on an artificial diet in wax artificial eggs were equal in size to females reared in *Manduca sexta* eggs, oviposited in a greater percentage of stretched plastic artificial eggs and deposited more eggs per oviposition arena than did those reared in *Helicoverpa zea* or *Sitotroga cerealella* eggs.

Overview of Artificial Rearing of Parasitoids and Predators: History, Current Status, and Prospects for the Future

P. GREANY[1] and S. GRENIER[2]

[1]USDA ARS Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL
[2]Laboratoire de Biologie Applique, INRA, INSA, Lyon, France

Abstract. The desire to rear insect parasitoids and predators on artificial diets for use in augmentative biological control has existed for many years. This is now a possibility for many species of egg endoparasitoids, some species of larval and pupal ectoparasitoids, and numerous predators due to significant advances made during especially the past 20 years. Less success has been had with *in vitro* culture of larval endoparasitoids, except for some endoparasitic tachinids. In a few cases, levels of production potentially suitable for commercial use have been attained. The use of artificial diets also provides unique opportunities for basic studies on the physiology and behavior of beneficial insects, for example by enabling experiments on parasitoids free from confounding interactions with the host. Some of the requirements for *in vitro* development of entomophagous insects are very specific, and remain unidentified. Accomplishments to date will be reviewed, and remaining research gaps will be identified. We will address the need to scale up production through development of efficient, mechanized systems, and to evaluate the efficacy of artificially-reared natural enemies relative to their naturally-reared counterparts.

QUALITY CONTROL
for STERILE INSECT TECHNIQUE

Evaluating the Quality of Released Sterile Codling Moths (*Cydia pomonella*)

S. BLOEM[1], A. L. KNIGHT[1] and K. A. BLOEM[2]

[1]USDA/ARS Yakima Research Laboratory, Yakima, Washington, USA
[2]Okanagan-Kootenay Sterile Insect Release Program, Osoyoos, British Columbia, CANADA

Abstract. The dispersal activity of both male and female sterilized codling moths was evaluated with the use of a grid of passive interception traps in three apple orchards. The flight activity of moths (1) reared under constant temperature and released after 12 hours of handling, (2) collected from a colony reared under fluctuating temperatures and released after 12 h or 36 h, and (3) collected from a colony that had been induced into diapause and released after 12 h, were compared in May and July. In general, moths released after only 12 hours of storage performed better than those stored for 36 hours. Moths reared through diapause out performed normal colony reared moths. The importance of these data to the implementation of an area-wide SIR program will be discussed.

Handling: A Forgotten Factor in Quality Control

K. A. BLOEM[1], L. S. FIELDING[1] and S. BLOEM[2]

[1]Okanagan-Kootenay Sterile Insect Release Program, Osoyoos, British Columbia, CANADA
[2]USDA/ARS Yakima Research Laboratory, Yakima, Washington, USA

Abstract. The Okanagan-Kootenay Sterile Insect Release Program's mass-rearing facility for codling moth eradication in British Columbia was designed with a production goal of 5.25 million insects per week. The current weekly production fluctuates between 7-10 million. Laboratory quality control tests and comparisons with published standards for both laboratory-reared and wild moths for parameters such as percent egg hatch, pupal and adult weights, and adult longevity indicate that product quality is excellent. Selection for good flight ability has been designed into the adult collection system. Unfortunately, field release-recapture tests indicate that storage, packaging, transportation and release procedures are decreasing the performance of the moths (as measured by trap captures) by 25-50 % over that seen when moths are released into the field directly from collection bins with minimal handling.

Field Evaluation of Quality for the Melon Fly Eradication Project in Japan

H. NAKAMORI

Okinawa Prefectural Agricultural Experiment Station, Naha, Okinawa 903, JAPAN

Abstract. Estimating the hatching rate of eggs oviposited by wild flies during the early to middle stages of a sterile release program (SIT) is a useful way of evaluating the effectiveness of sterile flies. We assessed the hatching rate of eggs from wild female flies captured with a sweep-net on Miyako Island where sterile flies were being released and wild flies were abundant. Field surveys were conducted in June, August and October 1985. The hatching rate of eggs oviposited by these flies was less at all sampling sites and times than it was for eggs oviposited by females captured in a non-release area (= control: Itoman-shi located in the Okinawa Islands) [except for the station Shimoji 1 in October (100%)]. The average hatch rate in each month was June: 30.5%; August: 35.3%; and October: 33.5%. These results suggest that:

1) The number of sterile flies being released was insufficient for the area where the population of wild flies was dense.
2) The reproductive rate of wild flies could be reduced by approximately 70%, since the rate of egg hatch did not increase during the assessment period.
3) The number of released flies was insufficient to reduce egg hatch to 0.

For successful application of SIT for eradication of a pest, it is essential to assess the quality of the sterile insect with respect to their reproductive behavior. Sexual competitiveness, which measures insect quality relative to that of wild males, reflects the accumulative effects of the fly strain, the mass-production process, and the exposure to gamma ray irradiation. Using the field survey data on egg hatchability and the M/U ratio (number sterile flies marked with fluorescent dye/number unmarked wild flies), we estimated the competitiveness value (C) for sterile flies as follows, Haisch (1970):

$$C = (Hn-Hc)/(Hc-Hs) \times Fc/(Fn-Fc) \qquad (1)$$

where Hn is the rate of eggs hatch in the non-release (control) area, Hc is the rate of egg hatch in the release area, Fn is the oviposition rate of females in the non-release (control) area, and Fc is the oviposition rate in the release area. Hn and Fn were obtained from females collected at the non-release site, Itoman-shi, Okinawa Island. Hc and Fc were obtained from females collected at Shimoji-cho, Miyako Island. In equation (1), Fc/(Fn-Fe) is the reciprocal of M/U as estimated from the oviposition data of females from both control and release areas. In estimating C:

1) We assumed that the age distribution of females collected at the treated and control were similar thus mating rates in both areas was similar. With this assumption, the oviposition and hatch rates could be compared between areas.

2) We did not calculate competitiveness if the M/U ratio differed much between the release and non-release sites.
3) The period during which the eggs were collected at the release and non-release site were the same.
4) We did not estimate competitiveness if the data were estimated from small sample sizes.

The estimated values for C ranged from 0.206 to 0.651. They were lower than the 0.8 estimated during the earliest stage of the eradication program at Kume Island, but they were higher than the 0.2 estimated just before eradication. From these results, we adopted a value of 0.4 for C as the quality control value for mass produced flies in our sterile release program and for the maintenance program thereafter.

Changes in Male Medfly Courtship Duration and Behaviour Associated with Crowding (Diptera: Tephritidae)

R. D. BRICENO[1] and W. G. EBERHARD[1, 2]

[1]Escuela de Biologia, Universidad de Costa Rica
[2]Smithsonian Tropical Research Institute, Balboa, Panama

Abstract. Pre-mounting courtship by male medflies was shorter under conditions of greater crowding. The courtship behaviour of males of three strains that had been raised under highly crowded conditions in mass-rearing facilities for approximately 75, 190, and 236 generations was shorter than that of wild males under conditions of equal crowding. Courtship was shorter in one of the two older strains than in the younger strain. Duration of head rocking showed greater differences than did wing buzzing or wing vibration. Head rocking was most often omitted by one old mass-reared strain, and least often omitted by wild flies. Shorter courtships are probably advantageous for males in crowded conditions because they reduce the likelihood of the courtship being interrupted by other flies. Abbreviated male behavior rather than earlier female acceptance was apparently responsible for shortened courtships: a) males of a mass-reared strain paired with wild females performed short courtships; b) reductions in courtship duration associated with crowding and mass-reared strains occurred both in courtships leading to mounting attempts and in unsuccessful courtships; c) female behaviour patterns associated with courtships that led to mounting occurred earlier under more crowded conditions, but apparently had no triggering effect on early male mounting behavior. This study documents the persistence of heritable variation in male courtship behavior in a wild population with relatively low genetic variability, and demonstrates the genetic assimilation of facultative variations in courtship behavior.

