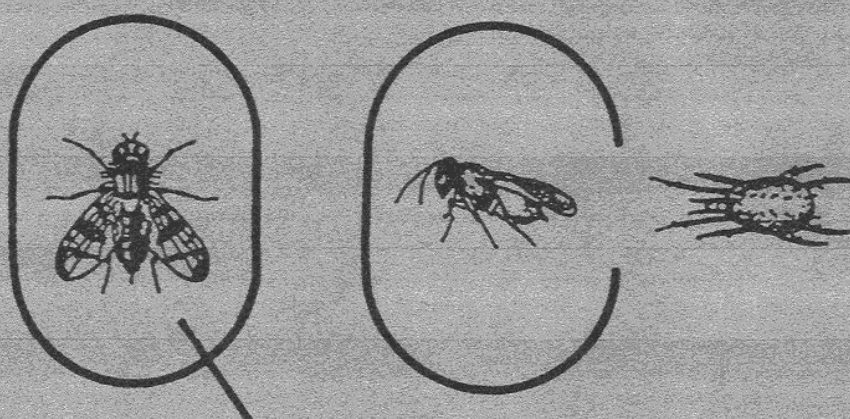


ORGANISATION INTERNATIONALE DE LUTTE BIOLOGIQUE  
CONTRE LES ANIMAUX ET LES PLANTES NUISIBLES



INTERNATIONAL ORGANIZATION FOR BIOLOGICAL CONTROL  
OF NOXIOUS ANIMALS AND PLANTS



## Proceedings

Seventh Workshop of the IOBC Global Working Group  
"Quality Control of Mass Reared Arthropods"

Rimini, Italy  
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Edited by G. Nicoli, M. Benuzzi  
and N.C. Leppla

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were observed in greenhouses and field plots at several sites in the Rimini area. The day concluded with a seminar at APOFRUIT, a fruit and vegetable cooperative that promotes the use of biological control.

An understanding of the historical significance of this VII Workshop is important to the future of quality control in mass-reared arthropods and to the development of pest management technologies that depend on the production of abundant and efficacious organisms at a cost that competes favorably with chemically-based methods. According to Ernst Boller's "History of Quality Control in Mass-Reared Insects" presented at the III Workshop in 1986, there have been four distinct periods in the development of the field; before 1969 (period of little concern), 1969-1975 (growing awareness, ideas and concepts), 1975-1979 (international collaboration and prototypes), and 1980 on (general acceptance and practical application). We combine the first three periods into the first era and extend the second era from 1980 to 1991. Thus, there have been two primary eras in the evolution of quality control in arthropod production and this meeting occurred on the threshold of a third.

The first era was characterized by determining the need and establishing basic definitions, developing working principles, designing simple tests for fruit flies, and providing some training capabilities. It began in the 1960's with ratio tests conducted in sterile insect technique (SIT) programs; the need for quality control in SIT and biological control being expressed in formative papers by Boller (1972), Manfred Mackauer (1972), and Derrell Chambers (1975), among others. At that time, many researchers were concerned about or actually opposed to using laboratory-reared insects for developing control technologies. Nevertheless, this era progressed as follows:

1971. An IOBC Symposium held at Rome, Italy resulted in the 1972 Boller and Mackauer papers focused respectively on fruit flies and natural enemies (Entomophaga, Vol. 17). Boller proposed a division of quality assessment and monitoring into production and product quality control. This symposium and associated publications helped to stimulate many insect rearing and quality control

research programs.

1973. An IAEA/FAO panel, "Controlling Fruit Flies by the Sterile-Insect Technique," included the 1975 Chambers paper and three other reports on quality control testing by Guy Bush, Boller and Ulrich Remund, and Ron Prokopy, et al., plus a description by Bill Butt of the need for quality control testing in SIT programs (1975, IAEA, STI/PUB/392).

1974. A "Workshop on the Genetics of Insect Behavior" was held at Gainesville, Florida. The objective of the workshop was to consider if "behavioral abnormalities are important factors in the disappointing performance of some mass-reared insects and, if so, what can be done to assure their adequate and reliable performance." A proceedings was circulated to the participants.

1976. At the XXV International Congress of Entomology in Washington, DC, Leppla moderated a symposium entitled "Characterization and Evaluation of Insect Colonies." The Congress also had a symposium on natural enemies with contributions by Mackauer and Boller on "genetic aspects" and "quality considerations" in mass rearing, respectively. In Boller's opinion, the field of quality control was threatened by the "imminent split into diverging, competing schools." He, Chambers, and others intensified their efforts to unify the field and ultimately developed this IOBC Working Group for that purpose.

1977. IOBC published "Quality Control, An Idea Book for Fruit Fly Workers" by Boller and Chambers. Chapters included Introduction, Concepts and Approaches, Measuring Overall Performance, Measuring Individual Performance Traits (Motility, Orientation to Habitat, Sexual Activity, and Sexual Physiology), Monitoring Production (Monitoring Production Characteristics, and Measuring Adaptation), and Implementation of Quality Control.

1978. The "RAPID" Quality Control System was developed by Boller and his colleagues, and used to compare wild and laboratory strains of the Mediterranean

fruit fly, *Ceratitis capitata* (Wied.) in Europe and Guatemala.

1979. An International Workshop and Training Course on Quality Control in Fruit Flies was organized by the IOBC Working Group on Fruit Flies of Economic Importance in collaboration with the USDA, IAEA/FAO, Spanish Plant Protection Service, and Swiss Federal Research Station at Wädenswil. The course was based on the RAPID Quality Control System.

The second era in arthropod quality control began in 1980 when the IOBC Working Group on Quality Control in Mass-Reared Arthropods was proposed by Boller and Chambers. "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." Key events for this era included the following:

1982. The I Workshop held in Gainesville, Florida was co-chaired by Boller and Chambers. The agenda included pests of man and animals, i.e., biting flies such as screwworm, *Cochliomyia hominivorax* (Coquerel), fruit flies, and Lepidoptera. Post-meeting excursions were conducted to Tuxtla Gutierrez, Mexico (screwworm) and Metapa, Mexico (medfly). Tours of operational pest management programs based on mass-produced arthropods continue to be an important component of the workshops, particularly to maintain a practical orientation.

1984. The II Workshop held at Wädenswil, Switzerland was again co-chaired by Boller and Chambers. Natural enemies were included for the first time, encouraged by Mackauer.

1986. The III Workshop held at Guatemala City, Guatemala was chaired by Carrol Calkins. This meeting was organized by subject rather than taxonomic group:

Insect Colonization and Strain Development, Colony Maintenance, Quality Control of Production and Products (laboratory bioassays), Irradiation, Shipment and Distribution, Field Assessment, and Management of Quality Control Systems. Colonization and strain development were emphasized, still critical subjects that receive minimal attention. Tom Ashley (1987) developed a computerized quality control system at Guatemala following the meeting.

1988. The IV Workshop held at Vancouver, Canada was again chaired by Carol Calkins. The general topics of the 1986 meeting were divided into two sections, "Quality Control of Pestiferous Insects" and "Quality Control of Entomophagous Arthropods." Along with the topics discussed at Guatemala, behavior of entomophages was added by van Lenteren and colonization by Mackauer. IOBC was represented by the Secretary of the Global Body, John Paul Aeschlimann. This and the previous meeting at Guatemala were very important in developing the concepts of total quality control.

This account of the first two eras in arthropod quality control, partially based on the brief history written by Boller in 1986, provides a general idea about the events that have transpired in quality control of mass-reared arthropods during the past 20 plus years. Also, many other very important quality control meetings and publications have been developed simultaneously with these IOBC Working Group workshops. However, the "general acceptance and practical application" third era, ambitiously anticipated by Boller more than a decade ago, is just beginning as exemplified by the following meeting:

1991. The V Workshop held at Wageningen, Netherlands was hosted by van Lenteren and chaired by Bigler. It concentrated completely on entomophagous arthropods and resulted in a very important proceedings that describes specific quality control tests for natural enemies. This workshop produced the special subject meeting at Horsholm, Denmark in 1992 that had four objectives: 1) Review and improve guidelines for product control of natural enemies, 2) Draft product control methods for additional organisms, 3) Discuss quality control costs and

resources, and 4) Determine quality control information for European Community labels.

The third era of "universal implementation" has been delayed because of unforeseen and unnecessary barriers to technology development and transfer but it is being accomplished by: 1) Determining the needs, resources, and actions required to provide quality control for colonization, production, delivery, and field evaluation, 2) Implementing quality control extending from production through delivery and field evaluation that provides uniform, quantitative assessment and optimization through feedback, 3) Performing periodic reviews to maintain quality control systems and improve them by incorporating new technologies, and 4) Developing comprehensive quality control programs, including reasonable investment of resources, training of employees and active participation of management. The time has come to advance from ideas and limited action to the routine application of quality control for both sterile insect technique and biological control.

N. C. Leppla, Chairman

M. Benuzzi, Co-Chairman



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## PAPERS



## **Principles of Quality Control in Mass-Reared Arthropods**

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### **Introduction**

*Great knowledge and judgement are required to determine the correct course of action but another dimension of effort must be expended to actually effect this action.*

The application of quality control theory to arthropod production has advanced to the point of general implementation. This era will require development of taxonomically-specific, uniform diagnostic tests for routine product monitoring. However, it is essential to not approach overall quality control from the inappropriately narrow perspective of product control, testing to determine if there is an acceptable arthropod product at the end of production. It is necessary to assess the competitiveness of colonized strains, monitor them for genetic deterioration, 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 variability), perform *product control* at the facility and at critical points to field application, measure field performance, and provide feedback to optimize production and field delivery (Fig. 1).

### **Applications in Mass Rearing**

Currently, there is an increase in biologically-based methods for suppressing

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## Total Quality Control in an Insect Pest Suppression System

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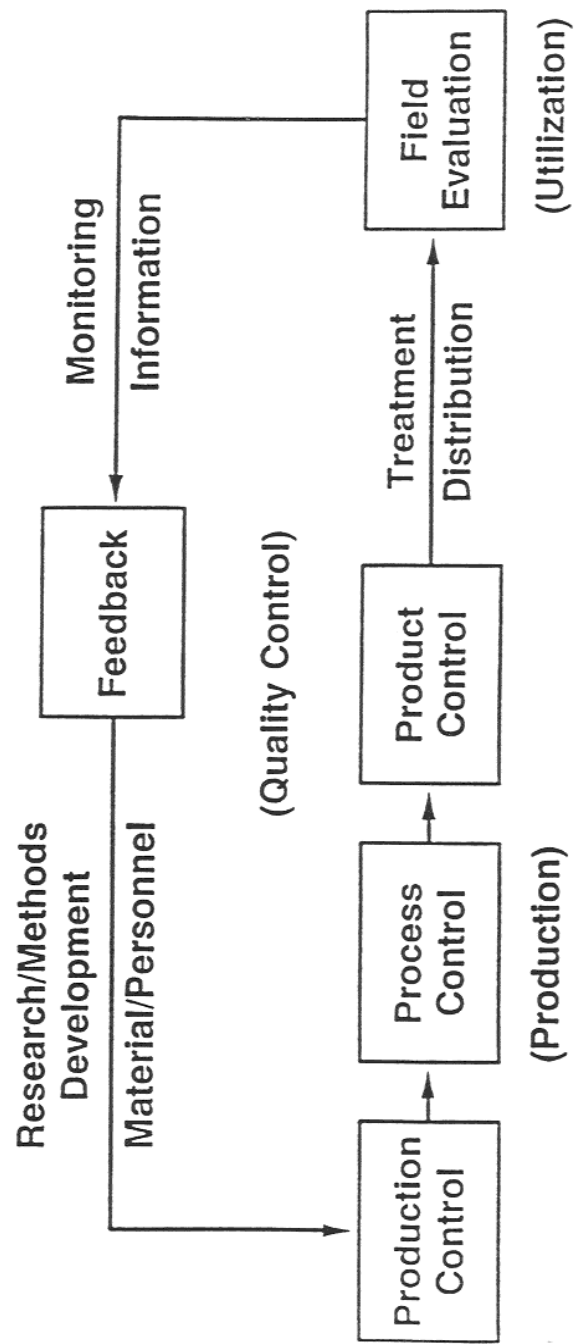


Figure 1. Total quality control system for production, utilization, and optimization (quality control) of arthropods mass produced for pest management.

pestiferous arthropods and weeds that will necessitate rearing of more species and numbers of arthropods, and assuring that their quality is acceptable (Parella *et al.*, 1992; van Lenteren, 1993; Williams and Leppla, 1992). Examples of expanding programs include the codling moth, *Cydia pomonella* (Linn.), in British Columbia; pink bollworm, *Pectinophora gossypiella* (Saunders), in Arizona; Mediterranean fruit fly, *Ceratitidis capitata* (Wied.), in Hawaii, Mexico and Guatemala; Mexican fruit fly, *Anastrepha ludens* (Loew), in Texas and Mexico; Caribbean fruit fly, *Anastrepha suspensa* (Loew) in Florida; screwworm *Cochliomyia hominivorax* (Coquerel), in Mexico and Guatemala; gypsy moth, *Lymantria dispar* (Linn.), in Massachusetts; *Trichogramma* spp. in Canada; and biological control agents worldwide. Growth is also occurring in colonies for testing biopesticides and control products from molecular biology. This is in addition to advances in contract research, particularly related to environmental assessment, in host plant resistance, and in other kinds of basic research.

#### Total Quality Control

Total quality control in arthropod mass production provides an organizational structure for developing and managing insect mass rearing and quality control systems (Leppla and Fisher, 1989, Fig.2). It is a hybrid developed by incorporating the experience of "troubleshooting" pest management programs based on mass-reared arthropods with the "Generic Guidelines for Quality Control" published by the U.S. National Bureau of Standards (Anonymous, 1979). The structure is composed of eight generic elements: Management, Research, Methods Development, Material, Production, Utilization, Personnel, and Quality Control. The elements and sub-elements are described as follows: *Management* determines policy, performs planning and administration, and controls program design as it evolves. *Research* is the source of knowledge and *Methods Development* creates new operational technologies. *Material* encompasses purchasing, establishing specifications and standards, assigning responsibility for quality materials, verifying compliance, and storage. The components of *Production* are facilities, equipment, rearing operations, and production control. *Utilization* is the treatment, handling and distribution of adult arthropods. Responsibility for the selection, training,

## Total Quality Control

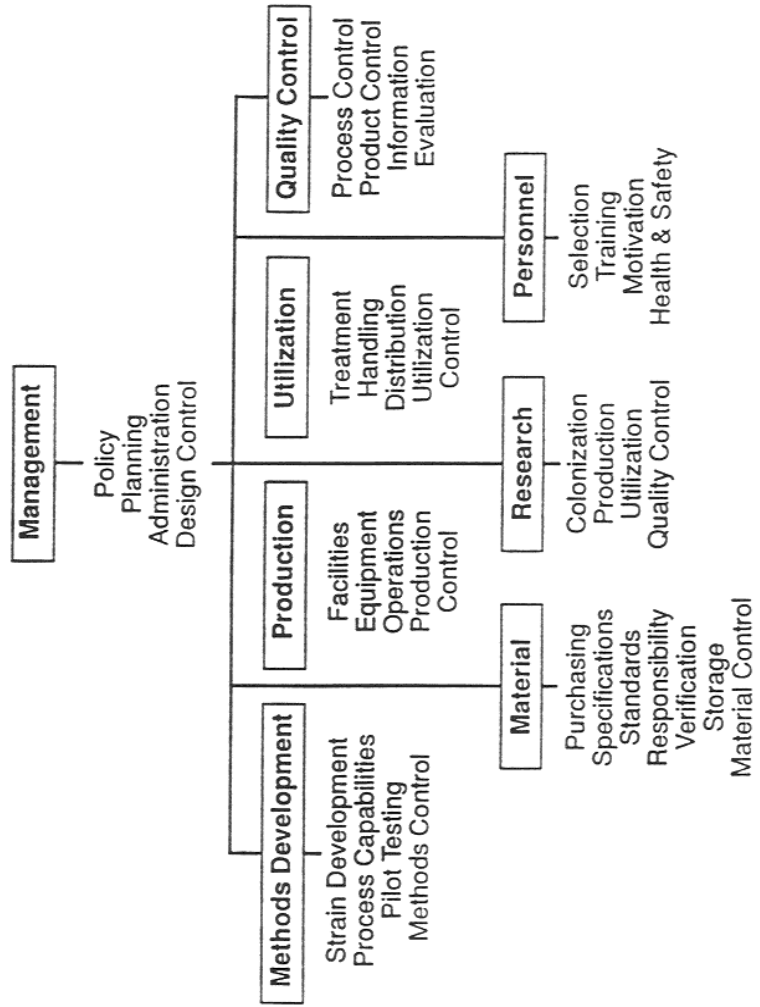


Figure 2. A total quality control system composed of generic elements for arthropod mass production.



motivation, and health and safety of employees belongs to *Personnel*. Accomplished personnel can overcome most difficulties that can occur in the entire system but no organizational structure can compensate for poor performance. *Quality Control* impinges on all program elements with primary emphasis on production, process and product control (Leppla, 1989). This element provides information management the other elements must have to optimize the entire pest management program (Webb, 1984).

### **Strain Development**

In practice, colonies are usually established with effective populations of much fewer than 200 specimens, 50% of each sex. Rarely, however, is there an attempt to determine which individuals reproduce and to characterize them in terms of relative fitness (Mackauer, 1976; Mangan, 1992). Males and females with different ecological and presumably genetic backgrounds should be crossed deliberately and maintained as isofemale lines to quantify their contributions to successive generations before the genetic composition of the final colony is determined. The strain must retain essential behavioral traits that assure its fitness in both the mass rearing facility and in the field, because it must perform in both environments (Chambers, 1975). Mass-reared arthropods must tolerate crowding and associated epizootics, and environmental variability caused by changing materials, equipment and techniques. Performance in the field depends on survival through delivery and release, ability to recover and seek suitable habitats, and success in finding and interacting with target populations (Chambers, 1977; Huettel, 1976).

### **Rearing Proficiency**

The interdependence of strain development and rearing proficiency is illustrated by a set of hypothetical colonization curves (Fig.3). These curves are based on years of experience in collecting, colonizing, and rearing phytophagous insects. Curve "A" plots the loss of a colony at the third generation, a common occurrence when one or more critical environmental factors are lacking and a decreasing number of individuals reproduce. In curve "B" the minimal requirements are provided but severe bottlenecks occur. This may result in major phenotypic changes or the loss

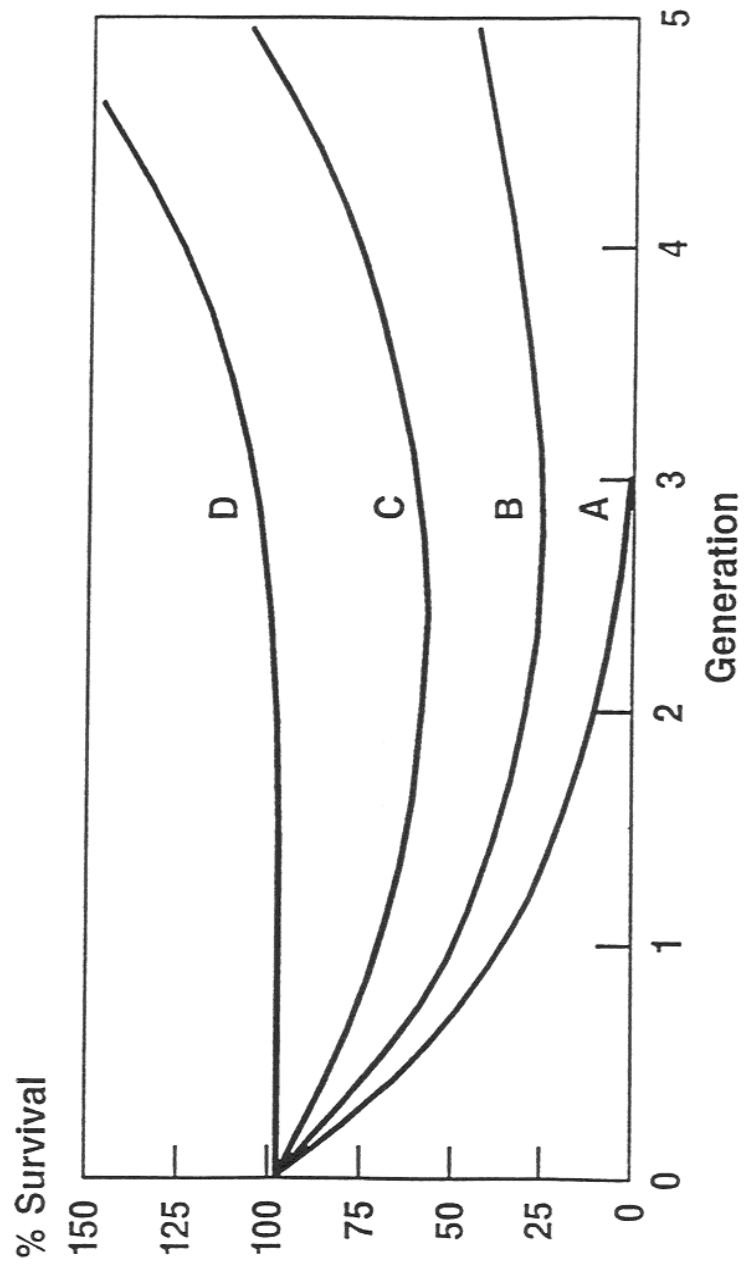


Figure 3. Hypothetical arthropod colonization curves based on increased rearing proficiency from A to D. Percentage survival for successive generations based on essentially 100% at colonization is potentially much greater than depicted for proficiencies C and D at generation 5.

of alleles essential for field performance. However, laboratory adaptation occurs after about five generations and large numbers are ultimately produced. Curve "C" represents the usual situation after experience has been gained with a new species. Limited adaptation occurs initially and the colony rapidly builds. Although curve "B" typically occurs, with experience a population can be removed from the field and established in the laboratory with no significant mortality.

#### **Strain Maintenance**

It is essential to optimize and stabilize all of the rearing operations because strains constantly readapt to each subtle change. Some changes are inevitable and perhaps useful; however, their effects on colonies should be minimized and quantified (Boller, 1992). If the adverse effects are great enough to prevent a strain from recovering in terms of performance, it will have to be restored or replaced. Genetic improvement of strains can be accomplished by collecting and colonizing specific biotypes, rearing them more proficiently, and practicing behavior-genetic selection. This process will produce more efficacious strains, rather than just maintain genetic variability and delay recolonization. However, a greater understanding of the biology of the strains will be required, along with much more precision in their rearing. Just attempting to rear more individuals per unit of space will not compensate for genetic losses.

#### **Implementation of Quality Control**

Quality control is necessary to produce a dependable supply of efficacious arthropods at minimal cost. It assures effective management of colonized strains, precluding genetic deterioration and required replacement. As total quality control, it incorporates all factors that affect strain quality with the elements of an interdependent pest management system, enabling it to be predictable and optimal. Immediate actions recommended to implement quality control are as follows:

1. Capitalize on investments the IOBC Working Group on Quality Control of Mass-Reared Arthropods has already made in developing quality control tests, data acquisition and analysis, and decision making systems (Bigler, 1992; Bigler *et al.*,

1991; Boller and Chambers, 1977). Also, provide quality control education and training, design and construct quality control test equipment for uniform deployment, specify uniform protocols for preconditioning and bioassay, recommend the best available rearing and delivery procedures, transfer technology from the food processing industry, incorporate process engineering, and improve field evaluation techniques.

2. Implement new or refine existing model quality control systems (i.e., Mediterranean fruit fly, Mexican fruit fly, Caribbean fruit fly, Screwworm, *Trichogramma*, and pink bollworm) that extend from production through delivery and field evaluation, and provide for quantitative assessment and optimization (Leppla and Ashley, 1989). Include specifications and standards of tolerance to assure that products are predictably acceptable. Design a procedure for periodically performing on site reviews to assure the consistency of quality control practices.

3. Develop quality control for governmental programs and commercial products, particularly the biological control industry but also contract rearing (Brazzel *et al.*, 1986; van Lenteren and Steinberg, 1991). Encourage cooperation and coordination without jeopardizing proprietary rights. Establish specifications and standards, and procedures and authority for quality control certification (Hoy *et al.*, 1991). Facilitate international shipment of natural enemies, pollinators, sterile insects, and other mass-reared arthropods by helping to establish workable laws and regulations.

4. Establish a subcommittee to review needs and activities for providing quality control to arthropod rearing systems. Inventory projects based on mass-rearing and quality control worldwide. Provide specific recommendations for improving products for use in the field.

#### **Future Action**

The prudent course of action is clear for effecting comprehensive quality control in

mass-reared arthropods. Underlying principles are well-established, its importance is universally appreciated, it is currently practiced in some form by all producers, production and process control stabilize rearing systems and reduce operating costs, and product control generally required by regulatory institutions provides a clear marketing advantage. For example, identity, purity and efficacy must be determined before natural enemies can be marketed in most European countries (Bigler 1992) and these markets are increasing rapidly (van Lenteren 1993). Product control with appropriate high standards will consequently provide a competitive advantage to conscientious producers and eliminate those who attempt to sell incorrect, impure or substandard products. Misapplication of natural enemies and other unethical practices will also be eliminated in the marketplace. Partnerships among governmental institutions and private companies will be needed to provide new biological control agents and contract rearing opportunities, establish routine identification services, conduct research leading to efficient product quality control tests, and implement procedures that satisfy local, national and international regulations.

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**Quality Control in Laboratory-Reared Codling Moth at Mt Albert Research Centre, Auckland, New Zealand.**

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**Abstract**

Methods for assessing quality in a laboratory (lab) strain of codling moth, *Cydia pomonella* are described. Case studies are outlined that show how quality control procedures identify, solve and prevent specific problems of low fecundity, low percent egg hatch and high larval mortality. The reduced response to pheromones and a larval attractant ( $\alpha$ -farnesene) of the lab strain is contrasted with a field collected strain which has been reared in the lab for three generations. Total quality control procedures that include routine monitoring of key biological and behavioural parameters are recommended.

**Introduction**

The concepts of quality as applied to insect rearing have evolved greatly during the last 20 years. Boller (1972) defined the quality of laboratory-reared insects in terms of their behaviour when released, compared to their counterparts in the field. This is essentially a "fitness-for-use" philosophy. Chambers and Ashley (1984) defined three aspects of quality control for insect rearing that maintain and improve quality: 1. Process control, "regulation of the production processes" so that problems are prevented, 2. Product control, "regulation of the quality of the insects" to a particular standard, and 3. Production control; "regulation of the

service" in terms of reliability, consistency and timeliness. Leppla and Fisher (1989) have since applied quality concepts (total quality control) to all aspects of insect mass production, which include the following eight elements: management, research, methods development, material, production, utilization, personnel, and quality control. This concept of quality control recognises that all aspects of a rearing programme need to be regulated to ensure success. Quality control as part of an overall Insect Rearing Management (IRM) philosophy (Singh and Ashby 1985) is practised at our HortResearch rearing facility at Mt Albert, Auckland, where insects are produced for pest management research programmes. Quality control as a component of IRM concentrates on product control, but other elements of IRM ensure production and process control are also regulated.

This paper reviews how quality has historically been assessed for a laboratory strain of codling moth, *Cydia pomonella* (Linnaeus). Case studies of successfully identified problems of diet deficiency, material contamination and a lack of response to pheromones and a larval attractant ( $\alpha$ -farnesene) are given. These case studies illustrate how IRM systems identified, solved and helped to prevent quality problems. Other modifications to improve quality control are suggested.

#### **Materials and Methods**

The larvae used to establish the laboratory (lab) strain were collected from Nelson, New Zealand, in 1967 and field-collected moths were introduced to the colony on a yearly basis until 1975. Since then no further field collected material has been introduced. In August 1993 the lab strain had completed 220 generations. This colony is maintained as two sub-colonies ("A" and "B") which are three weeks apart in development. At times the sub-colonies have been mixed especially when the colony was subject to increased demands caused by equipment malfunctions, disease, dietary problems or the demands for large numbers to supply research programmes. A more recent field-collected colony (wild strain) was collected from an organic apple orchard at Coroglen, Coromandel in March 1992, and in August 1993 had completed 10 generations. Both codling moth colonies are reared as described by Ashby *et al.* (1985) with some modifications including the following. To maintain the colonies larvae are reared individually in test-tubes. General

Purpose Diet (GPD; Singh 1983) was used for "colony maintenance" until generation number 173 when a modified diet of Brinton *et al.* (1969) was used. To produce codling moth adults for research programmes, as opposed to colony maintenance, large rearing containers are used (Ashby *et al.* 1985).

Fecundity, fertility and egg mortality are measured for 15 pairs of moths drawn from colony maintenance. Each pair is contained in a separate tube and measurements are recorded per pair. These parameters are measured for moths held for a 10-day period at 25°C, L:D 16:8 hours. A sample of pupal weights of 25 pairs from colony maintenance insects is also measured each generation. Larval establishment, percent larval mortality, percent pupation, sex ratio, deformities and presence of mould is recorded for all individually reared codling moths in the colony maintenance population. For larger scale production, adult yields per rearing container are recorded and observations are made of fecundity, egg hatch and larval establishment. Check lists, rearing records and information from researchers using codling moth provides additional information which assist with the regulation of quality. Virgin female and male codling moths were supplied to Dr. J.R. Clearwater for pheromone studies. Also codling moths were supplied to Mr. S.J. Bradley who examined the response of neonate larvae to  $\alpha$ -farnesene using video recordings of single larvae placed 2 cm from  $\alpha$ -farnesene impregnated papers. Larvae were scored for the following behaviour; head turning, head lifting, head direction and movement across a petri dish (Bradley and Suckling pers. comm.).

## Results and Discussion

### *Quality Control Procedures*

Fisher (1983) developed quality control charts for the fall armyworm, *Spodoptera frugiperda* (Smith) covering a range of developmental and behavioural traits. Using similar principles, control charts have been developed for the codling moth lab strain. Pupal weights, fecundity and percent egg hatch are displayed for sub-colony A of the lab strain (Figure 1). Each parameter is bounded by control limits, set at three standard deviations from the mean. Similar data is also collected and charted for sub-colony B. Charting these parameters shows natural variation

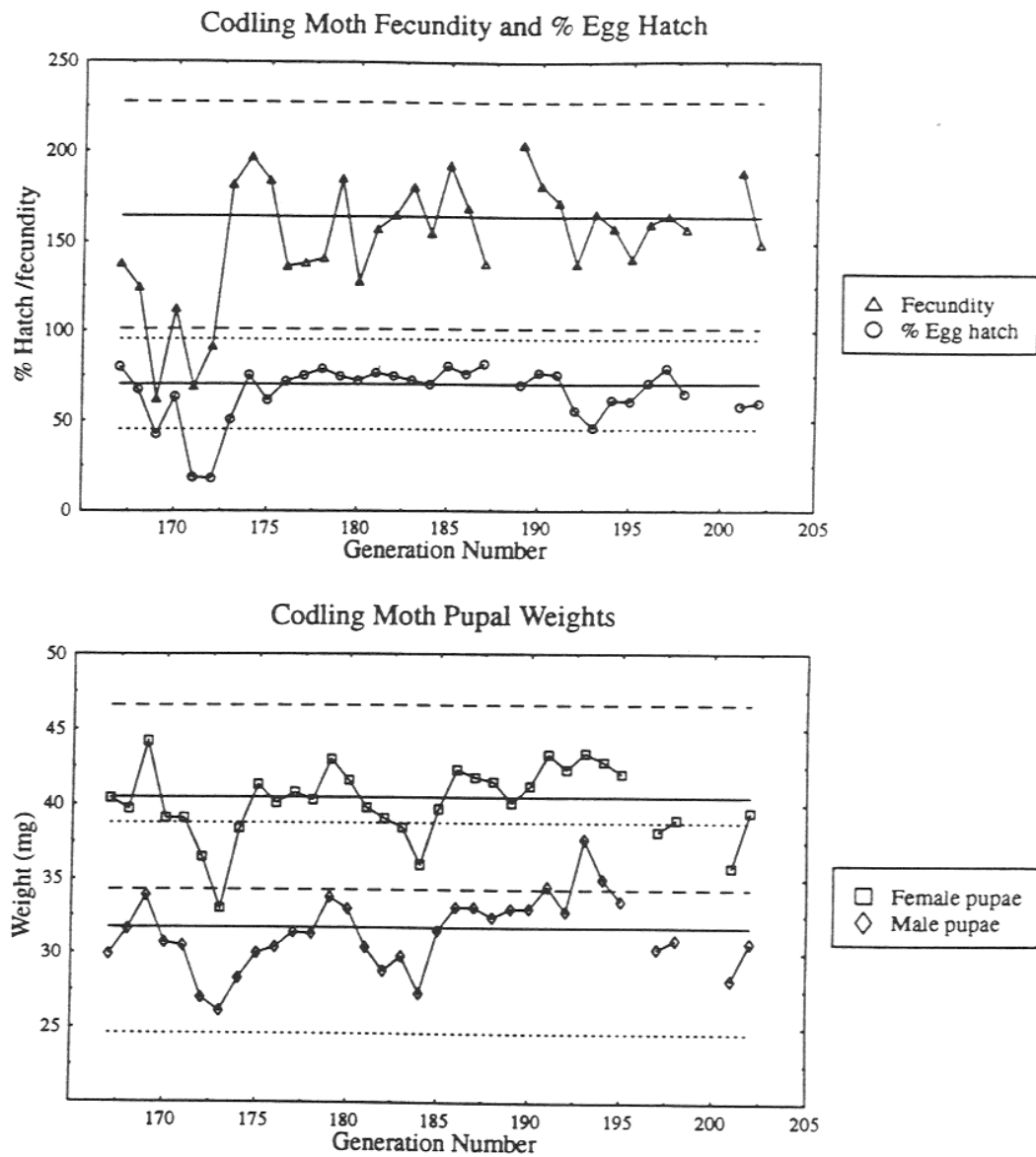


Figure 1. Codling moth fecundity, egg hatch and pupal weights (lab strain, sub-colony A). Means (solid lines) and control limits 3 standard deviations from the mean (dashed lines).

within the control limits, non-random drift towards one of the limits, and when a value has exceeded a control limit (Chambers and Ashley 1984). Table 1 includes data for the two lab sub-colonies from generation 173 to 202, a period of about 3.5 years. Apart from generation 188 the two sub-colonies have not been mixed. Mean values for the four important parameters listed are very similar.

The colonies have been successfully kept free of granulosis virus for the past five years by rearing larvae individually. This rearing method was initially used to eliminate low levels of virus but also prevents virus being introduced to the colony. In addition all eggs are surface sterilized in 10% formalin.

Table 1. Comparison of wild strain parameters with laboratory strains A and B (means  $\pm$  standard errors of the mean).

	Wild strain			Lab strain 173-202	
	Generation 5	Generation 8	Generation 10	Sub-colony A	Sub-colony B
Mean pupal weight of males (mg)	25.4 $\pm$ 0.5	26.4 $\pm$ 0.6	27.1 $\pm$ 0.6	31.7 $\pm$ 0.5	31.7 $\pm$ 0.4
Mean pupal weight of females (mg)	34.3 $\pm$ 0.8	36.0 $\pm$ 0.7	38.9 $\pm$ 0.6	40.5 $\pm$ 0.4	40.0 $\pm$ 0.5
Mean fecundity per female		184.7 $\pm$ 14.0	107.5 $\pm$ 22.6	164.3 $\pm$ 4.1	161.8 $\pm$ 4.6
Mean percent egg hatch		66.5 $\pm$ 7.8	80.0 $\pm$ 2.0	70.3 $\pm$ 1.7	69.5 $\pm$ 1.9

Process control is also regulated by check lists and colony records. For example, if low larval establishment is recorded, the insectary manager has information about who inoculated the larvae, which batch of diet was used, the rearing conditions and which generation the larvae came from. This data is then used to investigate quality problems. Also data and comments from researchers using insects provide specific information on production control and the product's "fitness for use" (see examples below).

### *Case Studies*

Diet deficiency. General Purpose Diet (GPD) was used for colony maintenance prior to generation 173. It was suspected diet deficiency was causing the large deviations from the mean (outside set control limits) around generation 170 on the fecundity, percent egg hatch and to a lesser extent pupal weight charts (Figure 1). Switching to the modified diet of Brinton *et al.* (1969) immediately increased both fecundity and egg hatch to an acceptable level but pupal weights on the new diet stayed low for one more generation (generation 173) before returning to normal. While this problem would have been obvious without collection of the data, quantifying the problem and subsequent plotting of the data provided solid information for decision making. The colony was maintained and insects supplied throughout this period from codling moths reared on trays containing a different modified diet of Brinton *et al.* (1969).

Material contamination. Data for generation 188 (Figure 1) is missing as part of the colony had 100% larval mortality. The wholemeal flour (a diet ingredient) purchased from a large health food company was probably contaminated with dichlorvos insecticide (used to control stored product pests around flour hoppers). As the contamination was not uniform, initially bioassays did not detect a problem with the flour. The colony was not completely lost and production only temporarily affected because fresh diet was immediately made with all new ingredients. In addition the sub-colony structure ensures a variety of stages are present and surplus eggs, pupae and adults are stored at lower temperatures. Carrying out bioassays on all diet ingredients is too expensive and in this instance was not very effective. To reduce the risk of future contamination, wherever possible natural ingredients are purchased from carefully screened suppliers, and the wholemeal flour used currently is organically produced Demeter™ certified (Anonym., 1993).

Response to pheromones. In orchards, lab strain tethered (held by a fine cotton thread) virgin females were able to attract and mate successfully with feral males. In field cages lab strain males did not fly from the release containers at dusk. Most were found still in the container the following morning. The reason for their



disinclination to fly is unknown. After considering these results, it was decided to establish a new colony of codling moths collected from organic apple orchards (wild strain). When laboratory-reared wild strain males were released in field cages, most flew from the release container; however, the level of mating with tethered virgin wild strain females was still low (Clearwater, pers. comm. 1993).

Response to  $\alpha$ -farnesene. Newly emerged codling moth larvae are attracted to  $\alpha$ -farnesene (Sutherland and Hutchins 1972), a fruit volatile released from the waxes on apples. Bradley and Suckling (pers. comm. 1993) have examined four behavioural attributes and quantified them for the lab strain reared for 211 generations, and the wild strain reared under the same conditions, three generations after field collection. The results show that the wild strain was significantly more active as shown by less random orientation, faster walking speed, more head turning and more head lifting. In the presence of  $\alpha$ -farnesene the wild strain was significantly more successful at finding impregnated papers than the lab strain and also showed a 40% increase in walking speed compared with only 8% for the lab strain. The lab strain has not been exposed to apples during rearing for over 200 generations which accounts for the decreased response to  $\alpha$ -farnesene, but lab strain neonate larvae are still able to establish on apples (Waddell, pers. comm. 1993). A simple test using  $\alpha$ -farnesene based on the work of Bradley and Suckling (pers. comm. 1993) will be investigated to monitor this behaviour as part of a quality control programme. It is also planned to introduce feral codling moths into the colony on an biannual basis to maintain quality.

#### *Quality Control Parameters for the Lab and Wild Strains.*

Table 1 displays wild and lab strain quality control parameters. The fecundity for generation 8 of the wild strain is within the expected range when compared to the lab strain, although the fecundity for generation 10 is lower. Percent egg hatch is similar for both strains. Pupal weights are lower for the wild strain for both males and females even though both strains are reared under identical conditions on the same diet. However pupal weights appear to be increasing for the wild strain. Each generation of the wild strain takes approximately five days longer to complete than

the lab strain using identical rearing methods. Since faster developing insects are used for rearing the next generation, this probably results in selection for quicker development. While measuring the above parameters indicates some difference between lab and wild strains, the most useful information for assessment of quality was obtained from the differences in behaviour to  $\alpha$ -farnesene and pheromones.

### Conclusion

Total quality control (Leppla and Fisher 1989) is a complete insectary management system that is driven by quality. Insect Rearing Management (IRM) is a very practical system that is more production oriented while maintaining an emphasis on quality. Thus IRM focuses on quality in terms of how the insects are used as well as measurements of fecundity, fertility and pupal weight. Heuttel (1976) suggests that quality should also be defined in terms of a particular set of behavioural traits, that could act as a suitable standard, independent of how the insects are used. These standards, once compared to feral insects (if possible), can then be used to monitor changes in lab populations. In the future, we plan to put a greater emphasis on total quality control principles that include monitoring a range of behavioural parameters.

IRM has (1) prevented many quality problems that could have arisen, by using check lists and good colony management (including virus prevention), (2) provided information so problems can be detected by charting performance parameters, communication with researchers using insects, and maintaining extensive colony records, (3) minimised the effects of problems through using sub-colonies which are at different stages of development and storage of insects, and (4) identified solutions by using quality control information to refine systems (e.g., organic flour), establishing the wild colony and recognising the need to monitor more behavioural parameters.

### Acknowledgements

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### **New Dosimetry Method to Assess the Quality of Irradiated Insects**

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#### **Abstract**

This report discusses a new production control method to accurately measure the dosimetry of irradiators used to sexually sterilize insects. Previous methods for calibration and dosimetry called for the use of ferrous ammonium sulfate (Fricke) solutions. This new method relies upon the use of radiochromic foils<sup>1</sup> that are commercially available. It provides a means of calibrating insect irradiators with a high degree of accuracy ( $\pm 10\%$ ). Advantages and disadvantages of this new method are compared with the previous one. In addition, implications associated with precision dose-mapping of insect irradiators and new ways of assessing the quality of irradiated insects are briefly highlighted.

#### **Introduction**

At the USDA-MOSCAMED mass rearing facility in San Miguel Petapa, Guatemala, quality control test results for mating propensity among factory-reared Mediterranean fruit flies (or "Medflies"), *Ceratitis capitata* (Wiedeman), were consistently lower than the minimum acceptable tolerance limit during most of 1990. Sterile flies were reported to be "sexually lethargic", and did not compete effectively with fertile flies. It was suspected that flies were

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<sup>1</sup> Mention of commercial products does not imply recommendation or endorsement by the United States Department of Agriculture or the National Institute of Standards and Technology, nor does it imply that the products identified are necessarily the best available for the stated purpose.

being overdosed with radiation, so production controls associated with the irradiator were critically examined.

At that time, Fricke dosimetry was used to measure the absorbed dose used to sexually sterilize the mass-reared flies. This dosimetry revealed that the minimum dose requirement of 145 Gy<sup>2</sup> was fully met, but gave indications that the flies received a dose much higher than the 145 Gy minimum necessary to achieve  $\pm 99.5\%$  sterility. Readings of 160 up to 185 Gy were recorded. However, there was no way to relate how much of the product actually received a given dose. It is noted in literature that dosage readings using Fricke solutions can vary as much as  $\pm 20\%$ .

One of the proposed solutions to poor insect quality was to alter the dose, but a question arose as to how. Taking into account that the dose could be  $\pm 20\%$ , a more reliable method of calibration was sought before making any changes. Whatever method is used, it must assure the customer that the insects have received the proper dose to achieve sterility, and at the same time show that the flies were not overdosed.

The search for a new method led the U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) to work with specialists at the National Institute of Standards and Technology (NIST), U.S. Department of Commerce (formerly the U.S. National Bureau of Standards) in Gaithersburg, Maryland, regarding standardized reference systems available for measuring radiation dose. Such systems can verify the dose administered and validate the production process used (McLaughlin, 1988). NIST chose to use radiochromic foils based on the dose range that commonly is used for insect sterilization, and that would provide assurance of the dose within  $\pm 10\%$ .

Through an interagency agreement, NIST agreed to dose map three USDA irradiators using radiochromic foils at a cost of \$1,000 per irradiator. The three irradiators are referred to as "Husman" irradiators after the USDA specialist, Chester N. Husman, who designed them specifically for insect sterilization. One of the units is located at the Medfly rearing facility in Guatemala, while two others are situated at the USDA facility in Hawaii. Isomedix, Incorporated of

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<sup>2</sup> The unit of absorbed dose = gray (GY) = 100 rads

Whippany, New Jersey, fabricated all three units in 1984. Approximately 42,000 curies of Caesium 137 were loaded into each irradiator.

#### **Previous Calibration Method - Fricke Dosimetry**

Fricke dosimetry measures absorbed gamma radiation dose using a ferrous ammonium sulfate solution. This method has been widely used over the years to calibrate industrial irradiators for medical and food processing purposes (Anonymous, 1959; Chadwick, K., D. Ehlermann and W. McLaughlin, 1977; Fricke, H. and E. J. Hart, 1966; Sehested, K., 1970). The American Society of Testing and Materials developed a standard method (E 1026-84) for preparation and use of Fricke dosimeters (ASTM 1984). Zavala, J. L. et. al.(1985) described the use of Fricke dosimeters in determining the absorbed dose to sterilize Medflies.

#### *Advantages of Fricke Dosimetry*

- Recognized as an industrial standard for calibration; used for calibration purposes in food irradiation for many years.
- Reliable at doses between 40 and 400 Gy.
- Solutions can be prepared and measured on-site.

#### *Disadvantages of Using Fricke Dosimetry*

- Ferrous ammonium sulfate solution is extremely sensitive to organic contaminants.
- Reagent grade chemicals must be used.
- Requires a properly calibrated high precision UV spectrophotometer to measure absorbance values.
- Resolution of the dosimeter is limited to the volume of solution contained in a vial rather than to a specific point. This gives a "ballpark" idea of dose administered, and only rudimentary dose mapping is possible.
- Fricke dosimeters are unstable ionic solutions that must be freshly prepared each time they are used.
- Solutions are acids and must be handled with care. They cannot be shipped easily because they are considered

#### *New Calibration Method - Radiochromic Foils*

A relatively new dosimetry film, GafChromic™ Dosimetry Media<sup>3</sup>, consists of radiosensitive film or radiochromic foil 6- to 7-  $\mu\text{m}$  thick with a sensitive emulsion coated on a 100- $\mu\text{m}$  thick polyethylene terephthalate base. As a dosimeter, it has been used for a variety of purposes including irradiator validation, dose mapping, and commissioning (McLaughlin, W. L., et. al., 1988). The following advantages and disadvantages are taken primarily from McLaughlin, W. L., et. al., (1991) and Soares, C. G., et. al., (1993):

#### *Advantages offered by foils*

- Color change proportional to absorbed dose.
- Reliable at doses ranging from 50 Gy to 1500 Gy.
- Can be read with a densitometer or visible light.
- Relatively easy to use; no mixing of acids. Available in small or large sheets that can be cut to desired size.
- At doses below 500 Gy, there is good consistency among different batches of film and different types of irradiation used for calibration.
- Emulsion requires no processing to develop image.
- Film stabilizes after 24 hours.
- Accuracy of dose interpretation  $\pm 5$  to 10% level (qualifies as Report of Special Measurement by NIST).
- Precision dose mapping is possible with resolution down to 100- $\mu\text{m}$  spot size stepped in 40- $\mu\text{m}$  increments in two dimensions, horizontally and vertically (Walker, M. L., et. al., 1992).

#### *Disadvantages and Limitations of Foils*

- At higher dosages (>500 Gy), foils respond more to electrons than to gamma rays.
- Sensitivity of the foil within any one batch may vary due to the variable thickness of the sensitive material coating.

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<sup>3</sup> Produced by International Specialty Products, 1361 Alps Road, Wayne, New Jersey 07470, telephone 201-628-4000



- Stability of the image is subject to variations in temperature (>55°C) and relative humidity.
- Optimum stability of unexposed and exposed films requires that they be stored in the dark at 22°C. Sunlight spontaneously colors the films.
- Care must be taken in positioning the foil during the irradiation and readout by a laser scanning densitometer or spectrophotometer. Film must be kept completely flat when read to provide optimal readings.

#### *New Procedures Using Foils*

ASTM (1993) established a standard practice for use of a radiochromic film dosimetry system. Radiochromic foil is available from commercial sources and it is produced in rolls or strips. The following outlines the general procedures that were used by NIST to map the Husman irradiators:

- Foils were cut into 125 mm squares, then circular discs were cut to the dimensions of the metal canister that is used to hold the insect pupae during irradiation. The canister is an aluminium cylinder 50 cm in length with a diameter of 13 cm. The leftover foil trimmings of the square were retained for quality assurance.
- Foils were placed horizontal to the canister's central axis at 16 different locations (Figure 1).
- Spacers between the films were cut and pressed from cardboard to a specific density equal to the insect pupa. These spacers filled the void and kept the foil horizontally flat and in a fixed position.
- Slight movement or rotation of the cylinder within the irradiator cannot be controlled externally; therefore, all films were analyzed with reference to the canister seam.
- NIST prepared the canister at their laboratory in Gaithersburg, Maryland. The prepared canisters were shipped to the Medfly rearing facilities in Guatemala (by mail) and Hawaii (via express carrier).
- Prepared canisters were placed into the Husman irradiators, then returned directly to NIST in Maryland using the same shipping method.
- Canisters were exposed for the time necessary to achieve ≥99.5% sterility of male and 100% sterility of female Medflies at a target dose of 145-150 Gy.

- NIST read the foils using a laser scanning densitometer.
- Each disk was sampled for 90 minutes to obtain approximately 240,000 readings that were reduced to 40,000 plotted points per disk. Sixteen disks required most of a week to read; review of data and rechecking took another week.

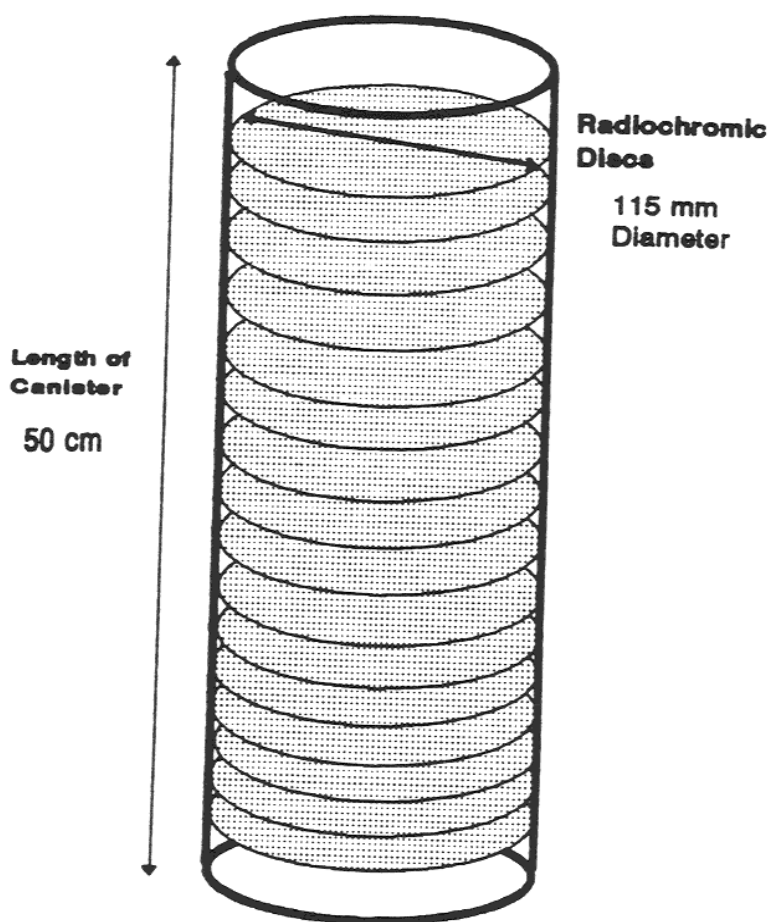


Figure 1. Irradiation canister. General position of radiochromic foil discs is shown. Discs were situated horizontal to the central axis of the canister.

### Results of Calibration/Dosimetry Outputs

A "Report of Special Measurement" was issued by NIST that included the following:

#### *Precision Dose Maps of the Irradiator*

The color-coded maps that are fairly easy to interpret (Figure 2).

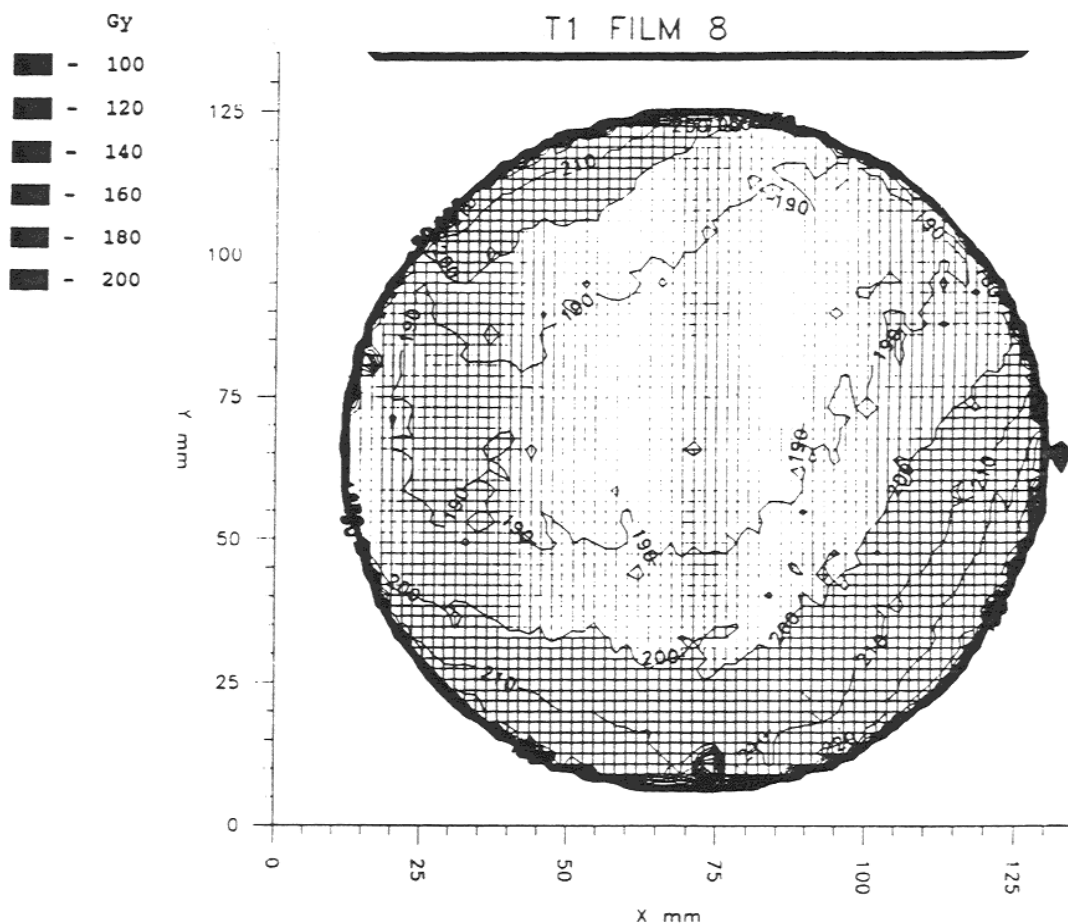


Figure 2. This is an example of a dose map generated by NIST. It graphically portrays the scanning results for one of 16 cross sectional discs used to map one irradiator. The intersection of each grid line represents the sum and mean of eight or more actual data readings. Contour lines show the division between various dose values. In color, it becomes easy to distinguish uniformity or asymmetry of dose.

This example shows only 1 of 16 cross sectional maps. Each map provides dose distribution within that specific plane; collectively, the maps give a picture of dose distribution throughout the canister. Isodose lines or curves are displayed showing the demarcation between different doses. Dose maps can be plotted in two- or three-dimensions. Each map represents thousands of measured data points.

#### *Frequency Tables*

The percentage of data points is shown within a range of doses for each film (Table 1). They are useful in examining how the dose is distributed within each cross section. These tables provide a rapid means of comparing the dose values within different portions of the canister. This table can be used to manipulate the position of the product within the irradiator to take advantage of desired dose ranges or to avoid undesirable ones.

#### *Histograms*

The percentages of data points are indicated that fall within a particular dose range for the entire irradiator (Figure 3). Although they do not show the actual distribution within the canister, they provide a quantitative picture of dose distribution for treated insects.