APPENDIX

IOBC Conference Participants
Santa Barbara, California, October 1995

Franz Bigler
Research Entomologist
Swiss Federal Research Station for Agronomy
Zurich 8046
SWITZERLAND

Beth Black
Beneficial Insectary
60 Taggart Crt.
Guelph Ontario
CANADA N1 6H8

Jake Blehm
Buena Bio Systems
PO Box 4008
Ventura, CA 93007

Ken Bloem
Program Coordinator
Okanagan-Kootenay Sterile Insect
Box 1080
Osoyoos, B.C.
CANADA V0H 1V0

Stephanie Bloem
Research Entomologist
USDA-ARS
Agriculture & Agri-Food Canada
Summerland Research Station
CANADA V0H 1Z0

Eliana Borges
Certified Quality Systems Lead Auditor
InterQual
1727 State St.
Santa Barbara, CA 93101

Regina Burger
Landi Basel
fenaco
Lyon-Str. 18 Dreispitz
4053 Basel
SWITZERLAND

Maclay Burt
Ex. Director
ANBP
10202 Cowan Hts. Dr.
Santa Ana, CA 92705

Daniel Cahn
President
Bunting Biological, North America
PO Box 2430
Oxnard, CA 93034

Carrol Calkins
Research Entomologist/Research Leader
USDA, ARS
3706 W. Nob Hill Blvd.
Yakima, WA 98902

Kent Daane
Asst. Spec.
Center for Biological Control, UC Berkeley
KAC
9240 S. Riverbend Ave.
Parlier, CA 93648

Patrick De Clercq
Laboratory of Agrozoology
University of GENT
Coupure Links 653
B-9000 Gent
BELGIUM

Jorge Diaz
Mexican Nat'l. Campaign Against Fruit Flies
9a Avenida Sur #5
CP 30700
Tapachula, Chiapas
MEXICO

Dennis Edwards
Research Manager
Novo Nordisk
1497 Drew Ave.
Davis, CA 95616

Donald Elliott
Managing Director
Applied Bio-Nomics Ltd.
11074 West Saanich Rd.
Sidney, BC
V8L 5P5 CANADA

Yuging Fan
Research Entomologist
"The Land", Epcot
PO Box 10,000
Lake Buena Vista, FL 32830

Lisa Forster
SRA
Dept. of Entomology
University of CA
Riverside, CA 92521

John Freeman
Manager
Sespe Creek Insectary
1400 Grand Ave.
Fillmore, CA 93015

Rick Frey
Arbico Inc.
18701 N. Lago del Oro Pkwy
Tucson, AZ 85737

Greg Gaffney
President
Bio Ag Services, Inc.
4218 W. Muscat
Fresno, CA 93706

Carol Glenister
President
IPM Laboratories Inc.
Main Street
Locke, NY 13092-0300

Elizabeth Gordon
BioLogic Co.
Springtown Rd.
PO Box 177
Willow Hill, PA 17271

Angela Hale
Nature's Alternative Insectary Ltd.
Box 19 Dawson Rd.
Nanoose Bay, BC
CANADA V0R 2R0

Christopher Hale
Nature's Alternative Insectary Ltd.
Box 19 Dawson Rd.
Nanoose Bay, BC
CANADA V0R 2R0

Man-Wi Han
Department of Entomology
University of California
Riverside, CA 92521

Norihide Hinomoto
Research Scientist
National Institute of Sericultural & Entomol. Science
Laboratory of Natural Enemy Breeding
Tsukuba, Ibaraki
305 JAPAN

Tomoko Iwasa
Kubota Corp.
5-6, Koyodai
Ryugasaki, Ibaraki
301 JAPAN

Kenjiro Kawasaki
Chief of Laboratory
National Institute of Sericultural & Entomol. Science
Laboratory of Natural Enemy Breeding
Tsukuba, Ibaraki
305 JAPAN

Mary Jane Kelleher
Health Canada
Pest Management Regulatory Agency
Nepean Ontario
K1A 0Y9 CANADA

Jun Kurihara
Tomen Corp.
14-27 Akasaka 2 Chome
Minato-Ku, Tokyo
JAPAN

Norm Leppla
Associate Director
USDA/APHIS/National Biological Control Institute
4700 River Road, Unit 5
Riverdale, MD 20737-1229

Jose Lopez
Mexican Nat'l. Campaign Against Fruit Flies
9a Avenida Sur #5
CP 30700
Tapachula, Chiapas
MEXICO

Robert Luck
Professor
Dept. of Entomology
University of CA
Riverside, CA 92521-0314

Keith Mason
MicroBio
56 Church St.
Thriplow
UNITED KINGDOM S98 7R5

John McLaughlin
Research Entomologist
USDA/ARS
17053 Shafter Ave.
Shafter, CA 93263

Carola Meierrose
Professora
Universidade de Evora
Departamento de Biologia
Apartado 94
7001 Evora Codey
PORTUGAL

Lee Merrill
President
M&R Durango, Inc.
PO Box 866
Bayfield, CO 81122

Rick Miller
Biosys
10150 Old Columbia Rd.
Columbia, MD 21042

Kazuki Miura
Lab. of Insect Pest control
Chugoku National Ag. Expt. Station
Fukuyama 721
JAPAN

Hiroaki Nakamori
Chief of Laboratory
Okinawa Pref. Agric. Exp. Stan.
4-222 Sakiyama-cho
NAHA
Okinawa 903
JAPAN

Brent Nelson
Rincon-Vitova
PO Box 1555
Ventura, CA

Steve Nelson
Plant Sciences
342 Green Valley
Watsonville, CA 95076

Len Nunney
Professor
Dept. of Biology
University of California
Riverside, CA 92521

Izumi Ohta
Researcher
Chugoku National Agricultural Experiment Station
6-12-1, Nashifukatsu
Fukuyama
721 JAPAN

Kevin Olsen
Mgr. - Technical Services
S&J Ranch
PO Box 3347
Pinedale, CA 93650

Libby Ouellette
Insectary Supervisor
Oxnard Pest Control
PO Box 1187
Oxnard, CA 93032

Phil Phillips
Area IPM Advisor
UC Cooperative Extension
669 County Square Dr., #100
Ventura, CA 93003

Noah Poritz
R&D Director
Biological Control of Weeds
1418 Maple Drive
Bozeman, MT 59715

Naomi Pye
BioLogic Co.
Springtown Rd.
PO Box 177
Willow Hill, PA 17271

W. J. Ravensberg
Head, Entomology Dept.
Koppert B.V.
Vellingweg 17
PO Box 155
2650 AD Berkel on Rodenrijs
THE NETHERLANDS

Richard Ridgway
USDA/ARS/BA/PSI/IBL
Bldg. 402, Barc-East
Beltsville, MD 20905

John Rogers
Buena Bio Systems
PO Box 4009
Ventura, CA 93007

Matthew Royer
Chief Operations Officer
USDA/ARS/PPQ
4700 River Road, Unit 133
Riverdale, MD 20737-1236

Glenn Scriven
President
Association of Natural Biocontrol Producers
425 La Cadena Dr. West #12
Riverside, CA 92501