#### *Issues/Questions Raised by New Method*

- As seen in the figures and table, the dose within the Husman irradiator is not uniform. Dose varies throughout the canister. A gradient exists that is highest nearer the sources.
- Different policies are followed by facilities with regard to irradiation schedule. In some instances, facilities use the average dose while others use a minimum dose.
- A random sample size of 100 individuals (the amount commonly used for various QC tests) would not accurately reflect the quality of the majority of irradiated insects.
- Should sampling of insects be skewed toward those regions that are more uniform in dose or closest to the target dose?
- Should samples be selectively taken or should the pool of insects be thoroughly mixed prior to sampling?

Table 1. This table describes the distribution of discs within the canister and the dose values recorded for each disc. Dose values relate to the position of caesium sources in the irradiator. Low doses at the extremes can be avoided by placement of styrofoam spacers in the canister.

T1 DOSE RANGE PERCENTAGE PER FILM, CANISTER DEPTH, cm																
Gy	4.1	5.9	8.1	10.2	12.2	15.5	19.1	22.5	25.9	29.2	32.5	35.8	39.1	41.2	43.2	45.2
100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
110	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
130	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33
140	24	1	0	0	0	0	0	0	0	0	0	0	0	3	1	32
150	32	38	0	0	0	0	0	0	0	0	0	0	0	48	35	17
160	19	26	0	0	22	0	0	0	0	1	0	0	5	22	27	11
170	13	17	32	21	36	9	0	0	0	43	11	16	43	15	17	4
180	5	10	29	36	20	45	37	32	25	25	42	37	20	7	12	2
190	3	4	17	18	12	19	28	33	35	16	19	18	14	3	4	1
200	2	2	11	13	4	14	18	19	21	7	15	13	8	1	2	0
210	1	1	5	5	2	6	10	9	11	3	5	8	3	1	1	0
220	0	0	2	2	1	3	3	3	4	2	3	3	2	0	0	0
230	0	0	1	1	0	1	1	1	1	1	2	2	1	0	0	0
240	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0
250	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
260	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
270	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
280	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
290	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

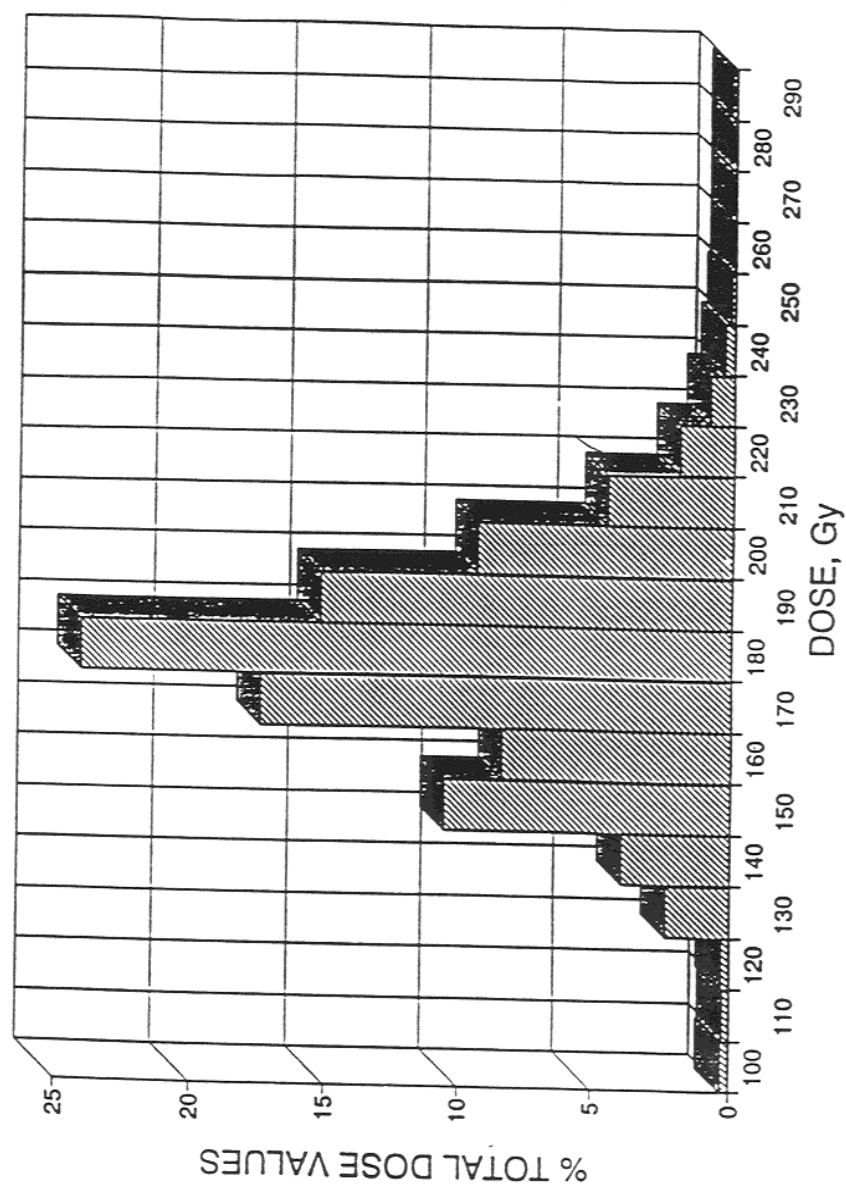


Figure 3. This histogram was generated from a frequency of dose values registered from all 16 cross sectional discs for one irradiator. Very few values fell below the target dose of 145 Gy; however, the mean dose values lie at 180 Gy which could adversely effect insect quality.

- Taking into account that the geometry of the irradiator cannot be changed, is it appropriate to correlate quality to dose administered and simply calculate how many insects received an optimal dose and what portion becomes either underdosed or overdosed?
- Can the canister be modified in shape or design to provide for a more uniform dose distribution? For example, reduce the circumference to eliminate the variation along the outer edges. Spacers already are used inside the canister at each end to compensate for the lower dose values. This reduces the maximum-minimum dose ratio, but lowers the throughput. The reduced volume could be significant at a mass production facility. Another option would be to rotate the cylinder using some sort of mechanical adapter.
- What should the tolerance range of acceptability be for irradiation of insects used in SIT programs? Is  $\pm 10\%$  acceptable? Is a lower tolerance possible? Would a higher tolerance suffice?
- Where should the indicator label be placed to assure the customer that the minimum dose level has been administered?

#### *Future Plans*

- Continue to work with NIST to develop quality assurance procedures that can be used locally.
- Correlate the effects of a specific absorbed dose to the quality of an individual insect.
- Quantify adverse effects of irradiation for each batch of insects sterilized
- Look at changes in irradiation procedures, schedules, policies, etc.
- Examine minor mechanical and geometric changes in the canister.
- Use dose maps to improve the design of new insect irradiators.
- Examine a lower dose to increase competitiveness of the sterile insect against the feral population.

#### **Conclusions**

Radiochromic film dosimetry met our expectations with regard to ease of use, stability, consistency, accuracy ( $\pm 10\%$ ), and traceability. Perhaps the greatest advantage stems from the detailed dose mapping that is possible along with the

dose frequency tables and histograms that provide a new, improved method for assessing quality of irradiated insects used in pest control.

This new method of dosimetry clearly indicates that existing quality control systems can be improved. Measurements of quality are based on relatively few individuals compared with the millions that are produced and irradiated each week. Scale of production limits the degree of sampling but, hopefully, the quality control test is sensitive enough that minor problems can be detected and corrected before they become severe. Quality control results should be indicative of the majority of insects that are mass produced and variability that results from procedures such as irradiation should be minimized.

#### Acknowledgements

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### **Methods Proposed to Apply Quality Control in the Mass Rearing of *Diachasmimorpha longicaudata***

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#### **Introduction**

The species *Diachasmimorpha longicaudata* (Ashmead) is an endoparasitoid that was taken to Hawaii to control the invasion of *Bractocera dorsalis*. After being released, it was one of the more frequent of three species of parasitoid caught (Bess et. al. 1961) and, as a consequence, it was mass reared.

The mass rearing of this parasitoid has allowed it to be release in different parts of the world to suppress fruit fly species (Greany et. al., 1976). Since the beginning, however, this parasitoid has been found parasitizing *Anastrepha* spp. more often than other genera and, at present, it has more potential of being used to suppress *Anastrepha* spp. populations.

In the Parasitoid Mass-Rearing Laboratory of the Mexico Fruit Flies Program, Metapa de Dominguez, Chiapas, Mexico, there is a five-year-old colony of *D.longicaudata*, with a projected weekly production of 50 million parasitoids which will be distributed along the whole country through mass releases.

The mass rearing methods are based on those used in Hawaii, by Wong et. al. (1991) and they are considered highly suitable. However, the methods lack production quality control, which would be useful for assessing their effectiveness and for evaluating the produced parasitoids. The main problem has been a need to monitor the development of immature parasitoids inside the puparia of their hosts. There are two relevant aspects which affect the development of methods to measure quality control: 1. Observations and measurements on the adult parasitoid

and 2. A lack on knowledge about the biological features of *D.longicaudata* which would serve as a basis to compare the quality of the produced parasitoid.

This paper describes some tests proposed to serve as the initial basis for developing quality control tests for *D.longicaudata* mass reared at the laboratory in Mexico. It reports results that have been suitable for the mass rearing process and for the more important biological aspects of the adult parasitoids produced.

### **Materials and Methods**

The methods achieve quality control of the processes and the adult parasitoids as final products of the mass rearing of *D.longicaudata*.

#### *Measurement of the Mass Rearing Process Quality*

Mass rearing of *D.longicaudata* has been carried out using host *Anastrepha ludens* larvae reared at the Fruit Flies Laboratory, in Metapa de Dominguez, Chiapas, Mexico. Larvae exposed to parasitoids were third instars, of eight, nine and ten days of age, on which the following measurements were taken: 1. A sample of 10 ml of larvae was taken by lot produced and exposed to the parasitoid, and the number of larvae in this volume was counted, 2. The same sample was weighted to obtained the average weight of each larva, 3. Naked larvae were counted taking the total volume by lot and the total number of larvae obtained by extrapolating the number of larvae in the sample to the total volume.

#### *Measurement of the Exposure to Parasitoids*

The *Anastrepha* naked larvae were mixed with diet to be exposed in the parasitization units. An amount of this mixture is taken to form the units. After this operation is ended, the number of units is counted to determine the density of larvae per unit.

#### *Measurement of Emergence and Parasitism*

After exposure, larvae complete their development in the diet, the puparium is formed, and the parasitoid matures. When larvae reach the age of 16 days, 24 hours before emergency, a sample of 1000 pupae is taken randomly per lot. The

volume of this sample is measured and to the total volume of pupae is extrapolated to obtain total production.

#### *Emergence and Parasitism Percentage*

The 1000 pupae sample per lot is placed in a container inside a Hawaii-type cage where parasitoids and/or flies emerge. After the productive period of the cage, 5 days of pre-oviposition and 10 days of oviposition, the number of live parasitoids, dead parasitoids, and emerged flies is counted. Sex-ratio and emergence percentage is determined. With the number of emerged insects, the parasitism percentage is obtained applying the following formula:

$$\text{Parasitism percentage} = \frac{\text{Number of emerged parasitoids}}{\text{Total number of emerged insects}} \times 100$$

#### *Quality Control of the Adult Parasitoid*

A sample of 70 recently-emerged adults (35 females: 35 males) is taken from each lot produced and placed in a Hawaii-type cage from the first day until day 20, then data on daily mortality is registered. This sample is also placed in each parasitization unit with 400 larvae of *A. ludens*. Larvae are held until emergence as adult occurs, in order to obtain data on production. The cage is discarded when females reach 20 days of age.

A sample of 20 adult parasitoids is taken (10 females: 10 males) from each lot of production and placed in a Hawaii-type cage. When female parasitoids reach the age of five days, 100 larvae of *A. ludens* are exposed to them in a parasitization unit. This exposure is carried out for four hours, during which observations are made every hour on the number of female parasitoids seen on the unit and the time required for 50% of the females (5 females or more) to start oviposition. Exposures are made during the three following days and every 24 hours the observations are repeated. Exposed larvae are held until they emerge. Results of parasitism are correlated with measurements taken through these observations.

#### *Specific Preference for the Host*

This measurement is done once a month with a production lot taken randomly. Adult parasitoids (200) are placed in four Hawaii-type cages, each with 50 parasitoids (25 females: 25 males). Larvae are exposed when female parasitoids reach five days of age. A parasitization unit containing 100 larvae of either *A.ludens*, *A.serpentina* or *C.capitata* is placed in one of the first three cages. In the fourth cage, three units are used, each with 30 larvae of one of the three fruit fly species. The exposed larvae are kept in diet and moved to closed containers with vermiculite where the parasitoids develop. From these same containers, the results of emergence are taken and used to differentiate percentages of parasitism from each host species.

#### *Data Analysis*

The mean and standard error are calculated from data obtained in the measurements. To detect differences between produced lots, analysis of variance and the Tuckey Multiple Range Test ( $P=95\%$ ) are calculated. Data obtained on rapidity to meet the host is correlated with percentages of parasitism.

### **Results**

#### *Quality of the Mass Rearing Process*

Results obtained during evaluation of the parasitoid mass rearing is in Table 1. These data correspond to average values with different ages of host larvae. There is an increase in larvae size and weight as age increases and, therefore, a decrease in the larval density by unit. The most indicative parameters of these differences are those related to pupation percentage, which increases as the larva becomes older. However, emergence percentages and parasitism decrease when larvae age. Pupation is favored when the exposed larvae are more mature and more prepared to pupate. Emergence and parasitism decrease when more mature third instar larvae are used because they are less preferred by the parasitoids. If oviposition takes place, conditions are not favorable for development of the eggs (Lawrence, 1982).

#### *Quality Control of the Adult Parasitoid*

Average data obtained in tests for longevity and reproduction are included in figures 1 and 2. Longevity of the colony decreases 10% at the fifth day when exposition starts, and mortality is close to 40% on the fifteenth day when parasitoids are no longer reproducing. Reproduction increases during the period of five to 15 days due to greater oviposition activity (Ashley and Chambers, 1979). However some recuperation starts after the fifteenth day (Personal observation). Conserving the colony after the 15-day period has some operational problems because maintaining the cages within the colony requires space.

The number of females observed on the parasitization unit by day and by hour are in Table 2. In general, there was a greater number of females posing on the units with larvae as the time passed, particularly the number of females posing by hour and by day. The activity was more remarkable during the second and third day, although in the last hours of these days a decrease in the frequency of posing started. It may be a consequence of the saturation of hosts available for oviposition. The average increase of the activity of female parasitoids on hosts is greater by hour every day. Lawrence et. al. (1978) found that *D. longicaudata* have more oviposition activity when larvae are exposed constantly, and at a younger age of the parasitoid. It may be due to the fact that there is a stimulus to develop eggs in females.

#### *Specific Preference of the Host*

Results on this parameter for *D. longicaudata* on the three Tephritidae species are in Table 3. This parasitoid strain has more preference for *Anastrepha* spp. as hosts with greater emphasis for larvae of *A. ludens*. Such preference may be a result of keeping *A. ludens* larvae as a host. They show more remarkable differences in size and movement compared with larvae of *C. capitata* and *A. serpentina*. Results obtained when different species were used as hosts in the same cage presented the same tendency and the percentages of average parasitism were similar.

Table 1. Quality control parameters (SE) of mass-reared *Diachasmimorpha longicaudata*

Parameter	Results		
	8	9	10
Larvae age (days)			
Larvae in 10 ml (No.)	355.6( 8.5)	380.6( 9.1)	361.1(14.7)
Larvae weight (mg)	12.2( 0.7)	25.9( 0.9)	26.6( 0.8)
Parasite density (larvae/unit)	846.6(45.4)	712.6(33.2)	679.4(69.1)
Pupation (%)	62.1( 4.2)	65.9( 2.1)	74.5( 4.2)
Emergence (%)	50.4( 2.3)a	50.5( 2.9)a	39.8( 5.5)b
Parasitism (%)	76.3( 3.4)a	68.9( 5.6)ab	54.6( 7.2)b
Sex ratio (Female:Male)	1.8:1	1.5:1	1.9:1

Numbers followed by the same letter indicate non significant difference (Tuckey Multiple Range Test, P = 95%)

Numbers without letter indicate non significant difference (Tuckey Multiple Range Test, P = 95%)

Table 2. Mean number (SE) of mass-reared *Diachasmimorpha longicaudata* females posing over the parasitism unit per day and per hour.

Day	1	2	3
Per day	0.86(0.2)a	2.02(0.3)b	3.53(0.6)c
1st hour	0.80(0.3)d	3.50(0.6)f	6.70(0.8)g
2nd hour	1.66(0.5)de	4.26(0.7)f	7.46(0.8)g
3rd hour	2.03(0.5)de	4.70(0.7)f	7.33(0.8)g
4th hour	2.46(0.5)e	4.66(0.6)f	6.76(0.8)g
Per hour/day	1.74(0.5)h	4.25(0.6)i	6.96(0.7)j

Numbers followed by the same letter indicate non significant differences (Tuckey Multiple Range Test P = 95%)



Survival ( $l_x$ )

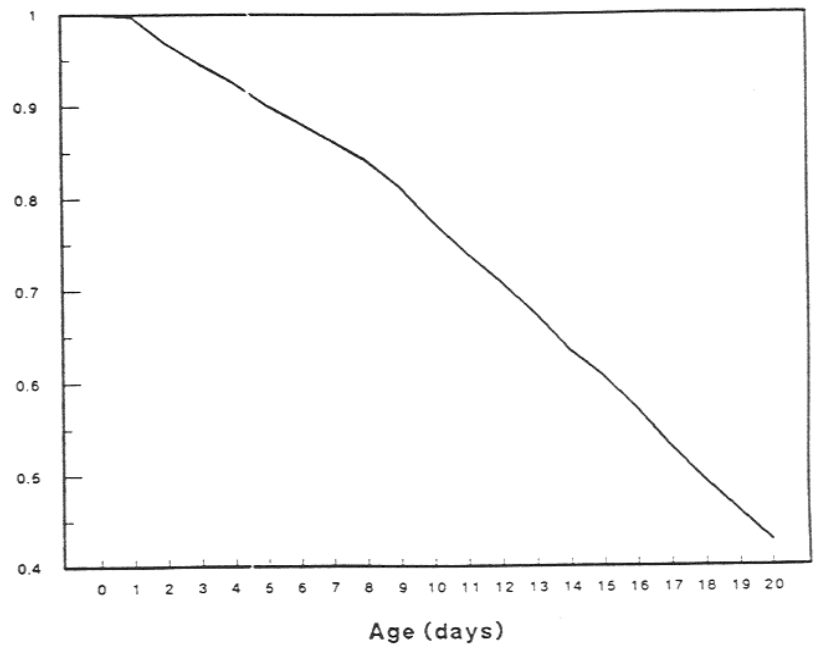


Figure 1. Survival of *Diachasmimorpha longicaudata*.

Offspring/female ( $m_x$ )

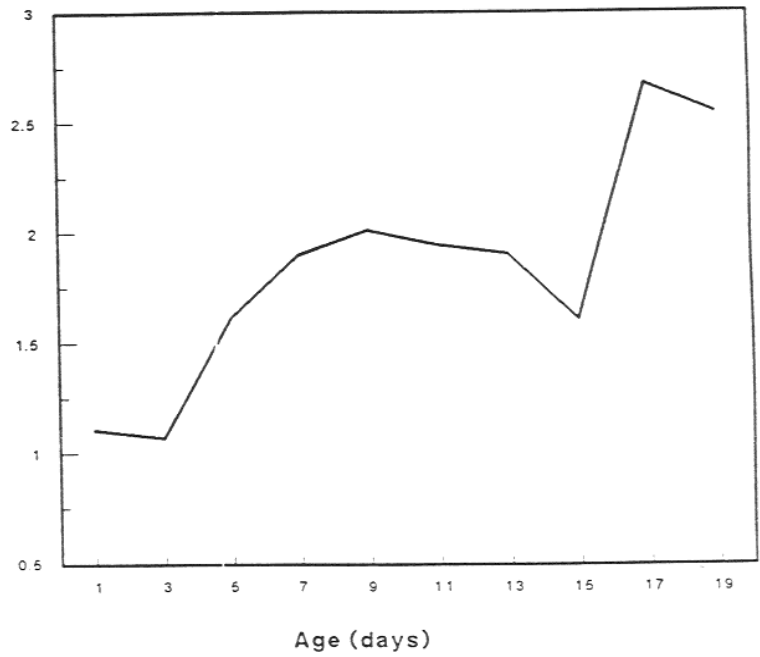


Figure 2. Female offspring per female of *Diachasmimorpha longicaudata*.

## Discussion

Methods proposed to evaluate the quality of *D. longicaudata* mass rearing process at the laboratory level were based on experiences obtained during five years of production of this parasitoid at the Parasitoids Mass Rearing Laboratory in Metapa de Dominguez, Chiapas, Mexico. They are also based on results of the different tests and routine production data. To verify operational feasibility, the biological aspects suitable for evaluating the parasitoid must be verified. Results obtained with these methods provide necessary and indicative information on the correct application of the steps of the mass rearing process and its consequences on the quality of the produced parasitoids.

Measurements of the mass-rearing processes allow optimization of the production of hosts and parasitoids. Parasitoids eight days of age are the best for production. However, it will be necessary to keep a constant control of variations in their weight. Results on evaluation of adult quality can be used to indicate the effectiveness of the adult parasitoid produced under laboratory conditions. Methods designed to evaluate longevity and reproduction by lot produced results similar to those reported by Ashley and Chambers (1979). However, some lots with low or high longevity and reproduction were also detected and this may be related to performance of adults in the field.

The method used to assess the rapidity to meet the host is directly related to reproduction (Fig. 3). The importance of assessing this ability in the laboratory is evident, since it is possible to obtain different responses with the evaluated lots. This ability increases as females are more experienced with the exposed larvae, as reported by Lawrence et. al. (1978). Rapidity to meet the host increases as the time runs until the end of the third day and then declines. It may be because females rapidly develop this ability, which allows most of the exposed larvae to be oviposited and females to finish eggs contained in their ootecs. They also may notice the high density of oviposited or over-parasitized larvae using the sting as a test (Greany et. al. 1977). The most important result of this method is that the time required to perform the test of rapidity to meet the host shows a range of ability to search the host.

Table 3. Percentage of parasitism (SE) in three Tephritidae species by mass-reared *Diachasmimorpha longicaudata*.

Unit	<i>A. ludens</i>	<i>A. serpentina</i>	<i>C. capitata</i>
No choice	77.8(4.2)a	66.1(3.9)a	43.1(4.8)c
Choice	87.1(2.5)a	63.2(3.5)b	43.2(5.3)c

Numbers followed by the same letter indicate non significant differences (Tuckey Multiple Range Test P = 95%)

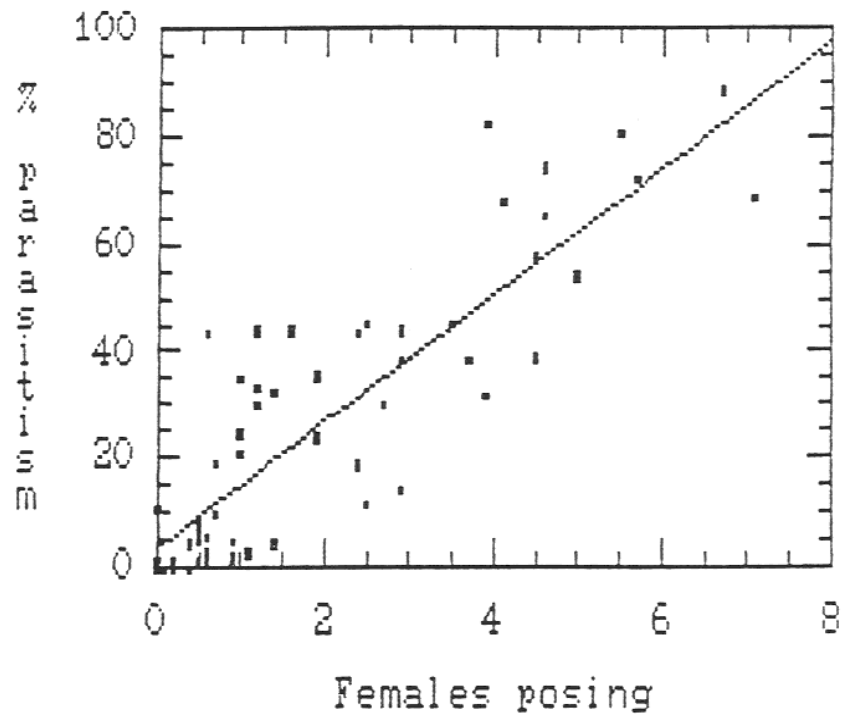


Figure 3. Relation between females posing and parasitism.

The proposed methods were designed to evaluate the quality of the *D. longicaudata* mass-rearing process and the adult parasitoid as a final product.

They included temporal evaluation of the colony through sampling to learn preferences of this parasitoid for different species of host larvae. This study, applying these methods, reports a preference for *A. ludens* as a result of keeping this strain exposed for five years to these larvae as hosts. Nevertheless, *D. longicaudata* exhibits considerable parasitism on larvae of *A. serpentina*, a pest of economic importance within the *Anastrepha* genus. The test should be applied using a sample of the colony taken randomly every month to maintain a reference on preference of the adult parasitoids to the host species. Based on these results it is possible to make some changes in the colony. However, it is necessary to work on the design of sampling methods to determine the size of the sample by lot produced and to obtain reference standards. However, it is also necessary to design methods to determine the effectiveness of the parasitoid in the field.

#### Acknowledgements

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### **Mating Propensity of *Diachasmimorpha longicaudata***

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#### **Abstract**

Two rapid and efficient techniques were developed to determine the mating propensity of female *Diachasmimorpha longicaudata*. The first method involved one hour observations of courtship in cages in the laboratory, and the second was by examination of the spermatheca of field released females. In the laboratory, the optimum age for mating females was  $54.2 \pm 14$  hours. Three days after placement of parasitized puparia in the release buckets, relatively high levels of mated females were observed (58 and 75% in the two field sites). The potential application of each method for future quality control protocols is discussed.

#### **Introduction**

The opiine parasitoid, *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) is a promising agent for biological control of more than 17 species of fruit flies (Knipling, 1992). Currently, the potential of opiine parasitoids for augmentative release is being studied in the United States (Hawaii: Purcell, unpublished, Florida: Sivinski, pers. comm., and Mexico: Wharton, pers. comm.), in Taiwan (Yao, 1989), Costa Rica (Camacho, 1990) and Spain (Jimenez and Castillo 1992).

A high level of mating would increase rates of population growth following augmentative or inoculative releases of parasitoids. This would be particularly important in areas where this parasitoid had not been established previously.

Relatively little information on mating behavior of *D. longicaudata* is available. In regard to reproductive strategies, studies have focused on males. For example, it has been shown that males use acoustical signals in courtship (Sivinski and Webb 1989). Ramadan *et al.* (1991) reported that male *D. longicaudata* copulated immediately upon emergence, but that mating activity significantly increased by four days of age.

One goal of the Hawaii pilot test with *D. longicaudata* for suppression of the oriental fruit fly, *Bactrocera dorsalis* (Hendel) was to evaluate the mating propensity of parasitoids released in the field. This parameter could be integrated with other quality control tests. Currently, quality control tests include determinations of sex ratio, emergence rates and flight ability (Purcell, *et al.* in prep.).

## Materials and Methods

### Source of Insects

*D. longicaudata* were reared at the USDA-ARS Tropical Fruit and Vegetable Research Laboratory, Honolulu, according to the methods of Wong and Ramadan (1992). Adult *D. longicaudata* were exposed to 5-day-old third instar *B. dorsalis* larvae for three hours. The larvae were held until pupation, then shipped to Kauai on a weekly basis for field release using standardized packing and shipping procedures (Purcell, *et al.* in prep.).