Sen Selvan
Ecogen
2005 Cabot Blvd. West
Langhorne, PA 19047

Alvaro Sequeira
Buena Bio Systems
PO Box 4009
Ventura, CA 93007

Miguel Serrao
Professor
Universidade de Evora
Departamento de Biologia
Apartado 94
7001 Evora Codey
PORTUGAL

James Smith
Div. of Phytosanitary Protection
Bioforest S.A.
Edificio Prales of 410
Valdivia
CHILE

Ross Smith
President
Activated Biological Control LLC
4312 Dwight Rd.
Elk Grove, CA 95758

Shimon Steinberg
Biological Control Industries
Sede Eliyahu
Mobile Post Bet Shean Valley
10810 ISRAEL

Peter Stephen
Research Technician
Outspan International
Outspan Citrus Centre
P.O. Box 28
Nelspruit 1200
SOUTH AFRICA

Clive Stinson
Ciba Bunting
Buntin Biological North America
P.O. Box 2430
Oxnard, CA 93034

Bruce Tate
Research Technician
Outspan International
Outspan Citrus Centre
P.O. Box 28
Neispruit 1200
SOUTH AFRICA

Don Vacek
Supervisory Geneticist
USDA/APHIS
Mission Biological Control Center
PO Box 2140
Mission, TX 78573

Joop Van Lenteren
Professor
Dept. of Entomology
PO Box 8031
6700 EH
THE NETHERLANDS

Jeroen Van Schelt
Koppert B.V.
Vellingweg 17
PO Box 155
2650 AD Berkel on Rodenrijs
THE NETHERLANDS

Jan Verineulen
Production Manager
Biobest NV
Ilse Velden 18
Westerlo
2260 BELGIUM

Jo Ann C. Wheatley
Professor
Cal Poly State University
Crop Science Department
San Luis Obispo, CA 93407

IOBC Conference Participants
Cali, Colombia, March 1998

Mohamed Samir Abbas
Plant Prot. Res. Institute
Dept. of Biological Control
Nadi El-Said Str., Dokki, Cairo
EGYPT
Tel.: 383-8698 - 573-3482
Fax: 383-8698

Alessandra Arzone
Dipartamento di Entomologia e Zoologia
Applicate all'Ambiente, Via L. Da Vinci
44-10095 Grugliasco – Torino
ITALIA
Tel.: 0039-11-6708532
Fax: 0039-11-6708586
e-mail: arzone@mbox.sicap.it

Tom R. Ashley
USDA, Aphis, IS
P.O. Box 3149
Laredo, TX 78044
Tel.: 52-961-414-33
Fax: 52-961-414-34
e-mail: art1941@acnet.net

Alias Awang
Malaysian Cocoa Board
P.O. Box 60079, 91010 Tawau, Sabah,
MALAYSIA
Tel.: 089-761722/761840
Fax: 089-761050

Cesar Basso
Faculted de Agronomia
Catedra de Entomologia Av. Garzon 780
12900 Montevideo
URUGUAY
Tel.: 598 (2) 309-7191
Fax: 598 (2) 309-30-04
e-mail: cbasso@eprove.edu.uy

Luz Adriana Lastra Borja
Cenicada, A.A. 9138
Cali, COLOMBIA
Tel: 57 (2) 6648025
Fax: 57 (2) 6641936
e-mail: cenicana@cali.cetcol.net.co

Denis Bouchard
Bio-controle 2300, Sherbrooke est., # 400
Montreal Qc H2K 1E5
CANADA
Tel.: (514) 528-9232 - 528-6849
e-mail: dboud@odyssee.ne

Regina Burger
LANDI Basel, Fenaco, Lyonstr. 18, CH-4053 Basel,
SWITZERLAND
Tel.: (41) 61-338-4040
Fax: (41) 61-338-4060

Alex E. Bustillo P.
Cenicafe, Apartado Aereo 2427
Manizales, COLOMBIA
Tel.: (968) 5065550

Maida Mollineda Caballero
Instituto de Investigaciones de Sanidad Vegetal
Calle 110 N^{o} 574 el 5ta B y 5 5ta F.
Miramar Playa C. Habana
CUBA
Tel.: (537) 22-2510-16
Tel. (home): 40-8777
Fax: (537) 24-0535 or 22-9366
e-mail: inisav@ceniai.inf.cu

Ludovit Cagon
Dept. Plant Protection, University of Agriculture
Lomonosovova 2, 94976 Nitra,
TSCHECHOSLOWAKEI

Jose L. Martinez Carrillo
CIFAPSON, CE - Valle del Yaqui
Apdo. Postal N^{o} 515, CD. Obregon
Son. C.P. 85000, MEXICO

Achille Casale
Dipto. Zoologia Univers., Via muroni 25
07100 Sassari, ITALY
Tel.: (79) 228661
Fax: (75) 228665

CIAT
Centro Internacional de Agricultura Tropical
A.A. 6713, Cali, COLOMBIA
Tel.: 57 (2) 445-0000
Bernardo Arias, e-mail: barias@hotmail.com
Ana Milena Caicedo V., e-mail:
amcaicedo@hotmail.com
Adriana Ortega G., e-mail: adriana@hotmail.com

Diego Fernando Monera, e-mail:
diegofdo@hotmail.com
Elsa Liliana Meb, e-mail: elmelo@hotmail.com
Elsie Glodys Burbano, e-mail:
egburbano@hotmail.com
Maria del Rosavio Manzano

CORPOICA - Corporacion Colombiana de
Investigacion Agropecuaria
Via Penitenciaria Palmira, A.A. 1301
COLOMBIA
Tel.: 57 (2) 2758161/66
Fax: 57 (2) 2714400
Aristebulo Lopez-Avila - Bogota, e-mail:
alopez@corpoica.org.co
Fulvia Garcia Roa - Palmira (Valle), e-mail:
fulviaga@telesat.com.co
Ana Elizabeth Diaz M. - Palmira (Valle), e-mail:
anadiaz@telesat.com.co
Carlos Alberto Vargas - Palmira (Valle)
Gustavo Trochez - Palmira (Valle)
Ana Teresa Mosquera E. - Palmira (Valle)
Adolfo Trochez – Palmira
Alberto Soto - Monteria, e-mail:
corpoica@monteria.cetcol.net.co
Guillermo Leon M. - Villavicencio (Meta), e-mail:
Guillermoleon@hotmail.com.co

Jorge Cancino Diaz
Segunda Avenida Sur # 5 Altos
30700 Tapachula Chiapas
MEXICO
Fax: 91 (962) 6-4955

Ana Lucia Alvarez Fernandez
Departamento de Control Biologico
Ministerio de Agricultura y Ganaderia
Apartado 10094 - 1000, COSTA RICA
Tel.: (506) 260-8291
FAX: 260-8301
e-rnail: protagro@sol.rcsa.co.cr

Francisco Ferrer
Servicio Biologica Carreta 5
N^{o} 4-76 URB del Este
Barquisimeto, VENEZUELA
Tel.: 0058-51-316-253
Fax: 0058-51-514318

Patricia Garcia
Dep. de Biologia, Universidade dos Acores
R. Mae de Deus, 9500 Ponta Delgada - Acores,
PORTUGAL
Tel.: 351 (96) 652089
Fax: 351 (96) 652044
e-mail: patricia@alf uac.pt

Marcos Gerding
P. & Ing. Agronomo Ms.
Instituto de Investigaciones, Agropecuarias
Estacion Experimental Quilamapu
Avda. Vicente Mendez 515 Casilla 426
Chillan, CHILE
Tel: (02-56) 42-211177 / 42-217852
e-mail: mgerding@quilamapu.inia.cl

Silvia Monteil Gomes
Escola Superior de Agri. “Luiz de Queiroz” Esalq
Universidade de S. Paulo (ESALQ/USP)
Depto. de Entomologia
CxP. 9, 13418-900 Piracicaba
S. Paulo, BRASIL

Luis Gomez
Cenicada, A.A. 9138, Cali, COLOMBIA
Tel: 57 (2) 6648025
Fax: 57 (2) 6641936
e-mail: lagomez@cenicana.org

Patrick Greany
USDA - ARS, 1700 SW 23 Dr.
Gainesville, FL 32608
Tel.: 352-314-5736

Simon Grenier
Institut National des Sciences Apliquees de Lyon
Laboratoire de Biologie Appliquue
20, Avenue Albert - Einstein Bat. 406
F-69621 Villeurbanne Cedex, FRANKREICH
Tel.: 472-438356
Fax: 472-438534
e-mail: sgrenier@jouy.inra.fr

Francisca Nemaura Pedrosa Haji
EMBRAPA Semi-arido, BR 428
Zona Rural, C. P. 23
CEP: 56300-000 Petrolina PE, BRASIL
Tel.: (081) 862-1711
Fax: (081) 862-1744
e-mail: nemaura@cpatsa.embrapa.br

S. A. Hassan, BBA
Institut fur biologischen Pflanzenschuzts
Heinrichstr. 243, 64287 Darsmstadt, GERMANY
Tel: 0049-6151-407223
Fax: 0049-6151407290
e-mail: Hassan.bba@t-online.de

Yolanda Gutierrez Hernandez
Incauca S.A., A.A. 6705, COLOMBIA
Tel.: (57-2) 8854545
Fax: (57-2) 8830360
e-mail: hcalderon@incauca.com

Jades Jimenez V.
Carrera 29 N^{o} 35-51 A.A.
1015 Palmira - Valle, COLOMBIA
Telefax: 22733719

Tancik Jonbo
Dept. Plant Protection, University of Agriculture
Lomonosovova 2, 94976 Nitra,
TSCHECHOSLOWAKEI

H. D. Kaushik
CCS Haryana Agricultural University
HISAR - 1250004, INDIEN
Tel.: 0091-1662-35880
Fax: 0091-1662-34952
e-mail: hau@hau.ren.nic.in

R. Kfir
Plant Protection Research Institute
Priv. B. X 134
Pretoria 0001 SUD-AFRIKA
Tel: +27-12-329-3269
Fax: +27-12-329-3278
e-mail: rietrk@plant2.agric.za

Norman Leppla
1641 Edgewater
Mount Dora, FL 32757-6944
Tel.: (352) 735-9715

Alejandro Madrigal C.
Universidad Nacional de Colombia Sede Medellon
Apartado Aereo 3940
COLOMBIA
Tel.: 421-9070
Fax: 260-4489
e-mail: amadriga@perseus.unalmed.edu.co

Juan Antonio Alemon Martinez
Ave. 57 # 4416 Of. 44 y 46 Playa
Ciudad Habana, CUBA
e-mail: censa@ceniai.inf cu

Hernando Mejoa
Probiocol, Calle 5 # 6 A 132, Zarzal - Valle,
COLOMBIA
Tel.: 220-7006
Fax: 220-8918

Luis Mario Mejoa
Pribiocol Zarzal - Valle, Calle 6^{a} # 14-54,
COLOMBIA
Tel.: 220-6948
Fax: 220-8918

Francois Meloche
Plant Research Centre, Ottawa, Ontario, K1A 0C6
CANADA
Tel.: (613) 759-1615
Fax: (613) 952-9295
e-mail: ottgw::In%“melochef@ncccot.agr.ca”

Juan Carlos Monje
Universitat Hohenheim - Institut f. Phytomedizin
70593 Stuttgart, GERMANY
Tel.: 49-711-4592398
Fax: 49-711-4592408
e-rnail: jcmonje@uni-hohenheim.de

Carlos Gomez Munoz
Institute Colombiano Agropecuario
A.A. 233, Palmira, COLOMBIA
e-mail: caengo@hotmail.com

Manuel Amaya Navarro
Carrera 4 # 4-69, Buga, Valle del Cauca,
COLOMBIA
Telefax: 57 (22) 366248

Ru Nguyen
DACS-DPI, P. O. Box 147100
Gainesville, FL 32614
Tel.: 352-372-3505
Fax: 352-955-2301
e-mail: runguyen@juno.com

Donald A. Nordlund
USDA, ARS, BCMRRU
P.O. Box 5367
Mississippi State, MS, 39762
Tel: 601-323-2230
Fax: 601-303-0476
e-mail: onn@ra.msstate.edu

Marco V. Neira Oviedo
Pontificia Universidad Catolica del Ecuador
Casilla 17-11-5157
Quito, ECUADOR
Tel.: 593-2-467-832
e-mail: mneira@uio.satnet.net

Jorge Pena
University of Florida
Institute of Food and Agricultural Sciences
Tropical Research and Education Center
18905 SW 280 Street, Homestead FL 33031
Tel.: (305) 246-6340
FAX: (305) 246-7003
e-mail: jepe@gnv.ifas.ufl.edu

Fernando Ponce
Riobamba, Escuela Radiofonicas Populares,
ECUADOR
Fax: 964946 / 961625

S. Prozell
BBA Vorratsschutz, Konigin - Luise-Strasse 19
14195 Berlin, GERMANY

Marco A. Puente
Carrera 62 Bis 6 A 78
Cali, COLOMBIA
Telefax: 5518362

Jorge Mejoa Quintanilla
Universidad de Cordoba - Km. 4 Via a Cereto;
Monteria,
Cordoba, COLOMBIA
Tel.: (947) 860111

Carlos Alberto Rodas
Smurfit Carton de Colombia
Apartado Areo 219
Cali, COLOMBIA
Tel.: (57-2) 4425800 or 6694073
Fax: (57-2) 6694073

Genny Rojas
Inbeagro, Carrera 26 A # 58-09
Palmira, COLOMBIA
Tel./Fax: (57-2) 2754009
e-mail: casaaved@pino.univalle.edu.co

Rodrigo A. Vergara Ruiz
Universidad Nacional Sede Medellon
COLOMBIA
Tel.: (94) 2607333
Fax: (94) 2300420
e-mail: rvergara@perseus.unalmed.edu.co

M. Schuller
BBA Vorratsschutz, Konigin - Luise-Strasse 19
14195 Berlin, GERMANY

S. Sithanantham
ICIPE, P. O. Box 30772
Nairobi, Kenya, KENYA
Tel.: 254-2-802501
Fax: 254-2-860110
e-mail: icipe@users.africaonline.co.ke

Joao Tavares
Dep. de Biologia, Universidade dos Acores
R. Mae de Deus, 9500 Ponta Delgada - Acores,
PORTUGAL
Tel.: 351 (96) 652089
Fax: 351 (96) 652044
e-mail: patricia@alf uac.pt

Lee Ming Tong
Malaysian Cocoa Board
Beg Berkunci 211, 88999, Kota Kinabalu, Sabah
MALAYSIA
Tel.: 088-212572
Fax: 088-239575/213037
e-mail: leemt@koko.gov.my

Julio Valdez
Calle 2 A # 66B - 86 Apto. 202 B
Cali, COLOMBIA
Tel.: 331-71-80
Cellular: 93-428-3656;

Luis Mario Varela
Calle 6ª 0 14 - 54, Barrio Ciudad Jarhin, Zarzal,
Valle, COLOMBIA

Diego Fernando Vargas F.
INTEP - Instituto de Educacion Tecnica Profesional
Calle 15 # 10ª - 14, A.A. 30, Roldanillo
Valle, COLOMBIA
Tel.: 229-7408 / 229-88425
Fax: 229-7226

Jose Galo Vivas P.
Calle 59 # 27-23 - Palmira,
Valle, COLOMBIA

ANBP (Association of Natural Biocontrol Producers) Product Profiles

History of the Product Profiles

The original Product Profile idea arose from the need to provide positive identification of beneficials to the distributor and the end-user. Originally identified as label guidelines, they were later termed Beneficial Insect Information Sheets because of the potential confusion of the term label with the EPA registered pesticide labels. Eventually, in October 1991, the title was shortened to "Product Profile."