### Laboratory Observations

During an 11-week period (between May 7 and July 16, 1993), 10 grams of parasitized puparia were randomly subsampled each week from a larger population, and transferred to a transparent Plexiglas cage (30 cm x 30 cm x 0.5 cm). The cage front had a 15-cm diam. opening, fitted with a nylon mesh sleeve and a 20-cm diameter top hole, covered with a #16 mesh screen (15 per inch = 6.3 per cm<sup>2</sup>). Caged puparia were held in the laboratory at 24.4±2°C, 67±7% RH and a photoperiod of L:D=14:10 hours. A vial of water with cotton wick was provided inside the cage, and a food source of spun honey was applied to the top screen of the cage.



Each day, newly emerged males were aspirated from the emergence cage into a separate cage with a food and water source. Occasionally, female emergence overlapped with males (typically, males emerge two days before females). When this overlap occurred, these individuals were discarded. Age of males was  $3.4 \pm 0.3$  days for mating studies. On the first day of eclosion from puparia, virgin females were aspirated into separate holding cages (20 females per cage), constituting three age categories. In group I, the mean age ( $\pm$ SD) was  $24 \pm 6$  hours, in group II, the mean age was  $41 \pm 2$  hours, and in group III, the mean age was  $54 \pm 14$  hours.

Three Plexiglas cages (identical to the above cages), one for each age category, were used for mating observations. Each experiment was initiated at approximately 0800 hr. First, ten males were aspirated into each cage, then allowed to "acclimate" for 15 minutes before introduction of 20 females.

Mating activity was observed for one hour, which was divided into six, ten minute segments, according to methods used to test mating propensity of Mediterranean fruit fly, *Ceratitis capitata* (Weidemann) (Brazzel et al. 1986). When a pair of wasps copulated, they were removed from the cage. Thus, mating pairs were only allowed to mate once. If copulation occurred within the first ten minutes, it was recorded in time segment I. A pair mating in the second ten minute time period was marked in time segment II. This continued for all six time segments to complete the one-hour test. A pair that mated within the first ten minute time segment was considered to have a higher mating propensity than a later mating pair. Therefore, each of the six time segments had a weighting factor associated with it. The number of pairs in time segments I to VI were multiplied by a weighting factor of 100, 50, 33, 25, 20, and 15, respectively. The values from all six time segments were added together for each cage. This became the index value for each age and cohort. The mating index (MI) was calculated by totaling the index values and dividing this sum by the total number of possible mating pairs. The formula (Brazzel et al. 1986) was as follows:

$$MI = \sum (MP_t \times WF_t) / TMP$$

where,  $MP_t$  = number of mating pairs at time  $t$ ,  $WF_t$  = Weighting Factor at time  $t$ , and  $TMP$  = total possible number of mating pairs.

Each one hour observation test was repeated with two subgroups in each cohort. Each cohort of parasitoids (constituting different generations of the colony) served as the replication unit; nine, five, and seven cohorts were tested for age groups I, II and III, respectively.

#### *Field Released Females*

On the same dates as the laboratory tests, the proportion of inseminated females was determined from two field release sites: the first, a 160 ha commercial guava orchard, and the second, a 65 ha. section of wild guava forest. Peak emergence of female *D.longicaudata* from puparia within the emergence buckets generally occurs three days after release (Purcell, unpublished data). Four to five females were collected from inside each of five containers, three days after the release. Assuming that females leave the bucket within a few hours after emergence, these females were presumably less than one day old, although some could have been up to three days old. These females were then kept alive for 24 hours before dissection to allow sufficient time for sperm to reach the spermatheca. (Ramadan *et al.* 1991). The females were then dissected in saline; the spermatheca of individual females was examined under a compound microscope at 40x magnification to determine the presence or absence of sperm.

The general linear model, ANOVA, was used to determine the effect of age group on the percentages mated and the mating index (MI). The least squared means *t* tests were performed to make pair-wise comparisons between age groups (SAS, 1988). In addition, the relative percentages of inseminated females in the two fields were compared using the same statistical tests.

### **Results and Discussion.**

#### *Laboratory Observations*

The age of females significantly affected their mating propensity in the cage studies ( $F = 13.4$ ;  $df = 2, 25$ ;  $P = 0.0001$ ). The percentage of mated females in age group III was 3.3 times higher than age group I and was 1.8 times higher than age group II (Table 1).

Table 1. Percentage of female *Diachasmimorpha longicaudata* mated in mating cages and mating index.

Age grouping	Mean age (hr)	n	Percentage mated	Mating index
I	24.2	90	17.8 a	11.4
II	41.4	50	32.0 a	17.6 a
III	54.2	70	58.9 b	36.7 b

Means within a column followed by the same letter are not significantly different (General Linear Models Procedure, planned *t* tests, least squared means option, SAS 1988).

In addition, the mating index of age group III exceeded the indices in age groups I and II ( $F = 9.1$ ;  $df = 2, 25$ ;  $P = 0.001$ ) indicating that mating speeds were faster. However, in all age groups, when counting only mated females, over 75% of these matings occurred within the first 30 minutes and over 90% of the matings occurred in 40 minutes (Fig. 1).

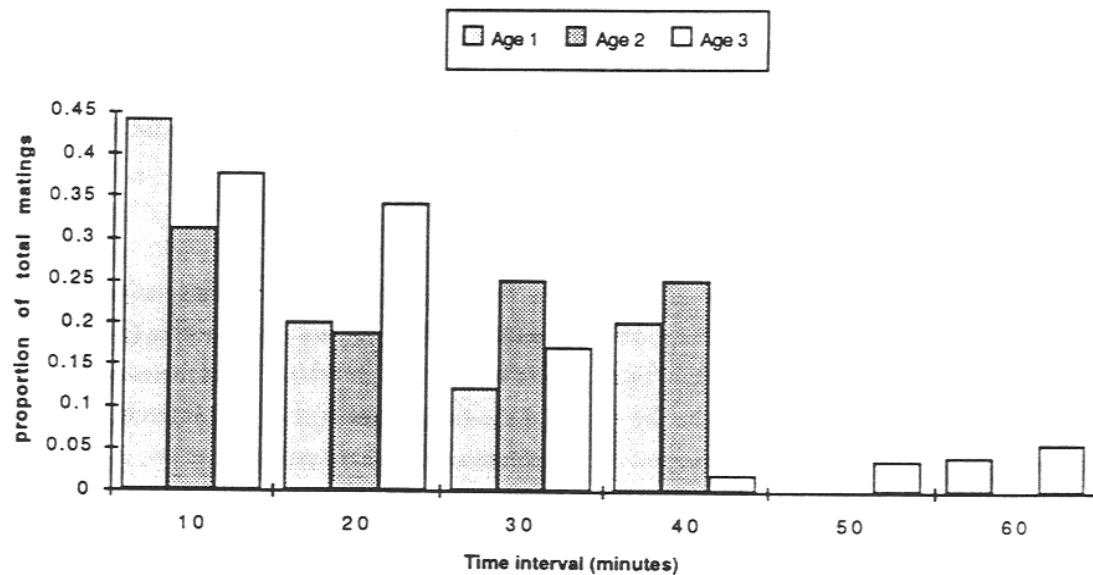


Figure 1. Proportion of mated females in three age groups versus time segment.

Therefore, when females mated, it occurred quickly. Although not quantified, most females in age group I actively rejected the males attempting to copulate by pushing them away or moving in the other direction. This suggests the optimal age range for mating in *D. longicaudata* females was between  $54 \pm 14$  hours. In contrast, Ramadan *et al.* (1991) reported that the majority of caged females were inseminated on the first day of eclosion (sperm was present in the spermatheca). However, a small percentage consistently rejected males until the third and fourth days. They concluded that the ideal age for 100% insemination was 3.3 days. In their study, females were exposed to males for 24-hour periods before they were dissected; thus the time for mating was much greater than in our studies (1 hour). This may explain the different outcomes.

We observed no differences in the percentage of mated females due to cohort tested in any age group. However, the mating index (reflecting speed of mating) differed by cohort for age group I ( $F = 4.9$ ,  $P = 0.05$ ) and age III ( $F = 23.1$ ,  $P = 0.04$ ). This may indicate that mating behavior of *D. longicaudata* does vary between different generations of parasitoids. Sex ratio (male:female) of resulting progeny collected in the fruit during these weeks was male biased in both the orchard guava (2.3:1) and wild guava (5:1), which indicates less than optimal mating activity. In the insectary, male to female sex ratio is generally female biased (0.6: 1). This indicated that mating levels in the field may have been lower, although further tests are needed.

#### *Field Released Females*

A higher percentage of females collected from inside the release buckets was inseminated in the wild guava (75%) versus the orchard guava (54 %) sites ( $F = 11.8$ ,  $df = 1, 4$ ;  $P = 0.03$ ). Only two cohorts were tested in the orchard; therefore, these differences may only be the result of the small sample size. No differences between cohorts were observed at either release site (orchard guava:  $F = 1.0$ ,  $df = 1, 8$ ,  $P = 0.3466$ ; and wild guava:  $F = 0.42$ ,  $df = 3, 13$ ;  $P = 0.75$ ).

The laboratory and dissection methods described herein were both efficient for quantifying the mating propensity of female *D. longicaudata*. These methods can also be used as a spot check in laboratory mass rearing colonies, to maintain

desired sex ratios. In addition, these techniques could also be used in conjunction with field release tests. We believe that the dissection of females was more useful than the laboratory test, because field released females were directly tested. However, the dissection method requires significantly more skill and equipment (i.e., a compound microscope, facilities for keeping parasitoids alive for 24 hours). This, could be a limiting factor in some cases. If a skilled person is available, the time to complete either the laboratory observations or dissection technique took about the same amount of time (ca. five hours per week).

The mating tests we describe in this paper should be incorporated into the routine quality control tests performed on mass reared parasitoids in the insectary (lab test) and on released wasps (dissection test). In the future, we plan to develop recommended mating indices for *D. longicaudata*. If mating indices of a particular cohort of parasitoids falls short of the recommended range, it could alert researchers to potential problems with a colony or release.

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### **Improving Quality Control of Mass Reared Opiine Parasitoids for Augmentation Against Tephritid Fruit Flies in Hawaii**

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#### **Abstract**

Three species of braconid parasitoids (*Diachasmimorpha longicaudata*, *D. tryoni*, and *Psytallia fletcheri*) were reared in insectaries in Honolulu, Hawaii and shipped to the island of Kauai for augmentation projects against tephritid fruit flies. Pre- and post-shipment measurements showed positive correlations between puparium weight and parasitoid emergence for all three species, and between puparium weight and sex ratio (percent females) for the two *Diachasmimorpha* species only. Shipping and handling significantly decreased percent emergence of all species. Fluorescent dyes applied to puparia decreased emergent adult longevity but increased the percent emergence from puparia. Reducing shipment time, decreasing shipment temperature, and modifying the design of field release vessels all improved parasitoid quality.

#### **Introduction**

Classical biological control programs have introduced many species of opiine braconid parasitoids into Hawaii to control three major tephritid fruit fly pests: the melon fly (*Bactrocera cucurbitae* [Coquillett]), the Oriental fruit fly (*B. dorsalis* [Hendel]), and the Mediterranean fruit fly (*Ceratitis capitata* [Wiedemann]). At least six of these parasitoids are quite effective at regulating fly populations throughout the state (Clancy et al. 1952). However, tephritids remain important

quarantine pests preventing export of Hawaiian crops, and research is still underway to develop better methods to eradicate or control fly populations.

There have been steady advances in techniques for insectary rearing of opiine parasitoids (Greany et al. 1976, Ramadan et al. 1989, Wong & Ramadan 1992), and several researchers in Hawaii are currently investigating augmentation of existing parasitoid populations with mass-reared insectary stock. Field trials on the island of Maui have shown the potential of one species, *Diachasmimorpha tryoni* (Cameron) to reduce damage from Mediterranean fruit fly in peaches and loquats (Wong et al. 1991, 1992). Other augmentation projects with opiine species, including *Psytallia fletcheri* (Silvestri) and *Diachasmimorpha longicaudata* (Ashmead), are also underway in Costa Rica (Camacho, 1990), Taiwan (Yao 1989), Florida (Sivinski, personal communication), and Mexico (Wharton, personal comm.), as well as Kauai, Hawaii.

Despite the importance of implementing quality control in mass production and field use of entomophagous species, previous augmentation programs in Hawaii using parasitoids of tephritids have not reported any inspection standards or protocols (Wong et al. 1991, 1992).

#### **Materials and Methods**

Parasitoids were reared in the insectary of the USDA-ARS Tropical Fruit and Vegetable Research Laboratory in Honolulu according to the methods of Wong and Ramadan (1992). Five 1-gm samples of puparia from each cohort to be shipped to Kauai were taken after formation of fly puparia, placed individually in screened wooden cages, and held at 25-27°C, 70-90% RH for emergence. The number of pupae in each one gram sample was counted, and the percentage of adult emergence and sex ratio of offspring in the laboratory were determined. Data from a total of 30 shipments of *D. longicaudata*, 26 of *D. tryoni*, and subsequently 16 of *P. fletcheri* are included in this paper.

To distinguish lab-reared from native parasitoids in field dispersal studies, 100g cohorts of parasitized puparia of each species were dyed with fluorescent powdered dyes before shipping. Dyed parasitoids were subsampled, packed, shipped, handled, and released in a similar manner as undyed parasitoids. Emergence, sex ratio, and



short range dispersal were measured in the same manner as described for undyed parasitoids.

Shipments from Honolulu to Kauai were made by air on the day each cohort was subsampled for emergence tests. Puparia containing parasitoids were packed in paper bags and placed in styrofoam boxes with frozen Blue Ice blocks. These boxes were placed in cardboard cartons and driven to the airport with instructions to place them on the next available flight. Time spent in transit was variable, ranging from ca. 4-28 hours.

Parasitoids received in Kauai were released in a grass field, in a commercial guava orchard, and in a 0.2 ha plot of mixed vegetables. Release vessels were retrieved after two weeks in the field. Percent parasitoid emergence was determined by inserting soft wide forceps into the paper bags at random angles and depths, and withdrawing a total of 50-150 puparia per bag for inspection. Workers were careful not to tilt, shake, or pour puparia from the bags to avoid settling and separation by weight. The number of puparia that were empty and the number that contained unemerged parasitoids were counted. The numbers of emerged but dead adult parasitoids of each sex inside the paper bags and outside the bags but inside the release vessels were also counted.

Two types of release vessels were compared for possible effects on in-field mortality and short range dispersal (= escape from the vessel). The first was the standard vessel used by Wong et al. (1991) in the augmentation projects on Maui, made from a 3.5 or 4.5-l, lidded white plastic bucket with six 10-mm diam. escape holes around the perimeter just under the rim of the lid. Buckets were suspended from wire coated with Tangletrap to prevent ant predation. Secondly, we tested a circular release platform constructed to hold and protect the paper bags but to allow the parasitoids to escape at any angle through a hardware cloth (1.2 cm<sup>2</sup> openings, height 10 cm, circumference 105 cm, total escape area = 1050 cm<sup>2</sup>). This platform allowed greater air circulation and provided more shade than the buckets. Percent emergence and the number of dead adult male and female parasitoids in each vessel were determined as described above, for 4-5 replications with each *Diachasmimorpha* species.

## Results

Subsamples of parasitized puparia held in the insectary had a mean emergence rate of  $68.6 \pm 0.6\%$  for *D. longicaudata* and  $26.0 \pm 0.6\%$  for *D. tryoni* (Fig. 1a). The mean female:male sex ratio was 1.8:1 for *D. longicaudata* and 1.4 :1 for *D. tryoni* (Fig. 1b). Cohorts of *P. fletcheri* had a mean emergence of  $67.0 \pm 3.5\%$  and a mean sex ratio of 3.8:1.

For both *D. longicaudata* and *D. tryoni*, there was a significant positive correlation between puparial weight and both the percent adult emergence and the proportion of females emerging on any given date (Fig. 2). For *P. fletcheri*, puparial weight was positively correlated with emergence, but was not correlated with sex ratio.

There were significant reductions in adult emergence in the field compared to the insectary for all three species (Table 1). The combined effects of shipping, handling, and field-exposure reduced *D. longicaudata* emergence an additional 26% below insectary levels, so that actual numbers of adults released in the field were slightly less than 50% of the parasitized puparia provided in the mass-rearing process. For *D. tryoni*, an additional 34% loss occurred during the handling and release stages, resulting in only 14.8% of the parasitized puparia emerging in the field.

Table 1. Decrease in emergence of mass-reared parasitoids due to shipping and handling<sup>a</sup>.

	Percentage emergence:		
	<i>D. longicaudata</i>	<i>D. tryoni</i>	<i>P. fletcheri</i>
Sampled before shipping	67.2 A	22.4 A	67.0 A
Sampled after release	49.5 B	14.8 B	54.6 B

<sup>a</sup> expressed as the percentage of puparia from which parasitoids emerged. Means within columns followed by the same letter are not significantly different (Tukey's studentized range test,  $P < 0.05$ ).

The type of release vessel used had a substantial impact on the number of parasitoids that died in the vessel before escaping. Female *D. longicaudata* suffered 29% higher mortality in buckets than platforms, and female *D. tryoni* had 83% higher

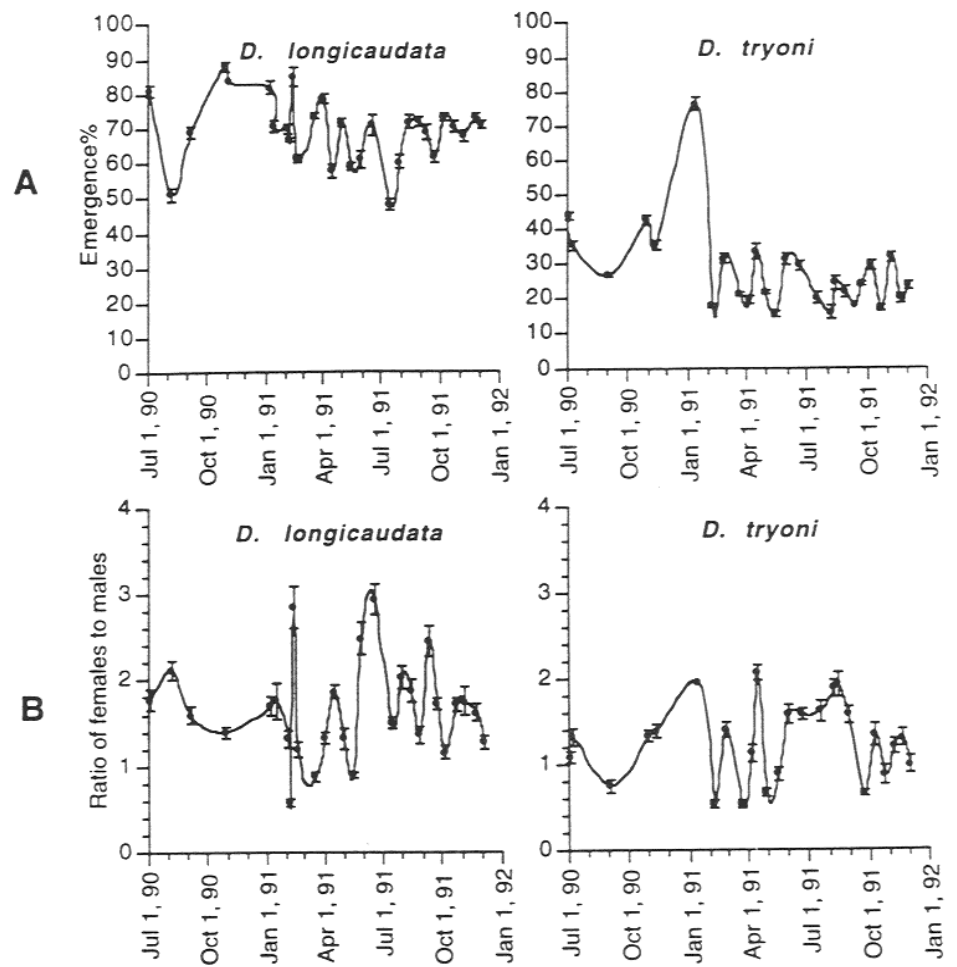


Figure 1. (A) Percent emergence and (B) sex ratio of mass-reared *D. longicaudata* and *D. tryoni* in the insectary over an 18 month-period.

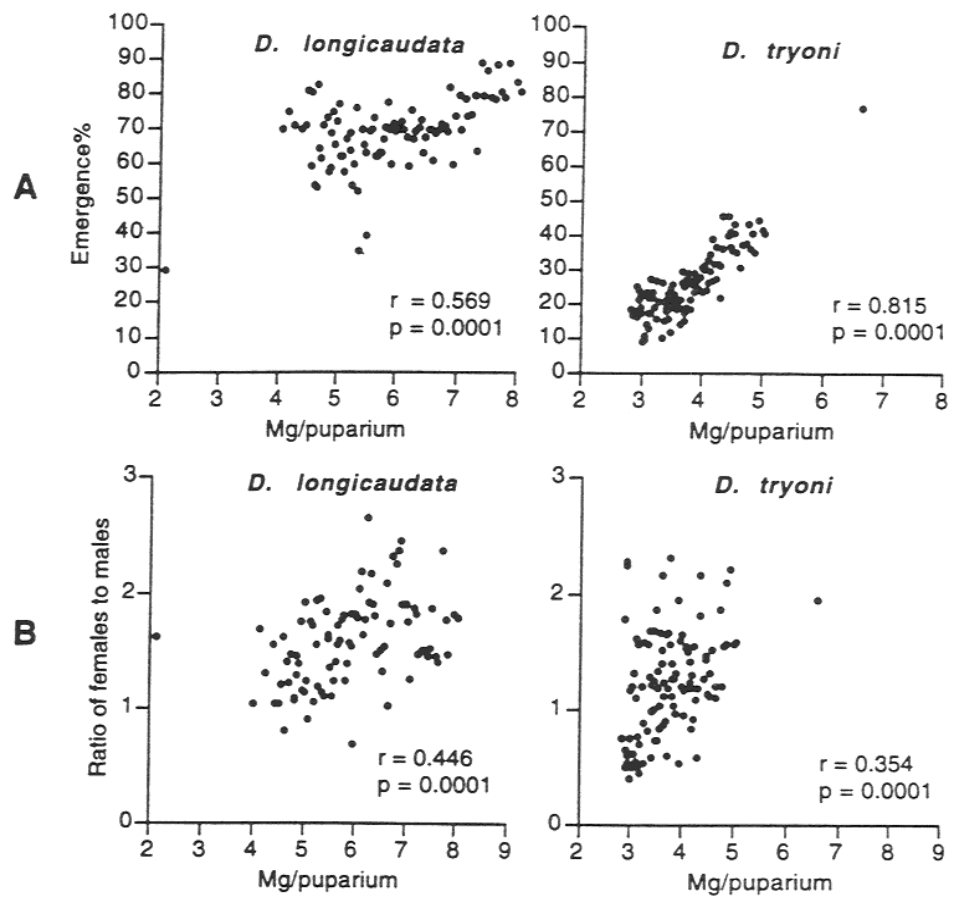


Figure 2. Relationship between puparium weight and (A) percent emergence and (B) sex ratio of *D. longicaudata* and *D. tryoni*.

mortality in buckets than platforms. Male mortality was not significantly influenced by vessel type in either species. (This may reflect the post-emergence behavior of males, which tend to remain in or near the vessel to intercept females as they emerge 1-2 days later). Differences in adult mortality within the release vessels were not related to differences in pupal emergence for either species, as the latter did not differ significantly between the two vessel types.

Applying fluorescent dyes to puparia significantly increased adult parasitoid mortality in release vessels in the field. However, the dyes appeared to have a positive rather than negative effect on pupal emergence for both *Diachasmimorpha* species, a trend which held true both in the laboratory and to an even greater extent in the field (Table 2). For *D. longicaudata*, adding powdered dye to puparia increased emergence by 2% (lab) and 9% (field); for *D. tryoni*, the corresponding increases were 20% (lab) and 85% (field). The reason for this improvement in emergence when puparia were dyed is not presently known, although it may be a function of the hygroscopic qualities of the powder influencing pupal moisture levels.

Table 2. Effect of fluorescent dye on parasitoid emergence from fly puparia.

	<i>D. longicaudata</i>		<i>D. tryoni</i>	
	Pre-shipment	Post-release	Pre-shipment	Post-release
Dyed	70.1 A	51.2 A	28.7 A	17.6 A
Undyed	68.8 A	47.0 A	23.8 B	9.5 B

<sup>a</sup> expressed as the percentage of puparia from which parasitoids emerged. Means within columns followed by the same letter are not significantly different (Tukey's studentized range test,  $P < 0.05$ ).

### Discussion

Even under stable laboratory conditions, only about one-fourth of the puparia of mass-reared *D. tryoni* and two-thirds of the puparia of *D. longicaudata* and *P. fletcheri* yielded live parasitoid adults in the Oahu insectary. This does not mean that (in the case of *D. tryoni*, for example) one out of every four fly pupae yielded a parasitoid; rather, flies are allowed to emerge earlier in the rearing process, empty puparia are removed, and only 22% of the remaining (parasitized) puparia yield parasitoid adults.

The feasibility of using parasitoids in augmentation programs is highly dependant upon the economics of mass-rearing, thus these data show the need to focus efforts on improving the yield of parasitoids per fly larva during insectary propagation of all three species.

The age and condition of tephritid larvae used during the rearing process is a critical factor influencing both emergence and sex ratio of opiine braconids. Our data show that larger fly larvae generally result in higher emergence rates, and also higher percentages of females for the *Diachasmimorpha* species. Wong & Ramadan (1992) have shown that, although relatively more female progeny are produced with advancing age of host larvae, a peak of overall emergence is reached sometime during the middle third instar, after which emergence rates again decline. During the year-and-a-half covered in this study, larval quality varied greatly from week to week, resulting in significant fluctuations of parasitoid quantity and quality. To successfully produce a reliable number of high-quality opiine parasitoids for field augmentation programs, it will be necessary to obtain (or produce) more uniformly large, third instar host larvae.

Shipping, handling, and release techniques result in even further significant reductions of the number of viable parasitoids released in the field. Although transport from Oahu to Kauai requires less than one hour flying time, airlines often delayed cargo loading for up to 24 hours, and temperatures in holding areas presumably reached  $>30^{\circ}\text{C}$  for several hours. Spot checks of temperatures inside the parasitoid-shipping coolers during transit using a maximum-minimum thermometer showed that temperatures occasionally reached a maximum of  $32.2^{\circ}\text{C}$ ., although it is not known if this was a result of ambient temperatures or metabolic heat (or both). Further heating due to intense sunlight on the release vessels in the field undoubtedly contributed to the overall reduction in viability of parasitoids.

The platform release vessels, with their greater shading and air circulation, significantly reduced in-field mortality of *D. tryoni* females, although the greater expense of constructing them must be weighed against the increase in viability obtained. Initial results for the *Diachasmimorpha* species did not distinguish between mortality which occurred during shipping and that which took place in the release vessels in the field. Subsequent releases of *D. longicaudata* and *P. fletcheri* suggest

that about 40% of the mortality occurs during shipping, and the other 60% in the field. Holding puparia at 19°C for ca. 12 hours before shipping, packing more Blue Ice into the shipping containers, and tightening schedules so that parasitoids were picked up within one hour of arrival at the airport significantly reduced post-insectary mortality.

Biological control through augmentative release of natural enemies in agricultural systems is considered by some to be "a strategy whose time has come" (Parrella et al. 1992). Undoubtedly, it is a technique with great potential to achieve environmentally-sound pest control. However, success is only likely to be achieved in those systems where sufficient resources are devoted to fundamental research in basic natural enemy biology, including not only rearing and quality control aspects but also dispersal, population dynamics, and behavior in the field. There is a danger that large-scale field tests of parasitoid releases (particularly in the context of "eradication" programs) without the required knowledge base may result in control failures and disillusionment with the strategy as a whole. Therefore, it is recommended that extensive basic research on rearing and behavioral ecology of candidate natural enemy species be conducted prior to, or at least in conjunction with, applied augmentation projects.

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Proceedings of the 7th workshop of the global IOBC working group "Quality control of mass reared arthropods". Rimini (I), 13-16 September 1993.

## **Designing and Implementing Quality Control of Beneficial Insects: Towards More Reliable Biological Pest Control**

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### **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. Conflicting requirements for natural enemies in mass-rearing programmes and field performance form another obstacle in obtaining a good biocontrol agent. These problems make 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 three years to develop quality-control programmes. The quality control project described in this paper is financially supported by a grant from the European Community.

### **Introduction**

Although biological control of pests in Europe has been known since around 1900, large-scale use of natural enemies of pests started only recently (van Lenteren 1990). In some areas of agriculture, such as apple orchards, maize, vineyards and greenhouses, it has been a very successful environmentally and economically sound alternative for chemical pest control (van Lenteren et al. 1992). Biological

pest control has no negative effects on the environment and improves safety of food and consumers. 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 presently on the market for insect and mite control, 120-180 are on the black-list in several European countries.

Success of biological control is primarily dependent on the quality of the natural enemies which are produced by commercial mass rearing companies and sold to farmers. Europe has about 25 natural enemy production companies. Only a few have more than 100 people employed, the others are very small, often not having more than ten persons contracted. Total employment in Europe is ca. 750 persons and growing. Total sales of natural enemies amounts to some 40 million US\$ (end user value). In addition to biological control, bumble bees used for pollination accounted for ten million US\$ in 1991. In the USA natural enemy sales amount to about 10 million US\$. 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 will be improved considerably when standards for acceptable quality are 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.

Scientists in Europe and North America have worked on quality control methods and natural enemy producers are starting to apply them quality control methods on a regular basis to be able to check and guarantee the effectiveness of their natural enemies. A first meeting of European scientists and commercial producers took place in Wageningen, the Netherlands, in 1991 (Bigler 1991). The participants concluded that in a united Europe, it would be highly requested to have uniform quality standards to be applied by all mass production companies. At the same meeting eight provisional quality tests were designed (Bigler et al. 1991, van Lenteren & Steinberg 1991).

At the second meeting in Hørsholm, Denmark, in 1992, the first results obtained with the provisional tests were discussed, tests were modified and three additional tests were designed (van Lenteren 1993). Because of the urgency to develop a full set of quality tests, the EC was asked to financially support a number of workshops. In May 1993 the EC has approved the grant proposal which is summarized below.

**Objectives of EC grant proposal "Designing and implementing quality control of beneficial insects: towards more reliable biological pest control".**

1. Develop realistic, simple and reliable quality control methods at research laboratories for the natural enemies which are widely used in biological pest control in Europe today.
2. Test quality control methods under commercial conditions.
3. Evaluate outcome of different quality control tests and improve the methods for practical use.
4. Implement quality control methods at mass production companies in Europe, develop proposal for EC standard.
5. Design training material for short courses for those who (will) work on quality control at production companies.

**Role of the Participants**

Participants from research institutes have been selected on the basis of their long-term experience in development of quality control methods (either because of ecological, physiological or genetical knowledge). Participants from the mass production industries have been selected for the specific natural enemy species they produce. All participants have been directly engaged in rearing natural enemies for a considerable period. The provisional roles of the participants are:

1. Denmark (private company). Develop, test and implement quality control for natural enemies of aphids in greenhouses (vegetables and ornamentals).
2. Denmark (public research). Design initial quality control tests and training material for natural enemies of aphids.
3. France (private company). Develop, test and implement quality control for

hypotheses related to performance of organisms are tested in an agricultural setting, and that performance criteria are critically tested. For agriculture the benefits are that the reliability of biological pest control will be improved considerably when good quality control standards are implemented by the biocontrol industry. When the pest control effect of natural enemies can be quantified and guaranteed, acceptance of biocontrol will be easier and implementation faster. A reduction in the use of environmentally hazardous pesticides is the result.

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- natural enemies of leafminers in greenhouses (vegetables and ornamentals).
4. France (private company). Develop, test and implement quality control for natural enemies of lepidopteran pests in the field (particularly cornborer in maize).
  5. Germany (public research). Design initial quality control tests and design training material for natural enemies of lepidopteran pests in the field (maize, fruit orchards and vineyards).
  6. Germany (private company). Develop, test and implement quality control for predators of aphids.
  7. Hungary (public research). Design initial quality control tests for natural enemies of thrips pests in greenhouses (vegetables and ornamentals).
  8. Italy (private company/public research). Develop, test and implement quality control for natural enemies of aphids, whiteflies and several other pests in greenhouses (vegetables and ornamentals) and in the field (vegetables).
  9. Italy (public research). Design initial quality control tests and design training material for natural enemies of aphids, whiteflies and several other pests in greenhouses (vegetables and ornamentals) and in the field (vegetables).
  10. The Netherlands (private company). Develop, test and implement quality control for natural enemies of whiteflies, leafminers and thrips in greenhouses (vegetables and ornamentals).
  11. The Netherlands (public research). Design initial quality control tests and training material for natural enemies of whiteflies, thrips and several other pests in greenhouses.
  12. Portugal (public research). Design initial quality control tests and training material for natural enemies of lepidopteran pests in the field (particularly of *Heliothis* in vegetables).
  13. United Kingdom (private company). Develop, test and implement quality control for natural enemies of leafminers and thrips in greenhouses (vegetables and ornamentals).
  14. Spain (public research). Design initial quality control tests and develop training material for predators of thrips, whiteflies and leafminers (field and greenhouse).

15. United Kingdom (private company). Develop, test and implement quality control for predatory mites used against spider mites in greenhouses (vegetables and ornamentals).
16. Belgium (private company). Develop, test and implement quality control for predators of thrips in greenhouses (vegetables and ornamentals).
17. Italy (private industry/public research). Design initial quality control tests and develop training material for predators of thrips in greenhouses (vegetables and ornamentals) and in the field (vegetables).
18. The Netherlands (private industry). Develop, test and implement quality control for parasitoids of aphids in greenhouses (vegetables and ornamentals).
19. Switzerland (public research). Design initial quality control tests and training material for natural enemies of lepidopteran pests in the field (particularly of cornborer in maize).
20. Switzerland (private industry). Develop, test and implement quality control for natural enemies of lepidopteran pests in the field (maize)
21. Israel (private company). Develop, test and implement quality control for natural enemies of spider mites and leafminers in the field (vegetables and fruit) and in greenhouses (vegetables).

#### **Expected results**

1. Report on large scale testing and evaluation of quality control tests under practical conditions.
2. Report with uniform quality control methods for all important natural enemies which are commercially applied in Europe (ca. 10 species).
3. Proposal for EC/IOBC standard for quality control of natural enemies.
4. Report with training material for short courses on quality control at production companies.
5. Implementation of quality control of natural enemies at the production companies.

#### **Expected benefits**

For the scientific community the benefit is that ecological, behavioural and genetic

### Product control of *Trichogramma brassicae* in Switzerland

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#### Abstract

The egg-parasitoid *Trichogramma brassicae* is commercially used in Switzerland against the European corn borer, *Ostrinia nubilalis*. Three different products of *T. brassicae* are presently registered and sold on the market. From 1991 to 1993, the quality of these products was assessed at the customer level. It is shown that the number of parasitized eggs per hectare is in all products between 20% and 40% less than stated by the distributors. The number of females per hectare was lower than required in three out of seven cases. Performance of the females was measured by assessing a number of parameters in the laboratory. Deformed wings, fecundity on eggs of the factitious and natural hosts, mortality and walking speed of females from the three products were compared to reference strains. Performance was in general good, except for strains stored and shipped under suboptimal conditions.

#### Introduction

The egg-parasitoid *Trichogramma brassicae* Bezdenko has been used inundatively against the European corn borer, *Ostrinia nubilalis* Hbn. in Switzerland since 1978. The parasitoid was applied in 1993 on a total of 6300 hectares of maize. The wasps used in Switzerland were mass-reared from 1978 to 1987 in one production unit in the country. Since 1988, a second producer in Europe has delivered its wasps to Switzerland and in 1993, a third producer distributed the parasitoids for the first time. With an increasing market, we do expect other producers of *T. brassicae* to have their parasitoids registered in Switzerland.

Presently, two delivery systems are used. One consists of cardboard cards holding the glued host eggs. Fifty egg-carriers per hectare are distributed twice a season (100 egg-carriers in total) and they are expected to hold at least 100,000 parasitized eggs per hectare and release. With the second system, cardboard capsules, containing the parasitized host eggs, are used. Two hundred capsules per hectare are distributed twice a season (400 capsules in total) with 100,000 parasitized eggs in the first and 200,000 in the second release.

Because of the public interest in biological control in general, the Swiss government promotes the use of *T. brassicae* in maize. Together with the producer and distributors of the wasps in Switzerland, we established over the last years a quality control system that makes production and use more reliable. The minimal standards of the products in terms of quantity and quality are designed to guarantee an acceptable efficacy if the products are used correctly. In order to improve the product's quality and our quality control system, we assessed the quantity and quality of batches delivered to farmers.

## **Material and Methods**

### *Numbers of parasitized eggs and females*

From 1991 to 1993, random samples from batches, supplied by the distributors to farmers, were taken and checked. The distributors are designated as products A, B, C, and are not otherwise identified in order to protect commercial interests. The material was incubated at 25°C, 70-80% RH and 16L:8D. Five to twelve units (egg-carriers) of both delivery systems (either cardboard cards or cardboard capsules) were confined individually in containers of 500 ml until all adults had emerged and died. Afterwards, the following parameters were assessed: number of parasitized (black) eggs per egg-carrier, emergence rate (200 parasitized eggs per egg-carrier), and percentage of females (200 adults per egg-carrier). From the parameters, the numbers of parasitized eggs and of females per egg-carrier and hectare were calculated.

### *Quality of females*

A number of quality parameters were measured on individual females. In 1991 we



assessed:

1. Fecundity on eggs of the factitious host, *E.kuehniella* and the natural host, *O.nubilalis*. For both tests, 25-one-day old females were confined individually in glass tubes together with eggs and a droplet of honey, and then left at 25°C, 70-80% RH, 16L:8D. *E.kuehniella* eggs were withdrawn after three days, egg-masses of *O.nubilalis* after 24 hours and thereafter incubated for four days until the parasitized eggs were black. Fecundity was determined by counting the number of black eggs.
2. Life-span was assessed on 25 females confined individually in glass tubes and fed honey. No host eggs were provided. Physical conditions were the same as for fecundity. Mortality was checked once a day.
3. Walking activity was measured on 25-one-day old females with a computerized image analysis system. The only parameter used to assess walking activity was walking speed measured during three minutes per female.

In 1993 we assessed:

1. Percentage deformed wings of 200 females.
2. Fecundity as in 1991. In addition we measured on 25 females the fecundity after seven days without renewal of host eggs.
3. Percent mortality after 7 days recorded from the fecundity tests. (We did not record average life-span and walking activity, methods are described in more detail in Cerutti and Bigler, 1991).

## Results and Discussion

### *Numbers of parasitized eggs and females*

The number of parasitized eggs and females per hectare are presented in Table 1. The number of females is calculated by taking into account the number of parasitized eggs per egg-carrier, emergence rate, percentage of females and the number of egg-carriers per hectare. Products A and C are registered in Switzerland for sale as 200,000 (2 x 100,000) parasitized eggs, product B for 300,000 (1 x 100,000 and 1 x 200,000) parasitized eggs. By comparing expected and actual numbers of parasitized eggs and females (Table 2), we observe with all products

Table 1: Numbers of parasitized eggs, females, emergence and sex ratio of *Trichogramma brassicae* from different products marketed in Switzerland from 1991 to 1993

Year	Product	Sample (=Release)	Number of egg-carriers checked	Number of parasitized eggs/egg-carrier mean $\pm$ SD	Emergence rate (%)	Sex ratio (% ♀)	Number of ♀/egg-carrier mean $\pm$ SD	Parasitized eggs/ha	Total number of ♀/ha
1991	A	1	6	1255 $\pm$ 105	94	66	779 $\pm$ 90	62'750	38'950
		2	6	1374 $\pm$ 116	94	76	982 $\pm$ 149	68'700	49'100
		1+2						131'450	88'050
	B	1	12	423 $\pm$ 140	95	78	313 $\pm$ 107	84'600	62'600
		2	12	638 $\pm$ 78	96	80	490 $\pm$ 74	127'600	98'000
		1+2						212'200	160'600
1992	A	1	5	1455 $\pm$ 95	95	68	940 $\pm$ 103	72'750	47'000
		2	5	1494 $\pm$ 103	94	71	997 $\pm$ 147	74'700	49'850
		1+2						147'450	96'850
	B	1	10	549 $\pm$ 114	94	71	366 $\pm$ 78	109'800	73'200
		2	10	610 $\pm$ 123	93	71	403 $\pm$ 85	122'000	80'600
		1+2						231'800	153'800
1993	A	1	5	1546 $\pm$ 90	80	72	890 $\pm$ 57	77'300	44'500
		2	5	1646 $\pm$ 187	90	73	1081 $\pm$ 163	82'300	54'050
		1+2						159'600	98'550
	B	1	10	320 $\pm$ 24	94	72	216 $\pm$ 20	64'000	43'200
		2	10	611 $\pm$ 158	94	71.5	411 $\pm$ 108	122'200	82'200
		1+2						186'200	125'400
	C	1	5	1099 $\pm$ 212	96	74	781 $\pm$ 127	56'049	39'831
		2	5	1106 $\pm$ 208	91	71	715 $\pm$ 151	56'406	36'465
		1+2						112'455	76'296

Products A and C are registered for 100'000 parasitized eggs delivered on 50 and 51 egg-cards per hectare and release respectively (200'000/ha in total)

Product B is registered for 100'000 parasitized eggs in the first and 200'000 in the second release delivered in 200 capsules per hectare and release (300'000/ha in total)

and years that the numbers of parasitized eggs are far too low with regard to the expected values. Despite the generally high emergence rates and percentage of females, the total number of females emerged per hectare is also too low, except in 1991 and 1992 with product B. From our experience we know that emergence under field conditions is most often less than 90%. Thus, the actual figures in the field will be lower than those we assessed in the laboratory. The figures show that there are generally negative deviations from the registered numbers in all products. The numbers of females per hectare, however, are unacceptable in three of seven cases where the negative deviations were higher than 10%.

Table 2: Number of parasitized eggs, females emerged and deviations from the required numbers (according to registration) of 3 products of *Trichogramma brassicae* marketed in Switzerland.

Year	Product	Parasitized eggs / ha	Deviation from required number <sup>1)</sup> (%)	Number of ♀ / ha	Deviation from required number <sup>2)</sup> (%)
1991	A	131'450	- 34.3	88'050	- 11.9
	B	212'200	- 29.3	160'600	+ 7.1
1992	A	147'450	- 26.3	96'850	- 3.1
	B	231'800	- 22.7	153'800	+ 2.5
1993	A	159'600	- 20.2	98'550	- 1.4
	B	186'200	- 37.9	125'400	- 16.4
	C	112'455	- 43.8	76'296	- 23.7

<sup>1)</sup> Products A and C are registered for 200'000 parasitized eggs, product B for 300'000.

<sup>2)</sup> with 50% females, products A and C are expected to deliver at least 100'000 females, product B 150'000 females.

#### *Quality of females*

Quality data assessed in the laboratory in 1991 and 1993 are presented in Table 3. The products are compared with a reference strain from our rearing that we know performs well in the field. All samples tested in 1991 were of good and even quality in the three products. Sample 1B had a low fecundity on eggs of the natural

Table 3: Quality of females of *Trichogramma brassicae* from different products marketed in Switzerland in 1991 and 1993

Year	Product	Samples	Deformed wings (%)	Fecundity/♀ on factitious host eggs		Fecundity/♀ on natural host eggs (24 h)	Average longevity (days)	Mortality after 7 days (%)	Walking speed (cm/sec)
				3 days mean ± SD	7 days mean ± SD				
1991	R <sup>1)</sup>			58.2 ± 19.5		10.5 ± 6.8	15.4 ± 7.0		0.242 ± 0.064
		1		46.9 ± 14.2		11.0 ± 4.8	9.1 ± 4.0		0.281 ± 0.039
	A	2		40.4 ± 12.9		13.6 ± 7.0	13.7 ± 4.6		0.247 ± 0.061
	B	1		41.3 ± 8.7		6.8 ± 4.9	11.8 ± 3.4		0.236 ± 0.042
		2		44.9 ± 9.9		10.8 ± 5.2	13.7 ± 3.7		0.274 ± 0.036
		3		48.7 ± 15.5		11.9 ± 5.9	15.6 ± 3.0		0.243 ± 0.066
		4		53.6 ± 13.6		9.1 ± 7.2	13.6 ± 3.8		0.217 ± 0.076
	C <sup>2)</sup>	1		46.8 ± 12.2		11.8 ± 5.3	11.5 ± 4.4		0.173 ± 0.061
1993	R <sup>1)</sup>		0	58.1 ± 10.6	73.3 ± 18.8	11.1 ± 5.7	15.6 ± 5.3	0	
	A	1	7	47.0 ± 10.9	48.0 ± 19.3	9.2 ± 5.7		8	
	B	1	1	47.1 ± 10.4	59.8 ± 7.7	11.2 ± 5.5		0	
	C	1	6	47.3 ± 19.9	53.8 ± 20.4	11.5 ± 6.6		0	

<sup>1)</sup> reference strain

<sup>2)</sup> this product was included in a preregistration procedure

host. This test is used as an indirect, rapid measurement of the natural host acceptance and suitability. It was shown by van Bergeijk *et al.* (1989) that egg acceptance and suitability of *O.nubilalis* is influenced by the number of generations *T.brassicae* is reared on factitious hosts.

Walking speed of the wasps was similar in products A and B. However, females of product C had a reduced speed. It was demonstrated by Bigler *et al.* (1988) that walking speed and field efficacy are related and, thus, slow wasps might have a weaker performance.

A relatively high percentage of deformed wings was observed in 1993 in products A and C. Wing deformations are unacceptable because these afflicted females are unable to fly and disperse in the field. Both, the 3 and 7-days fecundities, and the fecundity on natural host eggs, were high and in the same range for all products.

#### *Field efficacy*

Field efficacy was assessed regularly from 1980 to 1988 in order to compare different products, release systems, numbers per hectare and release intervals (Bigler *et al.*, 1989). Average field efficacies, assessed as parasitized eggs, ranged from 58.5 to 93% (mean 1980-88 = 77.7%). The reduction of larvae varied from 51.5 to 82.1 (mean 1980-88 = 74.6%). The three products of *T.brassicae* were registered in Switzerland against the European corn borer, based on equal efficacy data between A, B and C. Field efficacy was assessed in 1991 and 1993 for product A only. Average parasitism in 1991 was 83.3% (n=6) and in 1993 it was 77.9% (n=6).

#### **Conclusions**

Quantity and quality of *T. brassicae* marketed in Switzerland was checked by the Swiss Federal Research Station for Agronomy Zurich because the user (farmer) is technically unable to do it himself and the distributors of the products do not yet have a quality control system that includes product control at the enduser level. However, the results presented here show that this last control is absolutely necessary. The argument that the market will sort-out high and low quality

products is, to our experience, not valid in the case of *T.brassicae* in maize because low quality products with low efficacy may ruin the market totally. Customers of *T.brassicae* in Switzerland are still very sensitive to failures of biocontrol, and a few bad experiences may destroy the reputation and, as a consequence, the farmers may switch to chemical control. Therefore it is extremely important to market products only that fulfil the required standards. The results presented here demonstrate the need of a more elaborated product control system that increases reliability of the products. High quality products of *T.brassicae* is in the mutual interest of producer and customer. Since promotion of biocontrol is one of the targets in the Swiss agricultural policy, we try to improve quality in close cooperation with producers and distributors of *T.brassicae*.

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### **Fecundity and Host Acceptance Tests for Quality Control of *Trichogramma brassicae***

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#### **Abstract**

During a previous workshop of the IOBC working group "Quality control of mass reared arthropods", two questions were addressed: 1. could the laborious fecundity test of *Trichogramma brassicae* on eggs of *Ephestia kuehniella* be replaced by a quick test, and 2. how the fecundity and host acceptance test on eggs of *Ostrinia nubilalis* could be simplified and improved. Under standard conditions we performed 3-days, 7-days and total fecundity tests on eggs of *E.kuehniella* and fecundity tests at 1/4, 1/2, 1, 2, 4, 8 and 24 hours on eggs of *O.nubilalis*. We found significant correlations between mortality and fecundity on eggs of *E.kuehniella* and between fecundity on *E.kuehniella* and *O.nubilalis*. We also found that after four hours the fecundity on eggs of *O.nubilalis* is almost 92% of the fecundity after 24 hours. We conclude that the 3-day and the 7-day fecundity tests on eggs of *E.kuehniella* are of the same value; however, for economic and practical reasons, we recommend the 7-day test. We also suggest that mortality be assessed at the end of the 7-day test as an additional quality control parameter. As a simple and quick method for assessing the fecundity and host acceptance on eggs of *O.nubilalis*, we recommend the 4-hour test. Additionally, we suggest that the number of egg laying females be assessed at the end of the 4-hour test.

#### **Introduction**

The aim of product quality control methods is to obtain reliable results about the quality of the reared organisms. As most producers will not be able to perform large-scale and costly tests, it is important to develop methods which are quick, cheap and simple but nevertheless allow reliable predictions about the tested

organisms. To compare the results, the methods should be standardized and accepted by the producers. Nowadays, several standardized quality control tests of *Trichogramma brassicae* BEZDENKO (Hymenoptera: Trichogrammatidae) are established under the coordination of IOBC. During a previous workshop of the IOBC working group "Quality Control of Mass Reared Arthropods", it was decided to simplify the proposed methods and to evaluate their significance with the standard methods. The tests accepted so far are: Emergence rate, sex-ratio, fecundity and longevity on eggs of the factitious host, *Ephestia kuehniella* ZELLER or *Sitotroga cerealella* (OLIVIER), and fecundity on the target host, *Ostrinia nubilalis* HÜBNER, as an indirect measure of host acceptance. Other tests proposed are percent deformed wings, percent parasitization, and walking activity (BIGLER et al. 1988, BIGLER 1989, CERUTTI & BIGLER 1991). Other important parameters might be body size (BAI et al. 1992, BOURCHIER et al. 1993), flight propensity and perception of chemical cues (NOLDUS 1989, FORSSE et al. 1992). This paper answers the following questions:

1. Can the laborious and expensive fecundity test on eggs of *Ephestia kuehniella* be replaced by a quick 3-day or 7-day fecundity test ?
2. How can the fecundity and host acceptance test on eggs of *Ostrinia nubilalis* be simplified and improved ?

## Materials and Methods

### *Standard Conditions and Standardization of the Females*

All rearing and experimentation were performed in a climate chamber at a temperature of  $25 \pm 1^\circ\text{C}$ ,  $75 \pm 5\%$  RH and L:D=16:8 hours. From the strains to be tested, 0.1 g of parasitized eggs were put into a small plastic container ( $2 \times 2 \times 2$  cm) that was placed in a plastic cylinder of 15-cm height and 10-cm diameter. Tiny droplets of honey, about 2 per  $5 \text{ mm}^2$ , were placed around the inner side of the cylinder. The cylinder was closed with fine meshed organdie which was fixed by a flexible plastic lid with a central hole of about 7-cm diameter. From the beginning of emergence, distilled water was sprayed through this hole three times a day.

For all tests, only one-day-old females from the first day of emergence, with fully developed wings, mated and fed with honey and water, were used. The tests were carried out in glass vials ( $7.5 \times 1.5$  cm) containing a single female each and closed with a plastic plug with tiny holes for ventilation. Normally we used 25



females per test. The tests were started in the morning between 08.00 and 09.00 hours. We washed all materials with distilled water without using detergents.

#### *Fecundity on Eggs of Ephestia kuehniella*

In every glass vial, a tiny droplet of honey and distilled water was added, together with a piece of cardboard (about 0.5 x 2 cm) that carried about 250 fresh and UV-irradiated *Ephestia* eggs glued only with water. A droplet of honey was added to every cardboard. Fecundity was assessed by counting the parasitized (= black) eggs four days after the end of the exposure time. We carried out the following tests:

1. Total fecundity: A cardboard with eggs was put into the vial and exposed to one female for parasitization. The cardboard was replaced daily by a new one with fresh *Ephestia* eggs and a droplet of honey. A fresh droplet of distilled water was also added. This procedure lasted until the last female had died. We determined in this way the daily fecundity until death, the total fecundity and the lifetime.
2. 3-day test: A cardboard with eggs was put into the vial and exposed to the female for three days (no replacements).
3. 7-day test: The cardboard with eggs was exposed for seven days (no replacements).

Normally, 75 females per strain were used for one complete series (25 females for each test).

#### *Fecundity and Host Acceptance on Eggs of Ostrinia nubilalis*

Two fresh egg masses of *O. nubilalis* with at least 20 eggs each were presented to each female confined in the glass vial and exposed for parasitization for 1/4, 1/2, 1, 2, 4, 8 and 24 hours, respectively. For the complete test series, normally 175 females of every strain were used (25 females for each exposure time). For this test we did not add honey nor water. After five days, the parasitized (= black) eggs were counted. In addition, we determined for each time the number of egg laying females by counting the number of cardboards with at least one parasitized egg. As a measure of host acceptance, the number of tested and of laying females was compared by using the values of the 4-hour test of 20 strains that were either stored in diapause or quiescence or produced without storage. This test series gave

us a picture of the fecundity and host acceptance on the natural host of *T. brassicae* for the first 24 hours under standardized conditions.

#### *Trichogramma Strains*

For the different tests, we used strains from four commercial producers (in the following named as producers A - D) and strains that were reared in our research station. Most of the producers' materials were diapause and quiescence strains, a few were produced without storage. Our strains were not stored in general nor cooled; development was at most retarded in the 20°C climate room for one or two days. For the fecundity and lifetime tests we used the following strains:

1. OT (a, b, c, d): Strains reared on the natural host *O.nubilalis*, emerged at different times during the test period from spring until autumn 1993.
2. F1, F3, F10, F15, F20 and F21: Strains that were reared on their factitious host *E.kuehniella*, for the respective number of generations.
3. F400 (e, f): Strains reared on *E.kuehniella* since 1975, emerged at different times during the test period.

Not all strains were used for all tests.

#### *Evaluation of Data*

The mean values and the standard deviations (SD) of the measured parameters (lifetime, fecundity on eggs of *E.kuehniella* and *O.nubilalis*, etc.) were calculated. The Pearsons product-moment correlation (SNEDECOR & COCHRAN 1967) was used to evaluate the relationship between fecundity on *Ephestia* eggs and survival of the wasps at the 3-day and 7-day tests (n=23 strains) and between fecundity on *Ephestia* eggs at the 3-day and 7-day tests, and *Ostrinia* eggs at the 4-hour test (n=21 strains). We use scatterplot diagrams with regression lines. In addition, we give the equations of the regression lines, the coefficients of determination ( $r^2$ ) and the number of tested strains ( $p = 0.05$ ).

### **Results and Discussion**

#### *Fecundity on Eggs of Ephestia kuehniella*

Figure 1 shows two typical examples of the curves of the cumulated fecundity until death. In addition to this, we show the average fecundity of the 3-day and 7-day tests. The curves rise continuously until they reach a point where the females

no longer parasitize eggs, but still live for a few days. Out of seven strains tested, we found that the wasps of the 3-day tests (with no egg replacement) parasitized on average 7.7% fewer eggs than those with daily fresh eggs, and the wasps of the 7-day tests parasitized on average 18.0% fewer than those with daily fresh eggs (Table 1).