The Product Profile was intended to be geared toward end user and distributor informational needs. It was separate from the original QC guideline for in-house rearing. By February 1992, the QC committee had received guideline submissions for "live product arrival to distributor or supplier" from 2 producers. Their information included: product examination on arrival, identification, short-term storage, long term storage where applicable, shipping, and random sampling information. Also during 1992 the committee received "Product Profile" submissions from 5 producers which included: common name, origin, scientific name, environmental needs, biology, hosts, quantity, release instructions, producer name, compatibility with pesticides, warranty, and disclaimer. Eventually, 22 were covered by at least 12 producers.

At the same time, the committee was considering an ANBP certification and endorsement program and looking seriously at the IOBC Quality Assurance guidelines. The committee subsequently decided that the most important step was to require producers to list necessary identifying information on the product profile and help the market to police the products. The intent was that the profiles be concise, easy to read, one page documents geared to the distributor and end-user.

Acknowledgements

Lee Anne Merrill and Sinthya Penn initiated and compiled most of this project. Carol Glenister collated, edited and solicited some reviews. Glenn Scriven reformatted the profiles to their present form and entered them onto the internet.

Association of Natural Biocontrol Producers
Product Profiles as of July 1995

Aphid Parasitoid and Predator:
Aphidoletes aphidimyza
Chrysoperla rufilabris

Fly Parasitoids:
Muscidifurax raptor
Muscidifurax zaraptor
Spalangia nigroaenea

Mealybug Predators:
Cryptolaemus montrouzieri

Moth egg Parasitoids:
Trichogramma bactrar
Trichogramma brassicae
Trichogramma minutum
Trichogramma platneri
Trichogramma pretiosum

Navel Orangeworm, Bollworm and Codling Moth parasitoids:
Goniozus legneri
Pentalitomastix plethorica (Navel Orangeworm)

Scale Parasitoids:
Aphytis melinus (Red Scale)

Spider-Mite Predators:
Galendromus occidentalis
Mesoseiulus longipes
Neoseiulus californicus
Phytoseiulus persimilis
Orius insidiosus

Thrips Parasitoid and Predator:
Thripobius semiluteus
Neoseiulus cucumeris

Whitefly Parasitoid:
Encarsia formosa

Beneficial Nematodes:

Steinernema carpocapsae

Information on the use of natural enemies continues to be updated as research and experience indicate more effective ways to use them. Additional product profiles are being prepared.

Association of Natural Biocontrol Producers

PRODUCT PROFILE

Product: Predatory Midge of Aphids.

Net Contents: See container.

Common Name: Aphidoletes.

Family: Cecidomyiidae.
Genus: *Aphidoletes.*
Species: *aphidimyza*.

Origin: Western Canada.

Host Pest: Aphids, many species including green peach aphid (*Myzus persicae*) and cotton aphid (*Aphis gossypii*).

Host Plant: Pepper, cucumber, tomato, melon and many other vegetables, fruit and ornamental trees.

Life Stages Shipped: Parasitized aphids in carrier; adults will emerge within 7 days at 72^{o}F.

Sex Ratio: Approximately 1 Female to 1 Male.

Development: After mating, a female can lay up to 250 eggs over a 10-day period. Eggs are laid among aphids hatching in 2-3 days to larvae, which feed for 3-5 days and then drop to the ground to pupate. Adults emerge from pupae after 10-14 days.

Environment: Temperature range 60-85^{o}F, RH 50-75%. These predators work best in greenhouses or crops with soil or plant material on the floor for pupation and may also be used on outdoor plantings and trees. Diapause occurs under short day conditions (less than 8 hours of daylight) but can be prevented in greenhouses by low intensity supplementary light.

Pesticides: Some pesticides are toxic to this predator for long periods of time (detailed list available). Spot applications of insecticidal soaps are safe.

Storage: Perishable, apply to crop as soon as possible. May be held for a few days if necessary at 40^{o}F but the percentage of emergence and egg laying ability will decrease with cold storage time.

Augmentation: Release rates vary with plant type and size but should be made at the first sign of aphid damage and repeated weekly for 24 weeks or until predators establish. Peppers 1-2 midges/plant. Tomatoes-1 midge/6 plants. Roses-3-5 midges/plant. Trees- 5-10 midges/tree.

Association of Natural Biocontrol Producers

PRODUCT PROFILE

Product: Predator of many small insects.

Net Contents: See container.

Common Name: Green Lacewing.

Family: Chrysopidae.
Genus: *Chrysoperla*.
Species: *rufilabris* (Burmeister).

Origin: Southeastern United States.

Host Pest: Aphids, Thrips, Spider Mites, Sweetpotato and Greenhouse Whitefly, Mealybugs, moth larvae and other soft-bodied insects (also eggs of all listed above).

Host Plant: Most crops and flowers (indoors and out) including orchards and vineyards.

Life Stages Shipped: Eggs; some eclosion may occur during shipment. Food is provided, unless otherwise requested.

Sex Ratio: Approximately 1 female to 1 male.

Development: In a warm environment (75-80^{o}F.) the larvae will emerge in 3 to 4 days. These larvae will pupate approximately 14 days later. The adult lacewing emerges in approximately 5 days. The adult can lay up to 600 eggs. The eggs are laid individually, at the end of hair-like filaments on plant foliage (adults do not feed on insects).

Environment: Ideal temperature is 75-79^{o}F. Minimum temperature requirement for activity is 60^{o}F.

Pesticides: Although this insect has demonstrated tolerance to some pesticides, use may not be compatible, request technical information on specific products.

Storage: Not recommended; for best results use within 10 days of egg harvest. If absolutely necessary, store at 10^{o}C (plus or minus one degree) and 75% RH to minimize mortality. Mortality predicted to be at 20% 14 days from harvest and 50% 21 days from harvest under optimum storage.

Augmentation: Release rates vary: (1) size and type of plant, (2) number and type of pests, (3) other predator and parasitoid population, (4) temperature and humidity. Request technical information from supplier for specific use.

Association of Natural Biocontrol Producers

PRODUCT PROFILE

Product: A parasitoid that attacks fly puparia.

Net Contents: See contents on container.

Common Name: Fly Parasite.

Family: Pteromalidae.
Genus: *Muscidifurax*.
Species: *zaraptor*.

Origin: New York, Nebraska.

Host Pest: Houseflies and Stable flies.

Host Area: Confined livestock, poultry, horse and other animal operations.

Life Stages Shipped: Immature parasites developing inside fly puparia, with some emerging adults.

Sex Ratio: 1 Female to 1 Male.

Development: Eggs to adults requires 18 or more days depending on temperature.

Environment: (Optimum) decaying organic materials with fly larvae that are about to pupate. Keep out of direct sunlight. This species performs well over a wide range of climatic conditions.

Pesticides: Susceptible to pesticides, particularly those directed at the manure. Careful placement of pesticides will minimize harm. One tactic is to use poison baits for adult flies. Another tactic is to spray only fly resting surfaces with a long residual pesticide.

Storage: The best policy is to apply living beneficials immediately after receipt. Storage shortens beneficial insects useful life and may reduce their reproductive capacity. If immediate application is not possible, store at 50^{o}F with high relative humidity for a few days only.

Augmentation: Fly parasites are released to augment the beneficial populations already at work. Weekly releases of approximately 250 fly parasites per large animal from May to October. For chickens, release one parasite per 2 birds per week. Release rates vary greatly depending upon sanitation and manure management.

Association of Natural Biocontrol Producers

PRODUCT PROFILE

Product: An egg parasitoid of moths.

Net Contents: See contents on container.

Common Name: Trichogramma.

Family: Trichogrammatidae.
Genus: *Trichogramma*.
Species: *minutum*.

Origin: Northern California (check with Producer).

Host Pest: In the insectary, Trichogramma is normally reared on *Sitroga cerealella* eggs (50 eggs/gram); or *Ephestia kueniella*; it destroys the egg stage of many lepidopterous pests (moths).

Host Plant: Primarily used on field crops.

Life Stages Shipped: This insect is shipped in the pupal stage (last stage prior to adult emergence) within the host eggs. They may be glued on a paper substrate or simply as loose eggs, see container.

Sex Ratio: The sex ratio is normally 1 to 1.

Development: In the insectary, this insect is reared at 26^{o}C (80^{o}F) and 60% RH. Under these conditions, the life cycle is normally eight days.

Environment: In the field, the life cycle can be as few as eight days, at high temperatures 32^{o}C (90^{o}F) to as many as seventeen days at lower temperatures 15^{o}C (60^{o}F). Adults are most active (effective) between 23 – 29^{o}C (75 – 85^{o}F).

Pesticides: High mortality of adults occurs in the presence of virtually all pesticides. The egg, larval and pupal stages are afforded some protection against pesticides within the host egg, but there is very little scientific information on this.

Storage: Storage at reduced temperatures is not recommended. If it becomes necessary to delay adult emergence, check with the producer for conditions that will minimize yield losses.

Augmentation: The manner used and frequency of releases will vary greatly depending on the pest species, density, habitat and other pest control measures in use. Technical support may be obtained from your supplier.

Association of Natural Biocontrol Producers

PRODUCT PROFILE

Product: An egg parasitoid of Lepidoptera.

Net Contents: See contents on container.

Common Name: Trichogramma.

Family: Tricogrammatidae.
Genus: *Trichogramma*.
Species: *platneri*.

Origin: Most commonly collected from eggs of codling moth in California.

Host Pest: In the insectary, Trichogramma is normally reared on *Sitotroga cerealella* eggs. In the field it destroys the egg stage of most lepidopteran pests (moths) found on orchard crops.

Host Plant: Adapted to searching for host eggs on trees.

Life Stages Shipped: This insect is shipped in the pupal stage (last stage prior to adult emergence) within the host eggs. They may be glued on a paper substrate or simply as loose eggs, see container.

Sex Ratio: The sex ratio is normally 1: 1.

Development: In the insectary, this insect is reared at 26^{o}C (80^{o}F) and 60% RH. Under these conditions, the life cycle is normally eight days.

Environment: In the field, the life cycle can be as few as eight days at high temperatures 32^{o}C (90^{o}F), to as many as seventeen days at lower temperatures 15^{o}C (59^{o}F). Adults are most active (effective) between 23 – 29^{o}C (75 – 85^{o}F).

Pesticides: High mortality of adults occurs in the presence of virtually all pesticides. The egg, larval, and pupal stages are afforded some protection against pesticides within the host egg, but there is very little scientific information on this.

Storage: Storage at reduced temperatures is not recommended. If it becomes necessary to delay adult emergence, check with the producer for conditions that will minimize yield losses.

Augmentation: The manners used and frequency of releases will vary greatly depending on the pest species, density, habitat and other pest control measures in use. Technical support may be obtained from your supplier.

Association of Natural Biocontrol Producers

PRODUCT PROFILE

Product: An egg parasitoid of Lepidoptera.

Net Contents: See container.

Common Name: Trichogramma

Family: Trichogrammatidae
Genus: *Trichogramma*
Species: *pretiosum*

Origin: Texas (cotton, corn)

Host Pest: In the insectary, Trichogramma is normally reared on *Sitotroga cerealella* (Oliver) eggs (50 eggs/grarn); or *Ephestia kuehniella*; in the field it destroys the egg stage of most lepidopterous pests (moths).

Host Plants: Adapted to searching for host eggs on low growing plants.

Life Stages Shipped: This insect is shipped in the pupal stage (last stage prior to adult emergence) within the host eggs. They may be glued on a paper substrate or simply as loose eggs, see container.

Sex Ratio: The sex ratio is normally 1: 1.

Development: In the insectary, this insect is reared at 26^{o}C (80^{o}F) and 60% RH. Under these conditions, the life cycle is normally eight days.

Environment: In the field, the life cycle can be as few as eight days at high temperatures, 32^{o}C (90^{o}F), to as many as seventeen days at lower temperatures, 15^{o}C (60^{o}F). Adults are most active (effective) between 23 – 29^{o}C (75 – 85^{o}F).

Pesticides: High mortality of adults occurs in the presence of virtually all pesticides. The egg, larval and pupal stages are afforded some protection against pesticides within the host egg, but there is very little scientific information on this.

Storage: Storage at reduced temperatures is not recommended. If it becomes necessary to delay adult emergence, check with the producer for conditions that will minimize yield losses.

Augmentation: The manners used and frequency of releases will vary greatly depending on the pest species, density, habitat and other pest control measures in use. Technical support may be obtained from your supplier.

Association of Natural Biocontrol Producers

PRODUCT PROFILE

Product: A parasitoid of navel orangeworm.

Net Contents: See contents on container.

Common Name: Goniozus.

Family: Bethylidae.
Genus: *Goniozus*.
Species: *legneri*.

Origin: Argentina and Uruguay.

Host Pest: Navel orangeworm, pink bollworm and codling moth.

Host Plant: Almonds, walnuts, coral tree, dates, loquat, pecans, pistachio, prunes, etc.

Life Stages Shipped: Pupa and adult

Sex Ratio: 4 Females to 1 Male

Development: One adult can lay approximately 100 eggs during it's lifetime. Adults can live 70+ days with honey. The adult female permanently paralyzes the host larva and proceeds to lay her eggs on the larva. The eggs will develop in 12 days, hatch and the young parasitoids consume the host larva. (complete metamorphosis)

Environment: Ideal temperature is 80 to 90^{o}F and RH 20% to 85% (wide range).

Pesticides: Use may not be compatible. Request technical information on specific pesticides.

Storage: May be stored for 6-10 days at 50^{o}F and at 75% RH. Until release can occur, keep parasites cool (70^{o}F) with wet paper, ice packs, and feed honey. Keep away from sunlight and do not store in an enclosed vehicle or toolbox.

Augmentation: Release rate 1,000 per acre. Release method, every 5 trees, every 5 rows. Multiple releases, 2 - 3 releases per year (Navel Orangeworm).

Association of Natural Biocontrol Producers

PRODUCT PROFILE

Product: A parasitoid of the Navel Orangeworm.

Net Contents: Sold by mummy case, 750 -1250 wasps per mummy case.

Common Name: None.

Family: Encyrtidae.
Genus: *Pentalitomastix*.
Species: *plethorica*.

Origin: Mexico.

Host Pest: Navel Orangeworm.