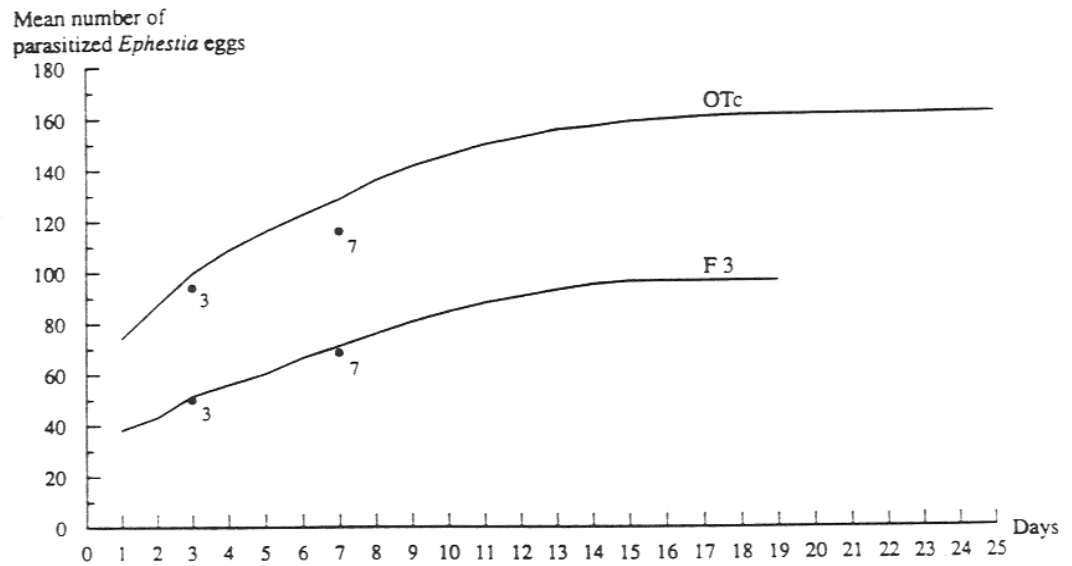


Figure 1. Cumulated fecundity on eggs of *Ephestia kuehniella* until death and mean values of the 3-day and 7-day tests. The described strains are OTc and F3. The mean fecundity includes both laying and non-laying females.

This is explained by the increasing age and therefore decreasing quality of the eggs (3 days compared to 7 days). Consequently the parasitization decreases compared to the curve of total fecundity with daily fresh eggs. We cannot explain why at strain F 15 there is a big difference between the fecundities with daily replacement of eggs and the 3-day and 7-day fecundities with no egg replacements, although we performed all tests at the same time with the same fresh egg material.

Table 2 presents a comparative list of the values of seven tested strains. Besides the total, 3-day and 7-day fecundities, we also list the average lifetime and the percentage of the 3-day and 7-day tests compared to the total fecundity. From

these values we found that the fecundities of the 3-day tests represent a mean of 51.1% whereas the 7-day tests represent 63.5% of the total fecundity. This shows that the females still have a remarkable potential to parasitize *Ephestia* eggs from seven days until death.

Table 1. Fecundity on eggs of *Ephestia kuehniella* after three and seven days, respectively. Comparison between the values of treatments with daily replacement fresh eggs and treatments without replacing eggs.

Strain	Fecundity after 3 days			Fecundity after 7 days		
	Daily fresh eggs Mean±SD	No egg replacement Mean±SD	Difference	Daily fresh eggs Mean±SD	No egg replacement Mean±SD	Difference
OT b	66.4±25.1	76.9±26.6	-15.8 %	93.4±35.6	101.2±26.	-8.4 %
OT c	99.5±21.7	94.0±28.8	5.5 %	128.0±23.6	115.5±18.8	9.8 %
F 1	59.2±19.6	58.1±10.6	1.9 %	86.5±29.9	73.3±18.4	15.3 %
F 3	51.4±14.8	50.0±17.6	2.7 %	70.8±20.8	68.2±28.3	3.7 %
F 15	57.0±13.1	24.3±16.8	57.4 %	83.6±21.1	30.3±25.9	63.8 %
F 20	60.9±17.1	63.0±10.1	-3.4 %	89.2±25.5	68.2±24.6	23.5 %
F400 f	73.3± 8.8	69.3± 6.9	5.5 %	99.7±15.4	81.6±12.9	18.2 %
Mean:			7.7 %	Mean: 18.0 %		

To compare the two tests by their accuracy for total fecundity estimation we use the percent values of the 3-day test (51.1% of total fecundity) and 7-day tests (63.5% of total fecundity), calculate the total fecundity and compare them with the measured total fecundity (Table 3).

As the values of mean and standard deviation of the differences of the two estimations show only little differences, we believe that the reliability of the 3-day and 7-day tests are similar.

#### *Relations between Fecundity and Survival*

We see in Table 2 that strains of good quality, such as OT strains, show high fecundities in the 3-day and 7-day tests have long lifetimes as well.

Table 2. Mean values of the total, 3-day and 7-day fecundity on eggs of *Ephestia kuehniella* and lifetime. N = number of tested females per strain.

Strain	N	Lifetime days Mean±SD	Total fecundity Mean±SD	3-days-fecundity Mean±SD	% of total fecundity	7-days-fecundity Mean±SD	% of total fecundity
OT b	20	18.2±5.1	120.7±49.1	76.9±26.6	63.7 %	101.2±26.8	83.9 %
OT c	20	20.3±2.9	160.9±31.9	93.9±28.8	58.4 %	115.5±18.8	71.8 %
F 1	15	15.6±5.3	129.3±51.0	58.1±10.6	44.9 %	73.3±18.4	56.7 %
F 3	20	12.4±5.6	95.8±32.4	50.0±17.6	52.2 %	68.2±28.3	71.1 %
F 15	25	8.3±2.3	87.2±22.9	24.3±16.8	27.9 %	30.3±25.9	34.8 %
F 20	25	21.2±6.9	142.0±42.4	63.0±10.1	44.4 %	68.2±24.6	48.1 %
F400 f	25	12.3±4.3	104.8±18.9	69.3± 6.9	66.1 %	81.6±12.9	77.8 %
Mean: 51.1 %					Mean: 63.5 %		

Table 3. Comparison between the measured total fecundity and the estimated total fecundity by using the mean values of the 3-day and 7-day tests.

Strain	Measured total fecundity	3-days-test (Estimator: 51.1% of total fecundity)		7-days-test (Estimator: 63.5% of total fecundity)	
		Estimated total fecundity	Difference	Estimated total fecundity	Difference
OT b	120.7	150.5	29.9	159.3	38.6
OT c	160.9	183.9	23.0	181.8	20.9
F 1	129.3	113.7	-15.7	115.4	-13.9
F 3	95.8	97.9	2.1	107.3	11.6
F 15	87.2	47.6	-39.6	47.7	-39.4
F 20	142.0	123.3	-18.7	107.4	-34.6
F400 f	104.8	135.6	30.8	128.4	23.6
		Mean:	1.7	Mean:	1.0
		SD:	27.4	SD:	30.4

To evaluate the most appropriate test, we compare the relationships between survival of the females and their respective fecundities after three and seven days (Figure 2). For this figure we used the values of 23 strains that were either stored in diapause or quiescence or produced without storage. The equations for the regression lines and the coefficients of determination are: 3-day test:  $y = 2.3x + 2.1$ ,  $r^2 = 0.50$ ; and 7-day test:  $y = 3.3x - 4.3$ ,  $r^2 = 0.55$ . In both tests the correlation between survival and fecundity is significant ( $p = 0.05$ ) according to the Pearsons product-moment correlation (SNEDECOR & COCHRAN 1967).

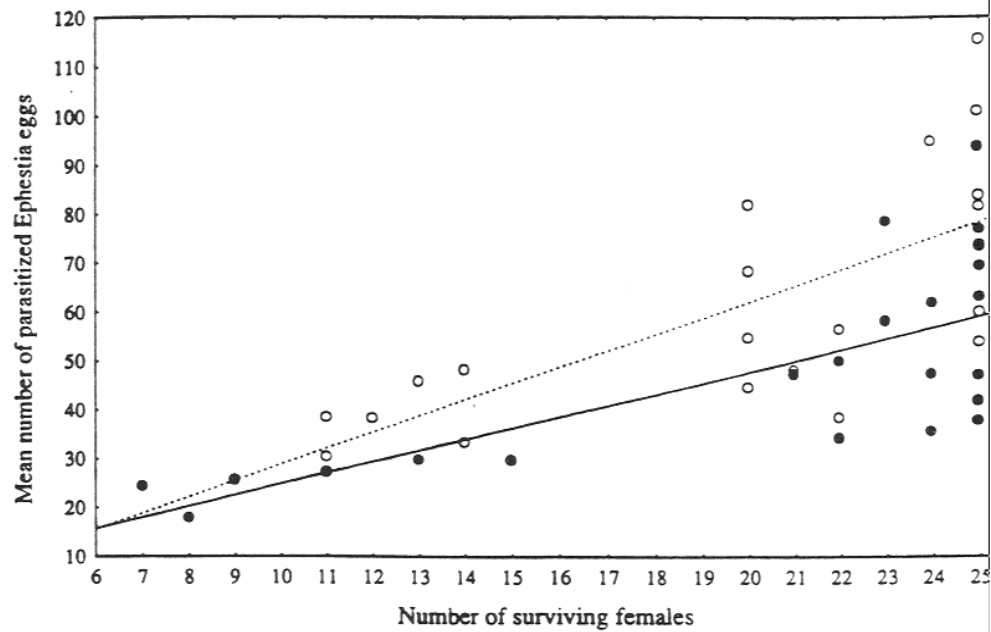


Figure 2: Scatterplot diagram and linear regression between number of surviving females and fecundity after three days (solid circles and line) and seven days (open circles and dotted line) on eggs of *Ephestia kuehniella*. N=23 strains each.

Because survival and fecundity are correlated we may use mortality after three or seven days as an additional quality control parameter for fecundity tests on eggs of *E. kuehniella*. We observed in most of the diapause and quiescent strains of commercial producers a higher mortality and lower fecundity than in fresh (not stored) material. This seems to be an indicator, besides the emergence rate, of suboptimal storage and/or long-range shipment (STINNER et al. 1974, CERUTTI &

BIGLER 1993).

As there seems to be a close relation between lifespan and total fecundity (Table 2), we think that the laborious total fecundity test could be replaced by assessing only lifespan, as a rough measure, as described in CERUTTI & BIGLER (1991). However, although lifespan could be used as a quality control parameter, it is not suitable for a quick test method, as it takes possibly several weeks until the last female of a tested strain has died.

#### *Fecundity and Host Acceptance on Eggs of Ostrinia nubilalis*

In Figure 3 we present two typical examples of curves of the fecundity on eggs of *O. nubilalis* within the first 24 hours of parasitization. The curves describe the average number of parasitized eggs for the whole test. The fecundity rises quickly until it reaches a certain level at the point of an exposition time of 4 hours. From this point, the females parasitize only a few additional eggs for the remaining 20 hours. In Table 4 we compare the fecundity of four and 24 hours using the values of 12 strains reared at our research station. We determined a mean difference between the 4-hour and 24-hour fecundity of 8.3%.

As we can see from Figure 3 and Table 4, the fecundity under standardized conditions after four hours is almost 92% of the total fecundity after 24 hours. This strategy of *T. brassicae* is very useful for field applications, because the shorter the time required for females to parasitize many *Ostrinia* eggs the higher is the probability of effective parasitization under changing natural conditions, such as wind, rain, etc. Figure 3 shows as well the decreasing fecundity, host acceptance and host suitability, for strains reared on the factitious host *E. kuehniella* (F3), as it was demonstrated by van BERGEIJK et al. (1989).

All results presented so far are the mean values, including both laying and non laying females. In Table 5 we compare the number of tested females and the number of laying females in the 4-hour test. We see that all OT strains have a high number of egg laying females and a high average fecundity as well, whereas all strains that were stored in diapause have a very low fecundity, even if the number of laying females is high. We must point out that the described method of assessing the fecundity on eggs of the target host, *O. nubilalis*, underestimates the realized fecundity (BOURCHIER et al. 1993), as we count only the number of black eggs, not considering the number of *Trichogramma* eggs per host egg. Van BERGEIJK et

Mean number of  
parasitized *Ostrinia* eggs

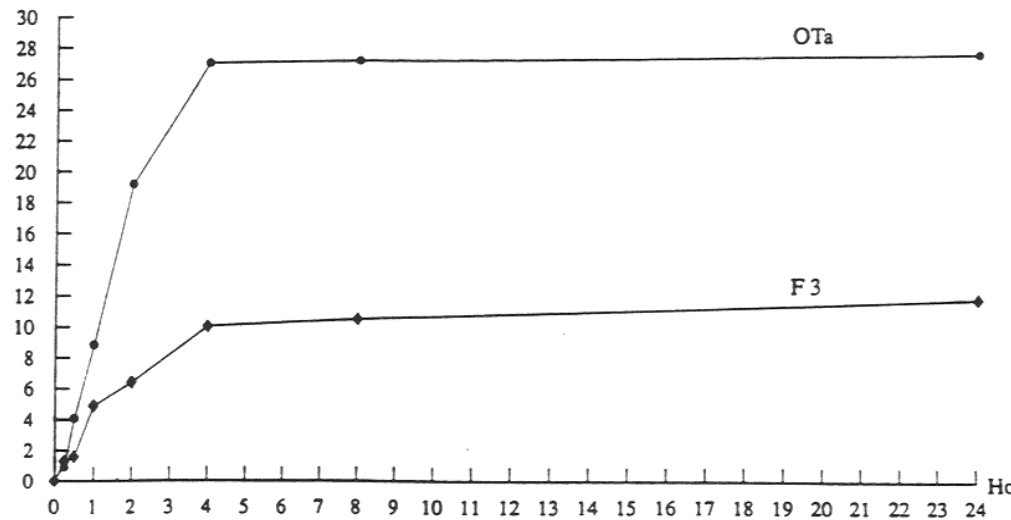


Figure 3. Characteristic curves of the mean values of fecundity on eggs of *Ostrinia nubilalis* within 24 hours exposure time. The described strains are OTa and F3. The mean fecundity includes both laying and non-laying females.

Table 4. Comparison between the fecundity on eggs of *Ostrinia nubilalis* for an exposure time of four and 24 hours.

Strain	Fecundity 4 hours Mean $\pm$ SD	Fecundity 24 hours Mean $\pm$ SD	Difference %
OT a	27.0 $\pm$ 11.2	27.7 $\pm$ 10.10	0.7 . 2.5 %
OT b	16.9 $\pm$ 9.4	19.1 $\pm$ 9.0	2.2 11.6 %
OT c	17.3 $\pm$ 10.1	20.4 $\pm$ 10.9	3.1 15.2 %
OT d	28.8 $\pm$ 9.0	27.4 $\pm$ 8.1	-1.4 -5.1 %
F 1	10.4 $\pm$ 5.7	14.1 $\pm$ 6.9	3.7 26.4 %
F 3	10.0 $\pm$ 6.8	11.8 $\pm$ 6.3	1.8 15.3 %
F 10	9.9 $\pm$ 6.2	10.8 $\pm$ 6.4	0.9 8.2 %
F 15	12.1 $\pm$ 6.4	11.4 $\pm$ 9.0	-0.7 -6.1 %
F 20	15.9 $\pm$ 6.2	16.8 $\pm$ 7.2	0.9 5.3 %
F 21	17.2 $\pm$ 7.0	17.2 $\pm$ 6.5	0.0 0.0 %
F400 e	9.2 $\pm$ 5.5	10.7 $\pm$ 5.0	1.5 13.8 %
F400 f	8.7 $\pm$ 6.0	10.0 $\pm$ 7.8	1.3 12.7 %
Mean:			1.2 8.3 %



al. (1989) demonstrated that the average number of eggs oviposited in one host egg of *O. nubilalis* is two on average for strains reared on the natural host (OT strains). It decreases, however, to only one if the wasps are reared for a number of generations on eggs of *E. kuehniella*.

Table 5. Fecundity on eggs of *Ostrinia nubilalis* at the 4-hour test. Comparison between the number of tested females and the number of egg laying females. A - D: Commercial producers, dia = diapause strains.

Strain	Tested females	egg-laying females	Difference	Fecundity Mean $\pm$ SD
OT a	25	24	96 %	27.0 $\pm$ 11.2
OT b	20	18	90 %	16.9 $\pm$ 9.4
OT c	20	18	90 %	17.3 $\pm$ 10.1
OT d	10	10	100 %	28.8 $\pm$ 9.0
F 1	15	14	93 %	10.4 $\pm$ 5.7
F 3	20	17	85 %	10.0 $\pm$ 6.8
F 10	25	24	96 %	9.9 $\pm$ 6.2
F 15	10	9	90 %	12.1 $\pm$ 6.4
F 20	25	24	96 %	15.9 $\pm$ 6.2
F 21	25	23	92 %	17.2 $\pm$ 7.0
F400 e	25	23	92 %	9.2 $\pm$ 5.5
F400 f	15	13	87 %	8.7 $\pm$ 6.0
A F6 dia	25	23	96 %	5.0 $\pm$ 4.0
A F6 dia	25	18	72 %	6.3 $\pm$ 5.4
A F6 dia	25	17	68 %	6.6 $\pm$ 5.7
A F5	25	14	56 %	7.0 $\pm$ 7.2
B F6 dia	25	16	64 %	5.3 $\pm$ 4.8
B F6	25	21	84 %	9.2 $\pm$ 8.7
C	25	23	92 %	11.5 $\pm$ 6.6
D	25	23	92 %	11.2 $\pm$ 5.5

In Figure 4 we compare the relationship of parasitized *Ostrinia* eggs after four hours and parasitized *Ephestia* eggs after three and seven days, respectively. We used the values of 21 strains that were either stored in diapause or quiescence, or reared without storage. The equations of the regression lines and the coefficients of determination are: Correlation 4-hour fecundity on *O.nubilalis* and 3-day fecundity on *E. kuehniella*,  $y = 2.58x + 22.17$ ,  $r^2 = 0.49$ ; correlation 4-hour fecundity on *O.nubilalis* and 7-day fecundity on *E. kuehniella*,  $y = 3.25x + 25.62$ ,  $r^2 = 0.50$ . In both tests, both correlations are significant ( $p=0.05$ ), according to Pearsons product-moment correlation (SNEDECOR & COCHRAN 1967).

This means that the observed fecundity on *Ostrinia* eggs depends about 50% on the fecundity realized on *Ephestia* eggs and 50% on the acceptance and suitability of *Ostrinia* eggs. The test on *Ostrinia* eggs is therefore a combination of the fecundity, host acceptance and host suitability. We observed in most of the diapause and quiescent strains low fecundities both on *Ephestia* and *Ostrinia* eggs. This is again a sign of suboptimal treatments.

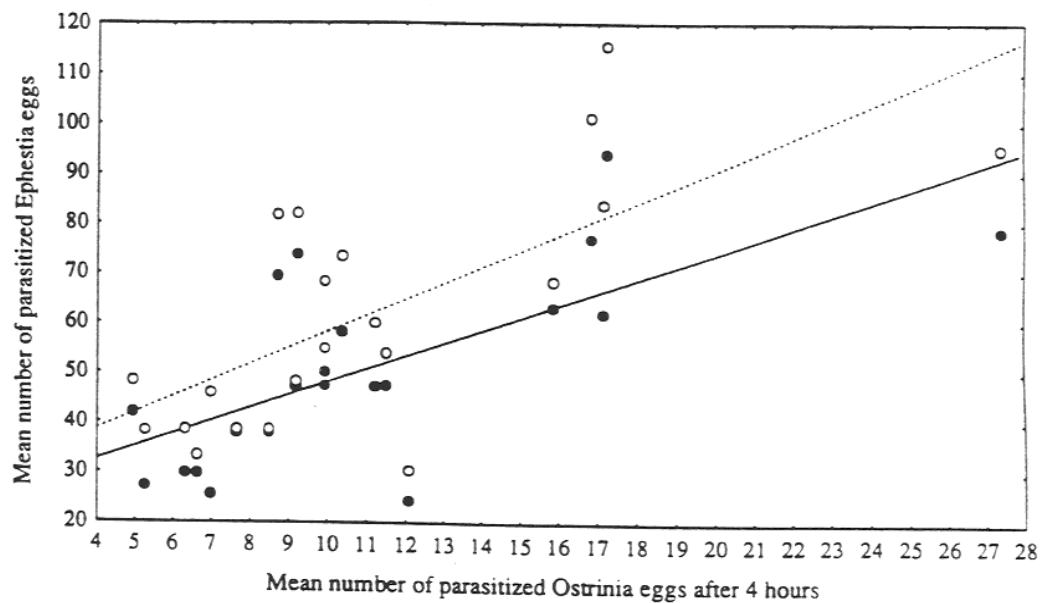


Figure 4. Scatterplot diagram and linear regression between the fecundity on eggs of *Ostrinia nubilalis* after four hours and the fecundity on eggs of *Ephestia kuehniella* after three days (solid circles and line) and seven days (open circles and dotted line) respectively. N=21 strains each.

### Labour assessment

The approximate total time producers must spend on the fecundity tests are presented in Table 6, not including the labour for *Ephestia* or *Ostrinia* eggs production. We must add the time of about 30-45 minutes for preparation and washing of the plastic cylinders for emergence and glass vials used for the tests.

Table 6. Expenditure of total time for the fecundity tests on eggs of *Ephestia kuehniella* and *Ostrinia nubilalis*.

Fecundity test	Expenditure of time for 25 females	Days from emergence to end of test (at 25°C)
3-days-test <i>E. kuehniella</i>	70-100 min.	8 days
7-days-test <i>E. kuehniella</i>	70-100 min.	12 days
Total fecundity <i>E. kuehniella</i>	350-1150 min.	14-35 days
4-hours-test <i>O. nubilalis</i>	80-115 min.	6 days

### Conclusions

1. From a scientific point of view the 3-day and 7-day fecundity tests on eggs of *E.kuehniella* are of the same value.
2. For producers we recommend the 7-day test to assign the fecundity on eggs of *E.kuehniella*. This method is more economical as costly work on weekends can be avoided.
3. As an additional quality control parameter, we suggest assessment of the mortality at the end of the 7-day test.
4. If results must be quickly available, the 3-day test may be performed.
5. We recommend the use of high quality *Ephestia* eggs that are UV-irradiated and stored at  $3\pm1^{\circ}\text{C}$ ,  $85\pm5\%$  RH for at most two days.
6. As a simple, quick and reliable method for assessing the fecundity and host acceptance on the target host *O.nubilalis* we recommend an exposition time for parasitization of 4 hours (4-hours-test).

7. In addition to host acceptance we recommend to note the number of egg-laying females at the 4-hours-test (by counting the number of cardboards with at least one parasitized egg).

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**Influence of Mass Rearing Techniques on the Oviposition of *Chrysoperla carnea***

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**Abstract**

An artificial diet for larval development of *Chrysoperla carnea* was tested to reduce the mass-rearing costs due to the extensive use of *Ephestia kuehniella* eggs. Cocoon weight, eggs laid per female, longevity, oviposition period and egg hatching ratio were recorded in 21 pairs of *C. carnea* adults reared on *E. kuehniella* eggs or artificial diet. No differences were found between the two diets except for cocoon weight, which was higher (taking into account gender) on the *E. kuehniella* eggs; fecundity also was unaffected. Even two *C. carnea* strains reared respectively on *E. kuehniella* eggs for about 100 generations and on artificial diet for about 60 generations showed no significant differences in the tested parameters except for cocoon weight and fecundity. These findings indicate that the tested artificial diet is suitable for mass-rearing *C. carnea*, even over many generations, is viable and without detrimental effect on the studied parameters.

**Introduction**

Inundative releases of the polyphagous predator *Chrysoperla carnea* (Steph.) that employ both eggs and larvae are used mainly for biological control of aphids (Principi, 1983). Yet *C. carnea* has not registered the same success rates in Europe, where it is primarily limited to protected crops (Celli *et al.*, 1991), as in the USA, where it is widely released in many, mainly open-field, crops.

This difference can be attributed to the larval cannibalism of released *C. carnea* and, mainly, to the higher costs of its mass-rearing, which are linked to the widespread use of Lepidoptera eggs as factitious prey instead of aphids. Indeed, more than a thousand eggs of *Ephestia kuehniella* (Zell.), one of the most common prey used in mass production, are needed to complete the larval development (Nicoli *et al.*, 1991).

Many researchers have attempted to overcome this drawback by developing artificial diets to replace, either completely or in part, the diet of live arthropods (Vanderzant, 1969; Ponomareva & Beglyarov, 1973; Bigler *et al.*, 1976; Yazlovetskij & Nepomnyashcaya, 1979). Biolab has mass-reared *C. carnea* since 1985, mainly for release as second instars against aphids in tunnel-grown strawberry over an area ranging from 60 to 80 hectares per year (Benuzzi, 1992). The aim of the present study was to evaluate whether the number of generations in laboratory and the use of an artificial diet can affect the performance of adult females in mass-rearing.

#### Materials and Methods

The tests were conducted in 1992 using three strains of *C. carnea*: (i) EK91, collected in the Cesena area of northern Italy during summer 1991 and mass-reared for about 15 generations on frozen eggs of *E. kuehniella*; (ii) EK86, collected in the same area during summer 1986 and reared for about 100 generations as a pure line on frozen eggs of *E. kuehniella*; and (iii) AD88, collected in Northern Europe during summer 1988 and reared on an artificial diet for about 60 generations. The artificial diet employed in the experiment was the same as that used in other mass-rearings, i.e. a meridic diet formed into small droplets coated by paraffin. Table 1 lists the biological parameters recorded for the three different strains as per diet.

The initial batch of eggs were collected by placing the adults of the three strains inside oviposition cylinders commonly used in our mass-production line. To minimise any differences, we used the eggs laid over 24 hours, which were then isolated in single vials for larval hatching to prevent cannibalism; the larvae were fed until cocoon spinning and the cocoons were held for adult



emergence. The newly emerged adults were sexed, paired and placed in a cardboard cylinder (7-cm diameter and 16-cm height) with a gauze cover for mating and oviposition. Each dead male was replaced by a newly emerged one. The adults were fed with a diet composed of honey and yeast extract. The pairs were kept at  $26\pm0.5^{\circ}\text{C}$ , 75 % RH and a L:D=16:8 hour photoperiod until the female died. The eggs were collected three times a week, counted and isolated for hatching ratio estimation.

Table 1. Strains of *Chrysoperla carnea* tested and larval food in rearing and during the experiment.

Strain	Larval food in rearing	Larval food in the experiment	Generations in rearing
EK91	<i>E. kuehniella</i> eggs	<i>E. kuehniella</i> eggs	15
EK91	<i>E. kuehniella</i> eggs	artificial diet	15
EK86	<i>E. kuehniella</i> eggs	<i>E. kuehniella</i> eggs	100
AD88	artificial diet	artificial diet	60

Cocoon weight (correlated to adult sex), female fecundity, longevity, oviposition period and egg hatching ratio were the parameters recorded.

The comparison of the number of eggs laid in the four trials was carried out by analysis of variance (ANOVA) followed by Tukey's test ( $P < 0.05$ ); because of variance heterogeneity, the Kruskal-Wallis test, followed by non-parametric multiple comparison (Dunn's Procedure), was employed for the other parameters.

### Results and Discussion

Table 2 summarises the results. Cocoon weight, for both sexes, differed significantly between the predators fed on *E. kuehniella* eggs and those on artificial diet; artificial diet produced significantly smaller cocoons. However, the number of eggs laid per female showed a significant difference only between EK86 and AD88, confirming that cocoon weight is not a good

Table 2 - Performances of *Chrysoperla carnea* strains related to larval food (means  $\pm$  sd).