Host Plant: Nutcrops.

Life Stages Shipped: Egg, larva, pupa and adult.

Sex Ratio: 1 Female to 1 Male.

Development: 45 days to emergence at 80^{o}F. The parasitoid lay eggs in the egg of the Navel orangeworm. It is poly embryonic and develops up to 1250 offspring from one Navel orangeworm mummy.

Environment: Ideal temperature is 75 to 80^{o}F.

Pesticides: Use may not be compatible. Request technical information on specific pesticides.

Storage: 50^{o}F at 75% RH for up to 7 days.

Augmentation: Rates vary; request technical support from supplier.

Association of Natural Biocontrol Producers

PRODUCT PROFILE

Product: A parasitoid of some scale insects.

Net Contents: Not less than 50,000 or quantity marked on container.

Common Name: The Red Scale Parasite.

Family: Aphelinidae.
Genus: *Aphytis*.
Species: *melinus*.

Origin: Pakistan/India.

Host Pest: California Red Scale (*Aonidiella aurantil*).

Host Plant: Citrus, various ornamentals.

Life Stages Shipped: Adult.

Sex Ratio: 1 Female to 1 Male.

Development: Egg to adult takes 12-13 days at 80^{o}F, 50% RH.

Environment: All citrus growing areas of California, (Coastal, inland southern California, San Joaquin Valley) Arizona and Mexico. Cold weather has an adverse effect on mating and reproduction. The ideal weather condition is 60-95^{o}F and a relative humidity of 30-50%.

Pesticides: Some Aphytis strains tolerate some pesticides. Before releasing Aphytis, bioassay citrus foliage from the grove, if the grove has a recent history of pesticide use. Consult a licensed PCA before spraying.

Storage: Maintain Aphytis at 60-70^{o}F with access to honey until release. Do not place container with Aphytis in direct sunlight, an enclosed vehicle or a tool box. In hot weather, it may require wrapping the release container in moist newspaper or placing it in a cool ice chest. Release Aphytis when they are between 12 and 48 hours old (after emergence).

Augmentation: 40,000-1,000,000 per acre per year, dependent on degree of infestation, geographical location and recommendation of a licensed PCA. Aphytis only attack the third instar of the scale, therefore, scale monitoring is important.

Association of Natural Biocontrol Producers

PRODUCT PROFILE

Product: A predator of spider-mites.

Net Contents: Depends on application method.

Common Name: Western Predatory Mite.

Family: Phytoseiidae. [fyto-say-id-de]
Genus: *Galendromus*. [gal-en-dro-mus]
Species: *occidentalis*. [auk-sid-den-tal-us]

Origin: Western North America.

Host Pest: All stages of Spider mites (not effective on eggs of European red mites).

Host Plant: Fruit trees, grapes, corn, cotton, ornamentals and strawberries.

Life Stages Shipped: Egg, larvae, protonymph, deutonymph, and adult.

Sex Ratio: Female predominant usually 2: 1 or 3: 1.

Development: Egg to Adult: 7 to 14 days depending on temperature.

Environment: Does best in warm weather (80 to 100^{o}F). Tolerates low humidity of inland valleys. Does not do well in cool coastal areas. Goes into diapause (hibernation) in colder temperatures.

Pesticides: Some strains tolerate Guthion, Sevin, Sulfer. Developing tolerance to Pyrethroids. Field tolerance will vary with spray timing, application methods, weather and crop. Avoid spraying one week before or after releasing predators. Some materials may be toxic for up to four weeks.

Storage: Highly perishable, should be applied to the crop as soon as possible. If storage is necessary refrigerate at 50^{o}F for up to five days.

Augmentation: Release rates on field crops range from 2,000 to 5,000/acre at the first sign of spider mites. Later releases will require much higher numbers to be effective. May be applied by hand, by tractor or airplane using specially designed hoppers.

Association of Natural Biocontrol Producers

PRODUCT PROFILE

Product: A predator of spider mites.

Net Contents: see contents on container.

Common Name: Longipes. [lon-gi-peez]

Family: Phytoseiidae.
Genus: *Mesoseiulus*. [mezo-sy-you-lus]
Species: *longipes*.

Origin: South Africa.

Host Pest: Spider mites.

Host Plant: Greenhouse crops.

Life Stages Shipped: Egg, larvae, protonymph, deutonymph and adult.

Sex Ratio: 4 females to 1 male.

Development: Completes a generation in about one week, depending on temperature.

Environment: Does best in warm to hot greenhouses. Tolerant to lower humidities (40% RH at 70^{o}F). Not recommended for outside crops.

Pesticides: Susceptible to pesticides. Field tolerance will vary with spray timing, application methods, weather and crop. Avoid spraying crop one week before or after releasing predators. Some materials may be toxic for up to four weeks.

Storage: Highly perishable, should be used immediately upon delivery. If storage is absolutely necessary, refrigerate at 50^{o}F (10^{o}C). Not to exceed 5 days to minimize mortality.

Augmentation: Release rates in greenhouses are highly variable. Release at least one per plant or one per square foot at the first sign of spider mites. Later releases will require much higher numbers to be effective.

Association of Natural Biocontrol Producers

PRODUCT PROFILE

Product: A predator of spider-mites.

Net Contents: See contents on container.

Common Name: Californicus.

Family: Phytoseiidae.
Genus: *Neoseiulus*. [neo-sy-you-lus]
Species: *californicus*.

Origin: California, Florida and Mediterranean.

Host Pest: Spider mites, Broad mite, Cyclamen mite.

Host Plant: Strawberries, corn, grapes, roses, ornamentals.

Life Stages Shipped: Egg, larvae, protonymph, deutonymph, and adult.

Sex Ratio: 4 females to 1 male.

Development: Completes a generation in one to two weeks depending on temperature.

Environment: Does best in warm humid conditions, minimum 50% RH and temperature up to 100^{o}F. Occurs along coast and inland valleys of California.

Pesticides: Some strains tolerant of pesticides. Field tolerance will vary with spray timing, application methods, weather and crop. Avoid spraying crop one week before or after releasing predators. Some materials may be toxic to predators for up to four weeks.

Storage: Perishable, should be used immediately upon delivery. If storage is necessary, refrigerate at 50^{o}F (10^{o}C). Not to exceed 5 days, to minimize mortality.

Augmentation: Release rates are being developed. Release at least 4 per plant or 4 per square foot in greenhouses at the first sign of spider mites. Later releases will require much higher numbers to be effective.

Association of Natural Biocontrol Producers

PRODUCT PROFILE

Product: Predatory mite for biological control of spider mites.

Net Contents: Bottles: 2,000 active stages mixed in vermiculite or corn grit. Leaves: 5,000 per bag.

Common Name: Persimilis. [pur-si-mil-us]

Family: Phytoseiidae.
Genus: *Phytoseiulus.*
Species: *persimilis*.

Origin: Mediterranean.

Host Pest: Two-spotted spider mite and other species in the family Tetranychidae.

Host Plant: Greenhouse crops, low growing field crops (strawberries), ornamentals.

Life Stages Shipped: Egg, larva, protonymph, deutonymph and adult.

Sex Ratio: 3-4 females/male

Development: Egg to adult: 7-8 days at constant 70^{o}F (20^{o}C)

Environment: Best suited to moderately warm humid climates, 70-85^{o}F (20-30^{o}C) at 70% RH. Ideal in protected cropping. Not appropriate in hot, dry environments.

Pesticides: Most organophosphate, carbamate and pyrethroid insecticides are toxic, some for extended periods of time. Tolerant of some fungicides and IGR insecticides. Some resistance to Omite. Tolerates Avid and Sulfer treatments. All pesticides should be used with caution within biological control programs. Avoid spraying crop one week before or after releasing predators.

Storage: Highly perishable, should be used immediately upon delivery. If storage is absolutely necessary, refrigerate at 50^{o}F (10^{o}C). Not to exceed 3 days to minimize to mortality.

Augmentation: Typical release rates are 1/sq ft., 1/plant or 20-40,000/acre. Rates are dependent upon pest levels and desired speed of control. Recommended pest/predator ratio at time of release 10/1. Avoid releases in temperatures below 45^{o}F or above 85^{o}F and during dry windy conditions. It is extremely important to release predators early, as soon as pest mites appear in the crop.

Association of Natural Biocontrol Producers

PRODUCT PROFILE

Product: A predatory bug.

Net Contents: 500 minimum (see container).

Common Name: Minute Pirate Bug.

Family: Anthocoridae.
Genus: *Orius*.
Species: *insidiosus/tristicolor*.

Origin: California, Holland and Israel.

Host Pest: Thrips, aphids, mites, scales, whiteflies and soft-bodied arthropods.

Host Plant: Many (most flowering plants) and commonly found in strawberries, corn, grapes, cotton, melons, orchard crops, and ornamentals.

Life Stages Shipped: Nymphs/adults (both predaceous)

Sex Ratio: Approximately 1 Female to 1 Male.

Development: 28 days from egg to adult at 70^{o}F. There are 5 nymphal instars. Females of *O. insidiosus* lay up to 130 eggs in their lifetime. Northern populations of *Orius* go into winter diapause (hibernation), which is broken around April.

Environment: Optimum field conditions: 75-90^{o}F, March-October.

Pesticides: Although this insect has demonstrated tolerance to some pesticides, use may not be compatible; request technical information on specific products.

Storage: Perishable, apply as soon as possible. May be held for a few days if necessary at 50^{o}F, but the emergence and egg laying ability will decrease with cold storage time.

Augmentation: Release rates range from 5,000-30,000/Ac when pest levels are low.

Special Instructions: Immatures are sensitive to short daylength (i.e. <13 hrs. daylight).

Association of Natural Biocontrol Producers

PRODUCT PROFILE

Product: A parasitoid of Greenhouse thrips nymphs.

Net Contents: Minimum 250 pupae per vial.

Common Name: Thripobius.

Family: Eulophidae.
Genus: *Thripobius*.
Species: *semiluteus*.

Origin: Brazil and Australia.

Host Pest: Greenhouse thrips.

Host Plant: Avocado, citrus and ornamentals.

Life Stages Shipped: Pupae.

Sex Ratio: Uniparental (all individuals female).

Development: Life cycle averaged 23.6 (range 22-25) days at 73^{o}F (23^{o}C).

Environment: Ideal temperature requirements are between 65-85^{o}F and a relative humidity of 50%. Adults live from 2-10 days. They are not very effective when ants are present on trees.

Pesticides: Not tolerant to any broad spectrum organophosphates or carbamates.

Storage: Perishable, apply as soon as possible. May be held for a few days if necessary at room temperature but the percentage of emergence and egg laying ability will decrease with storage time.

Augmentation: For Orchards: Release 250 pupae per tree on four trees in one acre. There are 250 pupae contained in one vial. Attach the open vial with masking tape horizontally on a branch as high in the tree as possible. This will allow emerging parasites to escape as they are ready. For Greenhouses: Attach vial with masking tape where thrips activity is present. If plants are small, put a stake in the ground and attach vial to stake horizontally.

Association of Natural Biocontrol Producers

PRODUCT PROFILE

Product: Thrips predators.

Net Contents: 25 mites per ml of bran.

Common Name: Cucumeris. [ku-ku-mer-us]

Family: Phytoseiidae.
Genus: *Neoseiulus*.
Species: *cucumeris*.

Origin: Western Europe.

Host Pest: Flower Thrips.

Host Plant: Vegetables, ornamentals.

Life Stages Shipped: Eggs, larvae, protonymphs, deutonymphs and adults.

Sex Ratio: 3 females to 1 male.

Development: From egg to adult takes one to two weeks depending on temperature.

Environment: Greenhouses (optimum) temperatures above 70^{o}F, high humidity.

Pesticides: Susceptible to pesticides, particularly those with long residuals. Not harmed by growth regulators such as neem and diflubenzuron.

Storage: The best policy is to apply living beneficials immediately after receipt. Storage shortens beneficial insects useful life and may reduce their reproductive capacity. If immediate application is not possible, store at 50 to 60^{o}F with high relative humidity.

Augmentation: Release rates for thrips predators vary depending on the number of pests present. Release rates for thrips at barely detectable levels range from 10 to 200 per plant.

Association of Natural Biocontrol Producers

PRODUCT PROFILE

Product: Parasite of some species of whitefly.

Net Contents: See container.

Common Name: A whitefly parasite.

Family: Aphelinidae.
Genus: *Encarsia*.
Species: *formosa*.

Origin: Western Canada.

Host Pest: Greenhouse and Sweetpotato Whitefly.

Host Plant: Cucumber, tomato, melon, poinsettia and many other vegetable and ornamental crops.

Life Stages Shipped: Parasitized scale attached to cards. The number of adults specified on the label will emerge within 10 days at 72^{o}F.

Sex Ratio: females only (parthenogenic reproduction)

Development: A female can lay up to 250 eggs at 7-15 eggs per day each of which develop to pupa within a single whitefly host, turning the parasitized whitefly pupa yellow to black in color, then emerging as adults after a period of 20 days at 72^{o}F. Adults live 10 days or more feeding on honeydew and body fluids of the host.

Environment: Optimum temperatures 65-80^{o}F, RH 50-85%. These parasites work best in greenhouses or crops grown under sunny, warm conditions.

Pesticides: Some pesticides are toxic to this parasite for long periods of time (detailed list available). Spot applications of insecticidal soaps are safe.

Storage: Perishable, apply to crop as soon as possible. May be held for a few days if necessary at 40 to 50^{o}F, but the percentage of emergence will decrease with cold storage time.

Augmentation: Release should be made when whitefly numbers are very low <1 adult whitefly per 100 plants). Release rates vary with crop type but should be continued weekly for 8-10 weeks or more until 80% of whitefly scales are parasitized. Tomatoes and Peppers- release 1 parasite per 4 plants. Cucumbers- release 1 parasite per 2 plants. Poinsettias- release 2 parasites per plant.

Association of Natural Biocontrol Producers

PRODUCT PROFILE

Product: Beneficial Nematodes.

Net Contents: One million minimum (see contents on container).

Common Name: Beneficial Nematodes.

Family: Steinernematidae.
Genus: *Steinemema*.
Species: *carpocapsae*.

Origin: UC Davis, California.

Host Pest: Fruit fly larvae, Colorado potato beetle, fungus gnat larvae, striped cucumber beetle, beet armyworm and other soil dwelling pests.

Host Plant: Used on many crops.

Life Stages Shipped: Infective juvenile

Sex Ratio: Unknown

Development: Egg, 4 juvenile stages, adult.

Environment: Avoid desiccation. Optimal soil temperature for activity is between 60 and 75^{o}F. Juveniles find host and enter through body openings. They then release an associated bacteria, which multiplies and kills the host.

Pesticides: Use may not be compatible. Request technical information on specific pesticides.

Storage: Not recommended. For best results apply immediately upon arrival. If absolutely necessary, store under refrigeration up to one week.

Augmentation: Release recommendations vary based on number and type of target pest. Evening application is advised to avoid exposure to ultraviolet light.