STRAIN	EK91	EK91	EK91	EK86	AD88
LARVAL FOOD					
		<i>E. kuehniella</i> eggs	Artificial diet	<i>E. kuehniella</i> eggs	Artificial diet
NO. PAIRS	21	21	21	19	20
COCOON					
Females	9.7 $\pm$ 0.8 c		7.5 $\pm$ 0.9 b	8.9 $\pm$ 1.3 c	6.1 $\pm$ 0.3 a
WEIGHT (mg)    Males	7.2 $\pm$ 1.0 b		5.9 $\pm$ 0.7 a	7.9 $\pm$ 1.1 b	6.0 $\pm$ 0.4 a
NO. EGGS LAID/FEMALE	614.7 $\pm$ 403.9 ab		549.8 $\pm$ 239.6 ab	438.8 $\pm$ 366.8 a	743.6 $\pm$ 400.9 b
LONGEVITY (DAYS)	37.3 $\pm$ 11.5 a		38.1 $\pm$ 9.0 a	37.0 $\pm$ 20.6 a	48.0 $\pm$ 16.4 a
OVIPOSITION PERIOD (DAYS)	30.7 $\pm$ 10.0 a		31.7 $\pm$ 8.2 a	29.2 $\pm$ 21.4 a	38.3 $\pm$ 14.6 a
HATCHING RATIO (%)	73.1 $\pm$ 8.7 a		73.0 $\pm$ 9.5 a	71.6 $\pm$ 7.7 a	74.6 $\pm$ 9.9 a
FERTILE FEMALES (%)	100 a		100 a	94.7 a	100 a

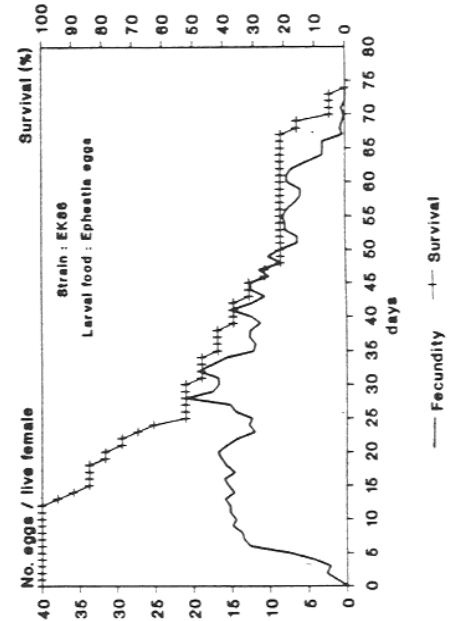
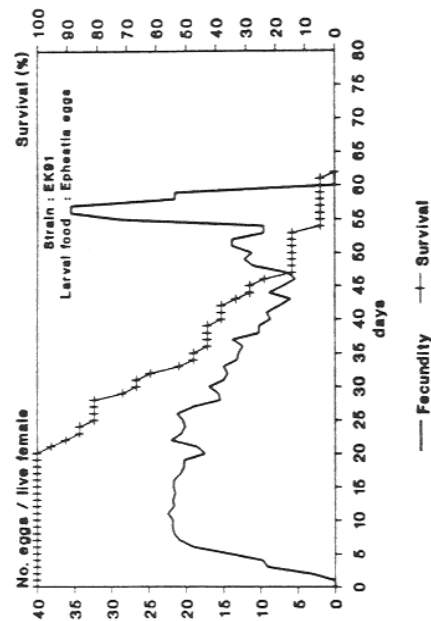
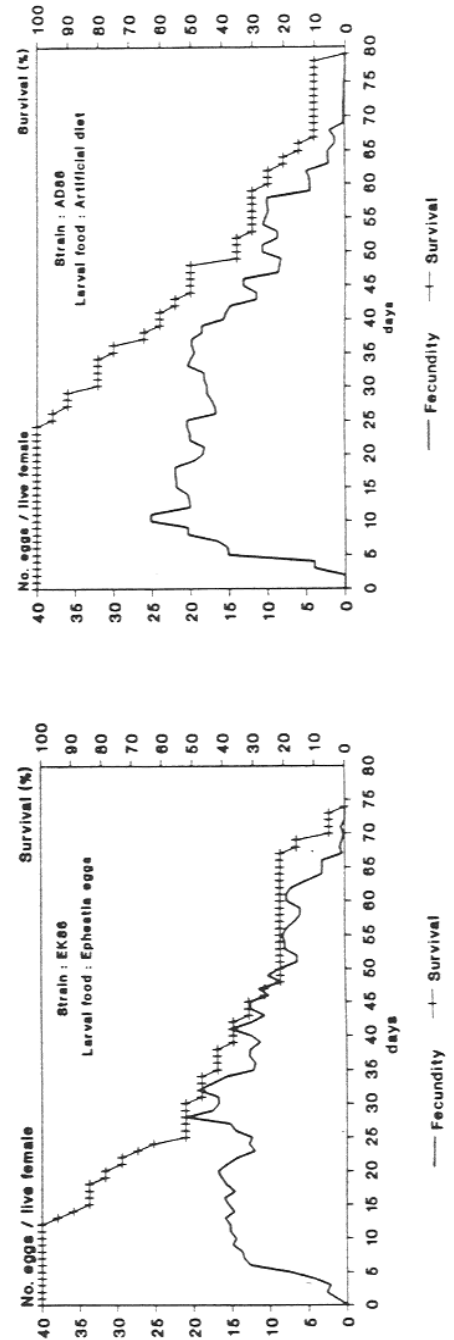
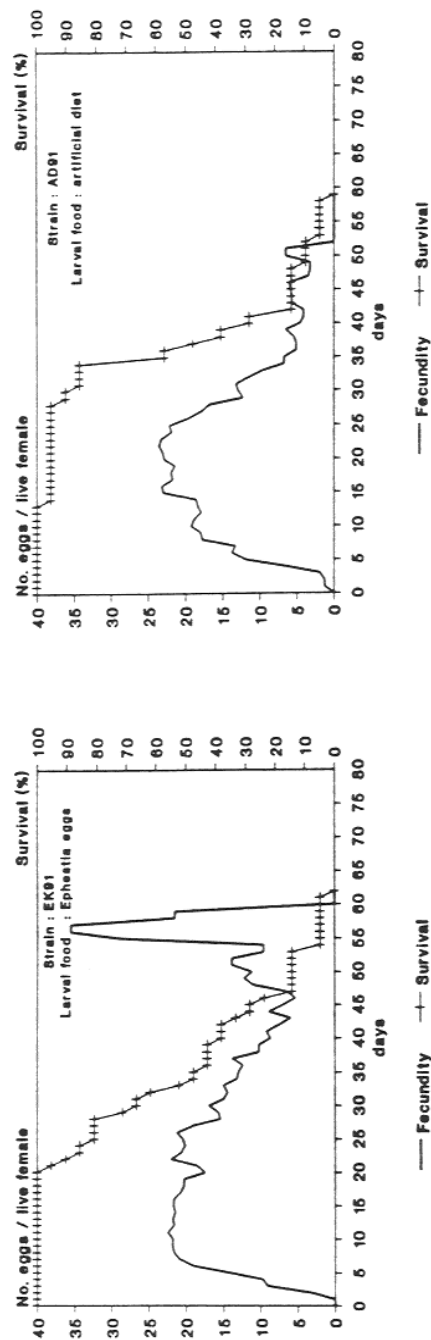


Fig.1. Fecundity and survival of *Chrysoperla carnea* strains related to larval food

parameter to predict the fecundity. Artificial diet appeared not to affect fecundity given that strain EK91 evinced no significant differences in this parameter when fed on *E. kuehniella* eggs or artificial diet. Nor were differences found as to longevity, length of oviposition period and egg hatching ratio among the four trial groups. The trends of the oviposition and survival rates of the four groups of females are shown in Fig. 1.

The tests showed that the egg prey can be replaced by an artificial diet, which was administered to *C. carnea* larvae as small droplets coated by paraffin (Hagen & Tassan, 1965). The biological parameters tested were not affected by a long period of mass-production using either the *Ephestia* eggs (about 100 generations) or artificial diet (about 60 generations), a very important factor for the mass-production of this predator. Further investigation is needed to determine whether the artificial diet affects the predation activity of the larvae.

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**Field Evaluation of *Chrysoperla* spp. in Augmentative Release Programs for the Variegated Grape Leafhopper, *Erythroneura variabilis***

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**Abstract**

Laboratory studies found green lacewings, *Chrysoperla* spp., to be voracious variegated grape leafhopper predators. Each *Chrysoperla comanche* larva consumed, on average, over 250 large leafhopper nymphs during a 9 to 10-day development period. Field results from some experimental plot and vineyard release trials showed a significant reduction in leafhopper numbers after commercial lacewing releases. However, results from these trials also suggest that lacewing release effectiveness can be greatly increased by improving release methodology, which currently can cause greater than 50% mortality of release material.

**Introduction**

Table, raisin, and wine grapes constitute one of the most important and widespread commodities in California agriculture. The variegated leafhopper, *Erythroneura variabilis* Beamer, has been the primary insect pest in San Joaquin Valley grape vineyards for the past decade (Kido et al. 1984, Daane et al. 1992). The leafhopper causes damage directly to the leaves through feeding, which reduces photosynthesis, and to the fruit through the excretion of honeydew, which leads to sooty mold growth. Adult leafhoppers damage the crop indirectly by disrupting harvest operations. To control leafhoppers, most grape growers rely on pesticide treatments; however, these treatments can disrupt natural control of spider mites

and mealybugs. Leafhopper resistance to commonly used insecticides has also been observed. For these reasons, improved biological controls of the variegated grape leafhopper are needed.

Parasitism of variegated leafhopper eggs by the wasp *Anagrus epos* is currently inadequate, by itself, to provide control; although this same wasp can provide excellent control of the grape leafhopper, *Erythroneura elegantula*, (Daane et al. 1992, Settle & Wilson 1990). To improve natural control, some grape growers are using augmentative releases of green lacewings, *Chrysoperla* species. Augmentative releases of green lacewings have been shown to effectively control numerous agricultural pests (Sundby 1966, Lingren et al. 1968, Shands et al. 1972, Adashkevich & Kuzina 1974, Ridgway et al. 1977, Canard et al. 1984). With the development of economically feasible artificial diets (Hagen 1950, Hagen & Tassan 1970, Hassan & Hagen 1978), evaluation of dietary requirements (Zheng et al. 1993a, 1993b), and mass culturing techniques (Ridgway et al. 1970, Morrison & Ridgway 1976) augmentative release of lacewings, specifically *Chrysoperla carnea*, as a commercial practice has become possible for a variety of insect pests. However, large-scale lacewing release programs for leafhopper control in vineyards are new and their proper implementation will require better guidelines than are currently available.

Here we present results from laboratory and field experiments which examined the effectiveness of lacewing releases in grape vineyards and, based upon this data, we suggest improvements to current programs.

#### **Lacewing predation**

A laboratory study of lacewing predation on the variegated leafhopper was conducted in 1990. Adult *Chrysoperla comanche* were field-collected and the eggs they deposited were placed individually in glass vials. After the lacewing larvae hatched, they were provided with fourth or fifth instar leafhoppers on freshly cut sections of grape leaves. Every one to two days thereafter the old leafhoppers were removed, their mortality was determined and fresh ones were added. Adults derived from the feeding study were supplied water and a protein diet, and their egg production was measured every two days. The feeding experiment was



This would equate to well over 1,500 of the smaller first or second instar leafhoppers in mass alone, although predation on these stages has not been tested. Average larval development time was 9.8 days at 26°C, with approximately 3.3 days for each larval stage. Development time in the field was observed to be longer. Just over 75% of the total leafhopper predation occurred in the third and final lacewing larval stage (Fig. 1). Comparing leafhopper mortality to lacewing development indicates that after field release there will be a 6 to 9-day period during which few leafhoppers will be killed while the lacewings develop to the third stage. Laboratory-reared lacewing larva killed on average 136.6 leafhoppers (Fig. 1) over a 14.2-day development period. Other researchers have reported that lacewing egg production and longevity deteriorate after only a few generations of inbreeding.

Results from the adult *C. comanche* fecundity trial show that females laid an average  $1,108 \pm 290$  eggs, over a  $53 \pm 10$  days period. Thus, there is a great potential for natural increase in lacewing numbers if gravid females can be attracted into the vineyard or if adults, which develop from the released lacewings, remain in the vineyard. For example, the egg production of 10 females would equal an augmentative release of approximately 10,000 eggs per acre. Further, the lacewing eggs are laid on stalks, providing some protection from other general predators. In a separate experiment, the grey ant (*Formica aerata*) was observed to readily prey upon lacewing eggs placed on the substratum.

#### Experimental plots

To determine lacewing predation in the field, a series of small-plot trials were conducted with varying lacewing release rates and schedules. Leafhopper numbers were monitored before and after release. In 1990, quarter-vine cages were used, set in a randomized block design with 12 replicates for each of three treatments: 1) no release, 2) 6 lacewings per cage, and 3) 18 lacewings per cage.

The work was repeated in 1991 and 1992 in 3-vine plots. Each plot was isolated by pruning border vines throughout the season and covering the exposed wire trellis with Tanglefoot® stikum. This procedure effectively prevented between plot movement of released lacewing larvae and leafhopper nymphs. In

repeated in 1991 with lacewings from a nine-month-old laboratory colony, reared on eggs of the Mediterranean flour moth and larvae of the potato tuberworm and navel orangeworm.

Results show field-collected *C. comanche* larva kill an average of 252.4 leafhoppers (Fig. 1).

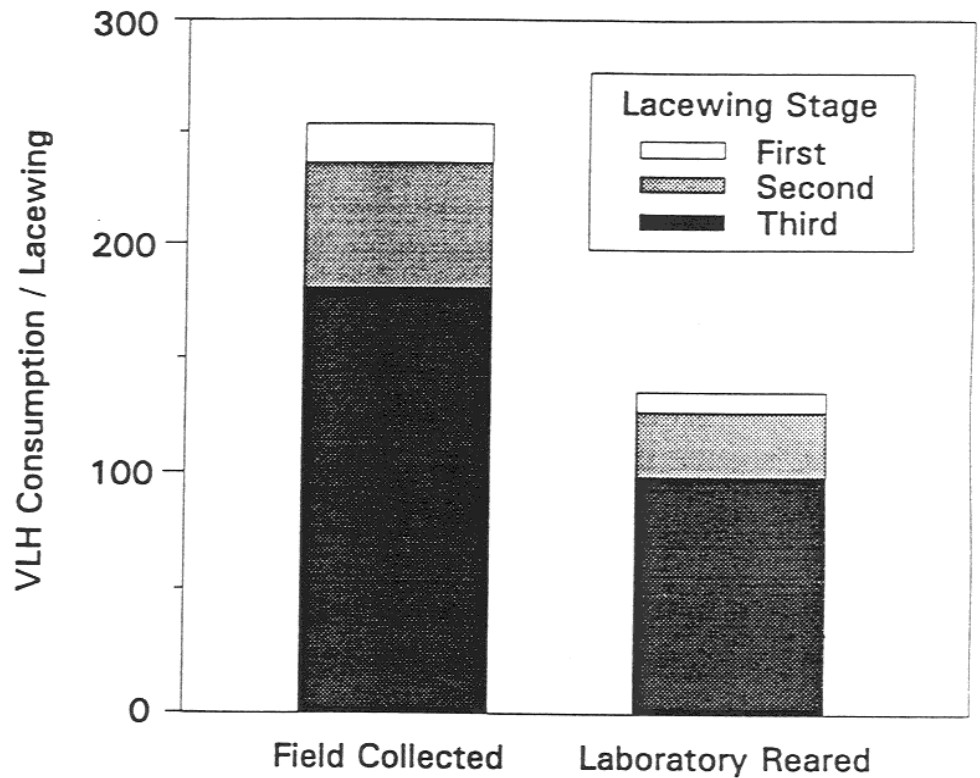


Figure 1. Average predation by first, second and third larval *Chrysoperla comanche* on fourth or fifth instar leafhoppers. Lacewing used were derived from either field-collected adults or a nine-month-old laboratory colony maintained at the Kearney Agricultural Center.

1991 treatments were: 1) no release; 2) 24 lacewings per plot, with releases timed by calendar-date; 3) 24 lacewings per plot, with a 'synchronized' release, timed at 50-70% leafhopper egg hatch to match released lacewings with leafhoppers phenology; and (4) 48 lacewings per plot, with a synchronized release. In 1992 treatments in the first leafhopper brood were: 1) no release, and 2) 48 lacewings per plot, with a calendar-date release. In the second brood treatments were: 1) no release, 2) 48 *C.carnea* per plot, 3) 48 *C.comanche* per plot, and 4) 48 *C.rufilabris* per plot, each with a synchronized release.

In all experiments first instar lacewing larvae were placed on leaves with a camel hair brush. Previous work had shown that releases of larvae, rather than eggs, provided greater control of release number due to variability in egg hatch and survivorship. Leafhopper densities were determined every two weeks from nymphal counts on 10 (1990 and 1991) and 20 (1992) leaves per plot. Data were analyzed with Analysis of Variance, using Tukey's HSD test to separate means.

Results for each year showed a pattern of lower leafhopper densities in lacewing release than in control plots. The difference between release and control plots was greatest in the 1990 cage trial, at a release rate of 48,000 eggs per acre, with a significant 42 and 28.7% reduction in leafhopper numbers in the first and second broods, respectively. However, at current costs for lacewing eggs (ca. \$3.00 US/1,000 eggs) the higher release rates may be economically prohibitive for the level of control achieved.

In 1991 the tested release rates more closely reflected commercial rates, typically near 3,000 to 4,000 eggs per acre with one to two releases each in the first and second leafhopper broods. At the higher release rate of 8,000 eggs per acre, a significant 33.6 and 31.4% reduction in leafhopper density was found in the first and second brood, respectively. Results were less consistent at the lower release rate of 4,000 eggs per acre. In the calendar-date release a 31.2% reduction and a 12.9% increase in leafhopper density was recorded in the first and second leafhopper broods, respectively, while in the synchronized release plots there was a 16% increase and a 12.9% decrease, respectively.

In 1992, there was no between treatment difference in first brood leafhopper densities. Lacewing releases in the second brood, comparing *C.carnea*,

*C.comanche* and *C.rufilabris*, showed only *C.rufilabris* to significantly reduce leafhopper density as compared to control plots. However, leafhopper density in the control plot was very low ( $<5/\text{leaf}$ ), far below any suggested economic injury levels, which we believe reduces the importance of this trial as a comparison of lacewing species. The data does, however, indicate that with similar release rates, there is a lower per leaf leafhopper mortality at low leafhopper densities than at high densities.

#### Commercial vineyards

Growers using commercial lacewing release programs have reported a reduction or elimination of pesticide applications for leafhoppers. As an initial evaluation of commercial release programs, eight Thompson seedless vineyards were monitored in 1990. Five vineyards had commercially-reared and grower-released lacewings, while three nearby vineyards had no release and no pesticide applications. The vineyards had similar cultural practices (pruned for raisins, no cover crops, furrow irrigation, vine-age, and fertilization practices). The lacewings were released at approximately 5,000 *C.carnea* eggs per acre, with two releases each in the first and second leafhopper broods, totalling 20,000 eggs per acre. Release timing was based on a calendar-date schedule. Leafhopper density in each plot was monitored by nymphal counts on 20 leaves every two weeks.

In 1991 and 1992, in three Thompson seedless vineyards, 150-vine plots were established in a randomized block design. Treatments in 1991 were: 1. no release and 2. machine release of lacewing eggs mixed with corn grit (see below). In 1992 a hand release of lacewing larvae, at the same rate as the machine release, was added as a third treatment. In both years, lacewings were released at approximately 3,500 eggs per acre, in one and two releases in the first and second leafhopper broods, respectively, totalling approximately 10,500 *C.carnea* eggs per acre. Releases were made on a calendar-date schedule. Leafhopper densities were monitored with biweekly nymphal counts on 20 leaves from the center 30 vines in each plot.

Results in 1990 show that the average leafhopper density in release vineyards ( $8.7 \pm 0.3$ ) was significantly lower than that recorded in control vineyards

( $12.5 \pm 0.5$ ). The 32.7% reduction in leafhoppers amounted to an average of 3.8 leafhoppers per leaf less in the treated vineyards. In this study, the effectiveness of lacewing releases, as measured by a per leaf reduction, may not return economic inputs in vineyards with low leafhopper densities.

In 1991 and 1992 the between-vineyard variation was removed by large, replicated plots within each vineyard. Figure 2a shows a trend of lower leafhopper numbers in lacewing release than in control plots in 1991. In the first brood there was a significant reduction in only one vineyard, however, the 34.9% decrease, from 23.2 to 15.1 leafhoppers per leaf, brought the average leafhopper density in release plots down to the suggested economic injury threshold of 15 leafhoppers per leaf. In the second brood there was a significant reduction in leafhoppers in each vineyard. While significant, the reduced leafhopper densities in vineyards one and two (Fig. 2a) remained above suggested economic thresholds. Thus, at leafhopper densities  $>30$  per leaf the release rates used, with machine release methods, appear to be too low to bring about the needed decrease ( $>50\%$  reduction) in leafhopper numbers. In vineyard 3, leafhopper density was below suggested economic thresholds in both release and control plots, and the average reduction in release plots amounted to only 2.4 leafhoppers per leaf.

In 1992 leafhopper densities were low, never exceeding the suggested treatment threshold (Fig. 2b). In only one of the three vineyards was a significant reduction in leafhopper densities recorded, a 36.7% reduction in leafhopper numbers in the hand release treatment as compared to control plots. The actual decrease was three leafhoppers per leaf. As seen in other vineyards, the effectiveness of lacewing releases is apparently correlated to the leafhopper density. At low leafhopper densities, lacewing consumption is lowered, presumably due to the adding searching time needed to locate sparse prey.

#### **Release methods**

From field release trials we identified areas in which current commercial release programs can be improved. Proper release timing and egg delivery to the vines are considered crucial to a successful program. Release timing is often based on a calendar-date schedule because lacewings must be ordered in advance to insure

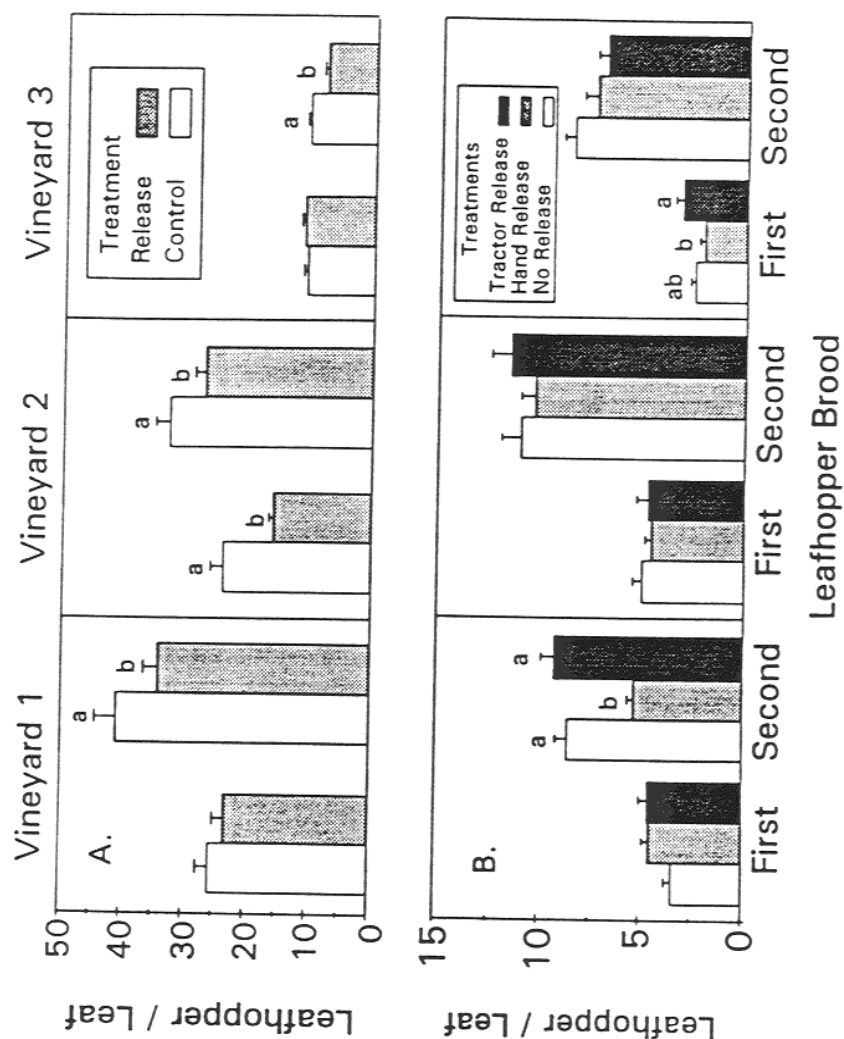


Figure 2. Leafhopper density in lacewing release and no release plots in three Thompson seedless vineyards in (A) 1990 and (B) 1992. *C. carnea* eggs were released at approximately 3,500 per acre in the first brood and 7,000 per acre in the second brood. Mean densities in each brood and vineyard separated by different letters are significantly different ( $P < 0.05$ ).

their timely delivery. Because leafhopper phenology is dependent on climate and not the calendar we often observed commercial releases falling between leafhopper broods, having little or no effect. Leafhopper phenology must be monitored to synchronize the release with leafhopper egg hatch. This will pair the newly hatched lacewings with the smaller stage leafhoppers. Similarly, after lacewings are shipped to the grower, the release schedule has to be matched to management practices, such as irrigation. Not only can a delay in the release place lacewing larvae in the vines at the wrong time, it can also reduce egg viability. As the insectary material sits the lacewing eggs hatch and, eventually, larvae feed on each other or die from starvation. Insectaries stress the importance of proper storage before delivery and work with their customers to deliver viable material at the proper time.

The most common lacewing release method is to mix lacewing eggs with a medium, such as corn grit, vermiculite, or rice hulls. The mixture is released over the vines, either by hand, from a tractor, or from an airplane. In the vineyards monitored, lacewing eggs were combined with corn grit and the mixture was placed in five gallon containers that had an adjustable funnel opening at the bottom. The containers were mounted on a platform above the vines and the mixture was spread as the platform moved down the rows, pulled by a tractor. By changing either the funnel size or the tractor speed, the release rate could be controlled.

A series of tests were conducted to determine the number and viability of eggs released with this method. The amount of mixture delivered to each vine was measured. At the same time, samples of the mixture were taken at the beginning and towards the end of a release batch. The samples were dissected in the laboratory, recording the number of live, hatched, crushed (from the mixing process) and dead (from the insectary) eggs and live or dead larvae. After delivery to the vines, egg survivorship can be affected by high temperatures. Egg versus larval releases were compared in 3-leaf plots, set in a randomized block design with seven replicates. Treatments were: 1. no release, 2. three fresh *C.comanche* eggs, and 3. three *C.comanche* first instar larva. Leafhopper and lacewing densities were determined every two to three days. To determine lacewing larval recovery in the field, leaves were sampled, five to ten days after release dates, in

commercial vineyards monitored in 1990-92. The number of lacewing larvae and live or hatched eggs (on stalks) were recorded.

Results from the egg number and viability test show that  $4.0 \pm 0.5$  ml of the corn grit and egg mixture was delivered to each vine. The average number of eggs in each 25 ml sample of was  $39.5 \pm 6.2$ , making egg delivery approximately 9.9 eggs per vine or 4,950 eggs per acre per release. This number corresponds well to the grower's desired release level of 4,000 eggs per acre. However, delivery of eggs throughout the vineyard was uneven. The average number of lacewing eggs per 25 ml samples taken at the beginning ( $55.3 \pm 8.6$ ) was significantly greater than that collected at the end ( $23.9 \pm 3.9$ ) of one release batch. The results imply that as the tractor moves the eggs sift down to the bottom of the release container and are delivered in greater numbers at the beginning rows. There was also a great range (min. 5, max. 119) in the number of eggs per sample, which indicates an uneven distribution of eggs in the field.

Results show that the method of egg dispersal used did cause some egg mortality, as can be expected with almost any delivery system. After dissecting the mixture, we found a 62% survivorship of the eggs delivered to the vines (live eggs + larvae), 35.5% crushed eggs (from the mixing process), and 2.5% dead eggs. The low number of dead eggs, is typical of material received from the insectaries, with most mortality occurring from storage and delivery methods after shipment. The percentage of live eggs delivered to the vines is acceptable considering other delivery methods. Air delivery of eggs to vines places much of the material on the ground, where the lacewing larvae remain. Using other mixing substances, such as vermiculite, have been reported to result in less egg mortality, but are difficult to use with the cooperative grower's system. Spreading the mixture over the vines by hand release is the most accurate way to deliver the eggs; however, costs of such an operation may be prohibitive. Currently, improved methods of lacewing egg delivery are being developed by university and insectary personnel.

Results of the egg and larval release comparison showed a great mortality of eggs in the field, with less than 20% egg hatch. There was no difference between egg release and control plots. After no lacewings were observed in the egg release



plots, leafhopper counts were discontinued. In plots receiving larvae, a significant reduction in leafhopper numbers was found as compared to the control (Fig. 3). From this work and field observations we concur with many insectaries and suggest that it is best to release lacewings when eggs are ready to or already have begun to hatch. For work in vineyards we suggest a 25 to 50% egg hatch at the release date. While there will be some cannibalism, the assurance of viable material delivered to the vines is increased.

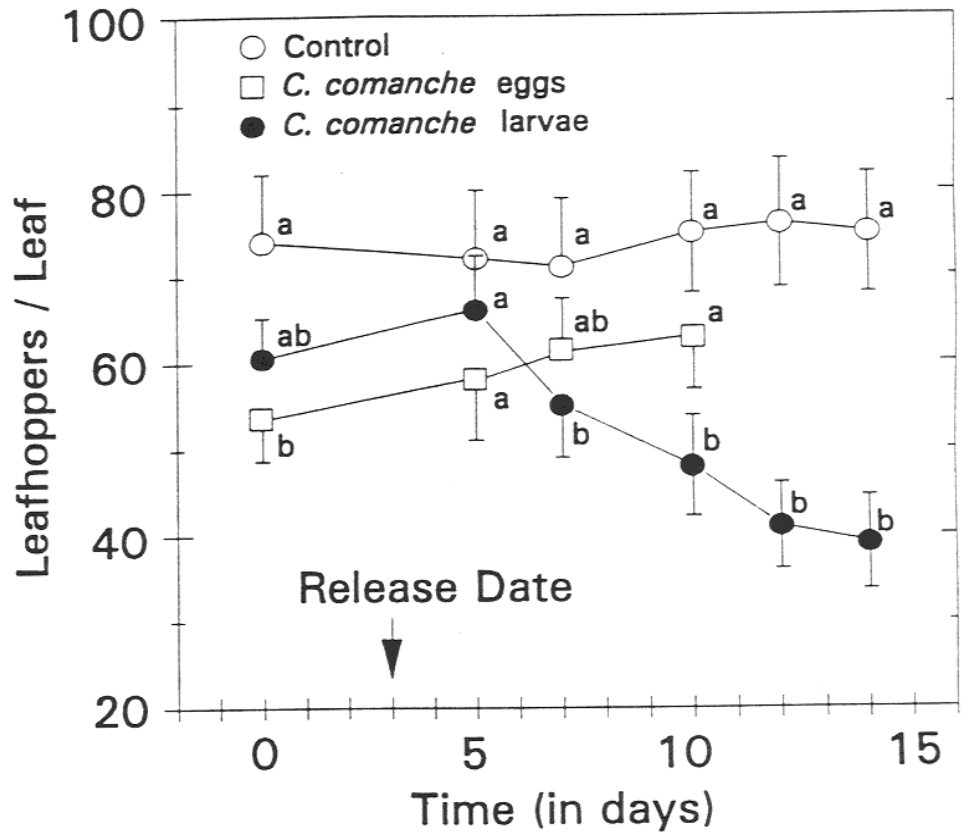


Figure 3. Leafhopper density in 3-leaf plots that received first instar *C. comanche* larvae was significantly lower than control plots or plots receiving fresh *C. comanche* eggs. Field hatch of released eggs was <20% in this study; ambient temperatures in excess of 36°C may have increased egg mortality.

Results of the larvae recovery test show no significant difference between release and control plots, both with less than 1 larva per 1,000 leaves sampled. The poor recovery does not implicitly correspond to low lacewing densities in the field, rather that sampling for lacewing larvae is difficult given their mobility and the low release rate in comparison to the number of leaves on the vine. Evidence of high natural lacewing populations is given by the number of eggs laid on stalks in both release and control plots. The highest densities of lacewing eggs recorded, 15 eggs per 100 leaves, were in vineyards adjacent to peach or almond orchards, which did not receive any commercial lacewings.

### Conclusion

Green lacewings are one of the most common commercial insectary-reared and released natural enemies. While release rates and efficacy data have been collected for some pest species, guidelines should be developed for each crop; for example, lacewings may be excellent for controlling aphids and good leafhopper predators but be of relative little value for use against scales. We believe that the level of leafhopper control with augmentative releases can be improved. Releases should be made as the leafhopper egg hatch is near completion but before leafhopper development progresses into the later stages. Field studies suggest that to improve effectiveness lacewing release rates must be better matched to leafhopper densities. For example, our data implies that leafhopper densities exceeding 40 leafhoppers per leaf can not be reduced below economic threshold levels with current release methods and at rates economically comparable to insecticidal treatments. Similarly, at low leafhopper densities (e.g., <5 leafhoppers per leaf) the resultant per leaf decrease in pest numbers will not be as great. Careful monitoring of the vineyard can help determine in which blocks lacewing releases can be most effective.

Finally, from surveys of lacewing larvae and adults naturally occurring in Central Valley vineyards, we identified five different lacewing species: *Chrysoperla comanche*, *Chrysoperla carnea*, *Chrysopa oculata*, *Chrysopa nigricornis*, and *Chrysopa coloradensis*. By far the most common adult collected was *C. oculata*; however, eggs and larvae on the vines are rarely this species.

*C. oculata* adults would fly as the cover crop was disturbed, giving the impression of a large lacewing population in the vines; however, the difference between *C. oculata* collected in the cover crops and the vines directly above could be as much as 100 fold. The most common larval species collected in the vines was *C. comanche* followed by *C. carnea*. While initial experiments found variability in the effectiveness of the different commercially available lacewing species, this difference is not nearly as important as the proper storage and delivery of the lacewing eggs, after insectary shipment.

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### A Fecundity Test for the Predatory Mite *Amblyseius cucumeris*

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#### Abstract

The predatory mite *Amblyseius cucumeris* is used in several glasshouse crops to control larvae of onion thrips *Thrips tabaci* and western flower thrips *Frankliniella occidentalis*. Two fecundity tests were compared: the arena method, consisting of a plastic tile surrounded by wet tissue paper, and the "petri dish-type" tray method, comprising of little trays with a sweet pepper leaf disc mounted on agar. The effect of three different food sources (*Tyrophagus putrescentiae* nymphal and adult stages, *Tyrophagus putrescentiae* eggs and *Ephestia kuehniella* eggs) on fecundity was tested in the "petri dish-type" tray method. The use of *Ephestia kuehniella* eggs as a food source in the "petri dish type" tray method appeared to give the most reliable results. Females used in this test produced a mean of 20 eggs over a 23-day period.

#### Introduction

*Amblyseius cucumeris* (Oudemans) (Acarina: Phytoseiidae) is used as a biological control agent of *Thrips tabaci* (Lindeman) (Thysanoptera: Thripidae) and *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) larvae in greenhouse crops (De Klerk & Ramakers 1986, Gillespie 1989). It has been produced commercially and used in sweet pepper and cucumber since 1985. In 1993 a diapause-free strain of *A.cucumeris* became available and it is especially useful for preventive introductions in early spring (Van Houten 1993). These predatory mites are mixed with wheat bran and grain mites *Tyrophagus putrescentiae* (Schrank) (Acarina: Acaridae) and applied by distributing the

material over leaves or as a small rearing system in sachets.

During the 6th meeting of the IOBC working group "Quality control of mass reared arthropods" in Horsholm (Van Lenteren 1993) it was generally felt that a simple fecundity test for *A.cucumeris* needed to be developed. This test could then serve as the basis of a criterion for product quality control of *A.cucumeris* for use in a periodically-conducted test. Fecundity has been investigated by several authors (Van Houten 1991) using the arena method with pollen as a food source. Baier (1992) tested fecundity with individual females in small chambers of synthetic glass. The chambers were covered on one side with a permeable material and on the other side with a cover glass.

In this study we investigated two types of fecundity tests, a "petri dish-type" tray method and an arena method. Three different food sources were used to select the easiest and most practical set up.

#### **Material and Methods**

Experiments were carried out in a climate room at a temperature of  $22\pm1^{\circ}\text{C}$ , RH  $70\pm10\%$  and 16:8=L:D photoperiod. These conditions were generally accepted guidelines on quality control of mass reared arthropods (Van Lenteren 1993).

##### *Arena method*

Rectangular arenas of thick white plastic (14x10x0.1 cm) were placed on a moist block of foam plastic. Wet tissue paper was wrapped over the edges, to serve as a barrier to prevent escape and as a water source. On the tissue paper an extra barrier was added which consists of a rectangle of insect glue. Folded pieces of transparent plastic and some cotton wool served as a shelter where mites tend to rest and oviposit (Overmeer et al 1985). An ample amount of grain mites (*T.putrescentiae*) were added every day as a food source. Twenty four mated females of *A.cucumeris* were individually tested from a commercial product. Egg production was monitored daily.

##### *"Petri Dish-Type" Tray Method*

Studies were conducted on individual female mites in small round plastic "petri



dish-type" trays (diameter 32 mm; height 15 mm) which could be closed very tightly. A nylon mesh was incorporated into the lid to facilitate air exchange. Trays were filled with agar solution (1%) to a depth of 5 mm. Just before the agar solidified a sweet pepper leaf disc was placed with its upper surface in contact with the agar. Care was taken to insure that a leaf disc had a vein and some hairs for egg deposition. Good contact between the leaf disc and the agar solution is necessary to prevent predatory mites from hiding. Thirty mated females of *A.cucumeris* were taken from a commercial product. Three food sources were tested: *T.putrescentiae* nymphal and adult stages (mobile stage), *T.putrescentiae* eggs and *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs. The mobile stages and eggs of *T.putrescentiae* were separated from wheat bran by sieving the raw material. An ample amount of food was added to the trays every day. The test was stopped after a period of 23 days. Trays were placed upside down in the climate room to protect the predatory mites from bright light. Every day eggs were counted and then removed. *A.cucumeris* females were transferred to a fresh tray twice a week. Every test began with 30 replicates. If a predatory mite died or escaped within one day it was not included in the test.

## Results and Discussion

### *Arena method*

It was time consuming to relocate the predatory mites because the arenas were relatively large for one mite. One day after introduction, only seven of the 24 females were found. After three days only one female was found. Some of the mites are likely to have drowned under the wet tissue paper. Also *T.putrescentiae* dispersed all over the arena causing it to become dirty and the tissue paper also went mouldy. The arena method is not very practical when testing individual females. Moreover, according to Van Houten (pers. comm.) rearing history of the mites influences the behaviour of the predatory mite in the arena. If they are not bred in the arena, they will try to escape.

### *"Petri dish type" tray method*

Results of the daily fecundity test are given in figure 1. The mean number of eggs

laid per female per day was not significantly different when using eggs of *E.kuehniella* and mobile stages of *T.putrescentiae* as a food source (Mann Whitney;  $p < 0,05$ ). In contrast, females fed on eggs of *T.putrescentiae* laid significantly fewer eggs than those fed on eggs of *E.kuehniella* (Mann Whitney;  $p < 0,05$ ).

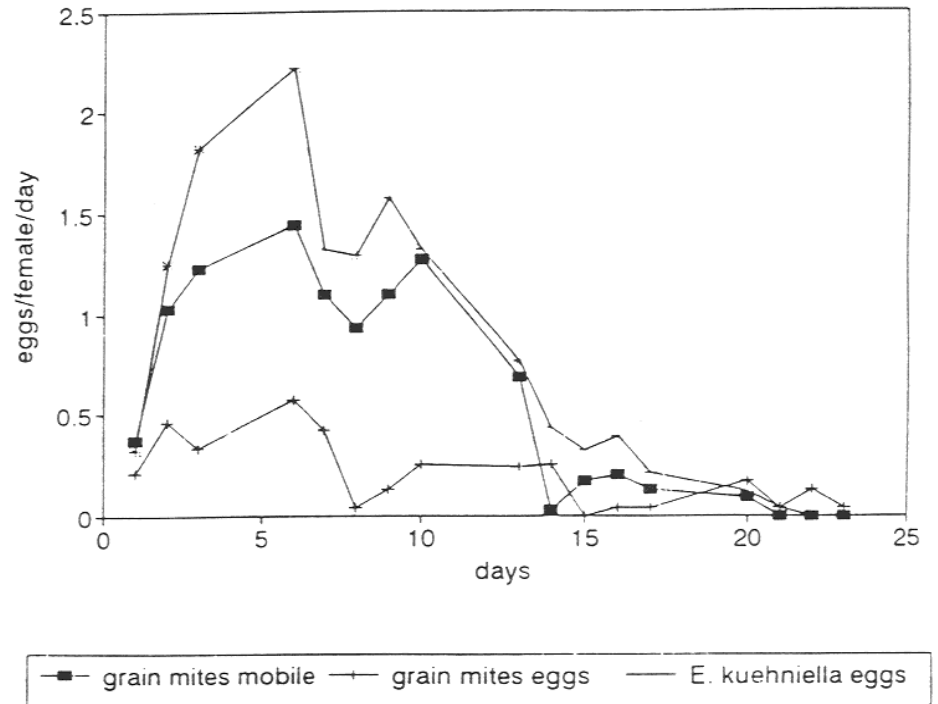


Figure 1. The mean fecundity of the predatory mites per day fed on different food sources (mobile stages and eggs of *T.putrescentiae* and eggs of *E.kuehniella*) during a period of 23 days.

It is possible that eggs of *T.putrescentiae* alone are not nutritionally adequate for the predatory mites. Thus, in preliminary tests quantity of food was found to be very important.

At the beginning of all the experiments, predatory mites laid a mean of 0.3

eggs. After two days, females laid about one egg each per day when fed on mobile stages of *T.putrescentiae* or on eggs of *E.kuehniella*. After 11 days the mean number of eggs laid per female per day decreased. Consequently, mortality or disappearance of females increased (Table 1).

Table 1. Mean number of days when 50% of mites tested died or disappeared using three different diets; three replicate tests.

food source	days	n
<i>T. putrescentiae</i> (mobile stages)	8.3 $\pm$ 4.0	3
<i>T. putrescentiae</i> (eggs)	6.7 $\pm$ 5.0	3
<i>E. kuehniella</i> (eggs)	17.0 $\pm$ 3.0	3

The mean time taken for 50% of the predatory mites to die or disappear when fed on mobile stages of *T.putrescentiae* was not significantly different from the time taken when fed on eggs of *T.putrescentiae*. However, significant differences were found between eggs of *T.putrescentiae* and *E.kuehniella*, and between mobile stages of *T.putrescentiae* and eggs of *E.kuehniella* (Mann Whitney). It took a longer time for 50% of the predatory mites fed on eggs of *E.kuehniella* to die or disappear. The effect of mobile stages and eggs of *T.putrescentiae* on mortality or disappearance is high, because these food sources make a mess in the trays. Also, it is difficult to adjust the amount of food per tray. If too much food is offered the trays become fouled; if too little food is used the mites die.

Total fecundity was assessed in 3 successive tests (Fig. 2). The fecundity of predatory mites fed on mobile stages of *T.putrescentiae* differed significantly from each other between tests (Kruskal Wallis;  $p < 0.05$ ). The same applied to predatory mites fed on eggs of *T.putrescentiae*. The *A.cucumeris* fed on eggs of *E.kuehniella* were not significantly different from each other between the tests (Kruskal Wallis). The results showed that the total fecundity of females fed on eggs of *E.kuehniella* was consistent. However, the results for the total fecundity of females fed on mobile stages and eggs of *T.putrescentiae* were very variable.

The mean total number of eggs per female fed on eggs of *E.kuehniella* over the 23-day period was 20. Using 25°C, RH 75% and *T.putrescentiae* as a food source, Baier (1992) reported that females laid a mean of 31 eggs each during their lifetimes period of 40 days. The total fecundity in this test is comparable to that determined by Baier (1992), because the age of the females was not known at the beginning of the test.

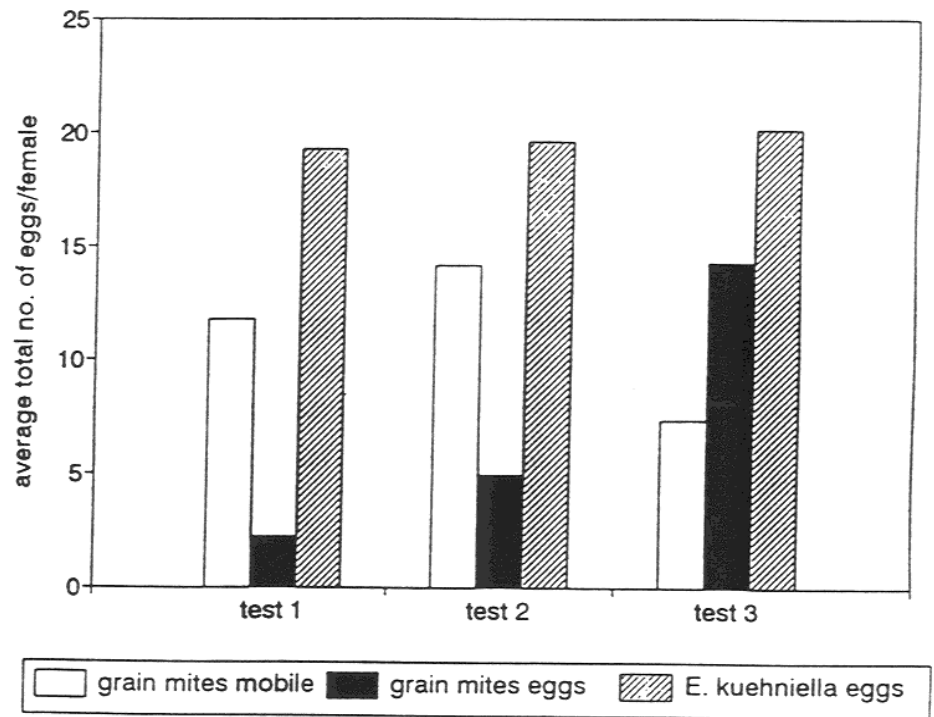


Figure 2. The total fecundity of the predatory mites fed on three different food sources (mobile stages and eggs of *T.putrescentiae* and eggs of *E.kuehniella*) in three replicate tests.

The "petri dish-type" method seems to be a reliable test of fecundity when the predatory mites are fed on eggs of *E.kuehniella*. The use of eggs or mobile stages of *T.putrescentiae* is complex and gives less consistent results. The arena method is considered to be impractical, time consuming and very unreliable.

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## **Quality of Predatory Bugs of the Genus *Podisus* Reared on Natural and Artificial Diets**

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### **Abstract**

Long-term laboratory rearing of the predatory stinkbugs *Podisus maculiventris* (Say) and *Podisus sagitta* (Fabricius) on natural and artificial diets is discussed. The pentatomids were easily reared on late-instar larvae of the greater wax moth, *Galleria mellonella* (L.), and on the beet armyworm, *Spodoptera exigua* (Hübner). When *P. maculiventris* and *P. sagitta* were reared on live prey during four and 11 years, respectively, the insects maintained good survival and high fecundity. To overcome occasional difficulties in food supply, an artificial diet based on bovine meat was evaluated as an alternative food source for rearing *Podisus*. Although development and reproduction on the meat diet were inferior to those on live prey, the nutritional value of the artificial diet was sufficient to produce consecutive generations in the laboratory. When returned to a diet of live prey after more than 15 generations on artificial diet, viability and predatory performance were similar to those of bugs continuously fed on live prey. The results suggest that prolonged rearing on artificial diet had no lasting deleterious effects on the quality of the predators.

### **Introduction**

Entomophagous insects reared under artificial conditions for research or biocontrol purposes should be of good quality. However, long-term laboratory cultures of an entomophagous insect may undergo adaptive and genetic changes. Such changes may alter the feeding preferences of the entomophage or may lead to impaired viability and performance, thus reducing its efficacy as

a control agent. The more artificial the rearing of an entomophage becomes (e.g., the use of an artificial diet), the higher the risk of deterioration of its behavioural, morphological and physiological qualities (Mackauer, 1976; Waage et al., 1985; Bigler, 1989; van Lenteren, 1991).

When starting a rearing programme for an entomophagous insect, it is often convenient to use natural hosts (or prey), particularly if there has been little previous work on rearing methods. For many reasons, however, the availability of an adequate artificial diet is highly desirable for the mass-production of many entomophagous insects.

This also applies to the rearing of predatory heteropterans. The predatory pentatomids *Podisus maculiventris* (Say) and *Podisus sagitta* (Fabricius) are potential biocontrol agents in the management of several lepidopterous and coleopterous pests (Clausen, 1940; LeRoux, 1960; Lopez et al., 1976; Khloptseva, 1991). In the United States, *P. maculiventris* is being reared commercially for biological control (Anonymous, 1992). Laboratory cultures of *Podisus* are mostly maintained on easily-reared lepidopterous prey, such as the pyralid *Galleria mellonella* (L.) and the noctuids *Spodoptera exigua* (Hübner) and *Trichoplusia ni* (Hübner) (Mukerji & LeRoux, 1965; Warren & Wallis, 1971; Drummond et al., 1984; De Clercq et al., 1988; Khloptseva, 1991; Biever & Chauvin, 1992). Given the voracity of these predators, however, extensive parallel cultures of prey insects are needed. The availability of an adequate artificial diet may offer an alternative to overcome occasional problems of discontinuity in food supply. However, only few successful attempts have been reported to rear predatory heteropterans on artificial diets. Using a modification of an all-beef diet developed by Cohen (1985) for the predatory lygaeid *Geocoris punctipes* (Say), we succeeded in rearing continuous generations of both *Podisus* species (De Clercq & Degheele, 1992a).

In the present paper, long-term rearing of *P. maculiventris* and *P. sagitta* on natural and artificial diets is evaluated, using developmental, reproductive and predation characteristics as quality parameters.

#### **Materials and Methods**

A laboratory colony of *P. sagitta* was established in 1982, with insects



originally obtained from Surinam. A culture of *P. maculiventris* was started in 1989 from eggs originating from Florida. Cultures of both insects were maintained in growth chambers at  $23 \pm 1^\circ\text{C}$ ,  $75 \pm 5\%$  RH and a 16:8=L:D photoperiod. Nymphs and adults were kept in plastic containers (18 x 11 x 6 cm, and 24 x 16 x 8 cm or 32 x 23 x 11 cm, respectively) with vented covers. The containers were furnished with several layers of absorbent paper towelling. Paper towels provided hiding places and oviposition sites, and ensured the absorption of excreta from predators and prey. Moisture was provided to nymphs and adults via a soaked paper plug fitted into a small plastic dish. Live prey were provided in excess from the second instar on (first instars are not predaceous). Last instars of the greater wax moth, *G. mellonella*, and of the beet armyworm, *S. exigua*, constituted the main diet of the predators. Occasionally, late instars of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), the cabbage moth, *Mamestra brassicae* L., and the cotton leafworm, *Spodoptera littoralis* (Boisduval), were offered as prey. Fresh prey were replenished and dead prey were removed every other day.

In 1989, nymphs of both species derived from insect-fed cultures were transferred to artificial diet. This diet was prepared as described by De Clercq & Degheele (1992a). It was composed of 200 g beef liver, 200 g fatty ground beef, 24 ml sucrose solution (5%), 1 g ascorbic acid, 2 g Wesson's salt mixture, and 20 g fresh hen's egg yolk. All ingredients were blended to a uniform paste. Cylindrically-shaped "artificial larvae" were produced by wrapping the paste into sheets of Parafilm. Artificial larvae were supplied in excess from the second instar on and were replaced daily.

The gross nutritional composition of the meat-based diet was compared with that of seventh-instar larvae of *G. mellonella* and fifth-instar larvae of *S. exigua*. Protein content was determined following the Kjeldahl procedure. Determination of fat content was done by direct solvent extraction (Weibull-Soxhlet). Water content was determined by drying in an oven at  $110^\circ\text{C}$  until a constant weight was achieved. Total ash content was determined by combustion in a muffle furnace at  $500^\circ\text{C}$ . The amount of carbohydrate was estimated by subtracting the sum of protein, fat, water, and ash content (in percent fresh weight) from 100. All analytical procedures were carried out as described in Egan et al. (1981).

Predators on both food sources had been in continuous culture until this study. To evaluate the quality of the predators produced on each diet, their developmental and reproductive traits were monitored, following the methods described in De Clercq & Degheele (1992a). Nymphal development was studied by confining 40 nymphs in groups of ten into nine or 14 cm-petri dishes, furnished with a strip of paper towelling. Oviposition and longevity was studied for six adult pairs placed singly in 14-cm petri dishes. Nymphs and adults were provided with moisture and fed either larvae of the greater wax moth or artificial larvae in excess. Fertility of egg batches collected from communal cultures on each diet was also recorded.

To determine possible deleterious effects of prolonged rearing on artificial diet, the predators were returned to a diet of live prey, i.e., larvae of *G.mellonella*, after different generations on the meat diet. Development on live prey was monitored as described above after eight and 17, and after six and 15 generations on artificial diet for *P.maculiventris* and *P.sagitta*, respectively. Eggs of each species were collected from cultures of adults on artificial diet in each of the above-mentioned generations. From the second instar on, nymphs were presented with seventh-instar larvae of *G.mellonella ad libitum*. Reproduction of females obtained from nymphs returned to a diet of insect prey after eight and six generations on artificial diet for *P.maculiventris* and *P.sagitta*, respectively, was studied in more detail.

Predation by bugs returned to live prey after 17 and 15 generations on artificial diet for *P.maculiventris* and *P.sagitta*, respectively, on larvae of *S.exigua* was measured in the laboratory. Nymphs from eggs laid by females of above-mentioned generations on artificial diet were allowed to develop to adulthood on a diet of wax moth larvae. When reaching the fourth instar, 20 nymphs of each species were placed individually into 9-cm petri dishes, lined with absorbent paper. Each nymph was offered ten early fourth-instar larvae of *S.exigua*. Each dish was supplied with castor bean (*Ricinus communis* L.) foliage to provide food for the prey and moisture for the predators. Each day throughout the fourth instar, the number of larvae killed was recorded, and dead larvae were replaced by fresh ones. Predation rates were also determined for female adults of which the nymphal stages were fed on live prey after extended rearing on artificial diet. Predation by such females on fifth-instar

larvae of *S. exigua* was investigated. All females used in these experiments were reproductively active (10-30 days old). Prior to testing, females were isolated for 24 hours without food, but with access to moisture. For each species, 20 starved females were placed singly in 9-cm petri dishes. Each dish was provided with ten early fifth-instar larvae of *S. exigua* and with castor bean foliage. Numbers of attacked prey were recorded after 24 hours. Predation rates of nymphs and adults fed in previous generations on artificial larvae were compared with those of bugs randomly selected from stock cultures maintained on live prey.

## Results and Discussion

### *Natural diet*

Given the simplicity of the applied rearing methods, *P. maculiventris* and *P. sagitta* were easily reared on natural hosts in the laboratory. Since their introduction, 6-8 generations of both pentatomids were raised per year without apparent loss of vigour. For both species, eggs hatched after six days (De Clercq & Degheele, 1992b) and nymphal development took about three weeks at 23°C (Table 1). More than 80% of the first instars reached adulthood. At a moderate temperature of 23°C, the proportion of female progeny was somewhat higher, with sex ratios ranging from 1:1 to 1:1.4 (male:female). Weights of newly emerged females averaged 85 and 65 mg for *P. maculiventris* and *P. sagitta*, respectively. Both male and female adults lived 2-3 months. Following a preoviposition period of about one week (De Clercq & Degheele, 1992a), females of *P. maculiventris* and *P. sagitta* produced an average of 700 and 900 eggs, respectively, with a rate of ca. 12 eggs per day. About 65% of the *P. sagitta* eggs hatched successfully; egg hatch of our *P. maculiventris* strain was lower, averaging only 55%.

Mean fecundity of *P. maculiventris* and *P. sagitta* in our laboratory cultures was considerably greater compared with that reported for *Podisus* bugs in the literature. Only Couturier (1938) mentioned a similar fecundity of 500-600 eggs for *P. maculiventris*, with an oviposition rate of 14 eggs per female per day. Other authors, however, reported a total fecundity of only about 300 eggs per female for this species in the laboratory (Mukerji & LeRoux, 1965; Warren & Wallis, 1971; Baker & Lambdin, 1985; Wiedenmann & O'Neil, 1990).

Table 1. Development and reproduction of *P. maculiventris* and *P. sagitta* on natural (live prey) and artificial diet, and on returning to natural diet after prolonged rearing on artificial diet

Diet	Nymphal period (days)	Nymphal survival (%)	Sex ratio (♂:♀)	Weight of females (mg)	No. of eggs per female per day	Total no. of eggs per female	Longevity of females (days)
<i>P. maculiventris</i>							
Natural	22.9 ± 0.7a*	82.5	1 : 1.4	86.5 ± 5.0a	12.2 ± 2.0a	710 ± 413a	64.7 ± 32.8a
Artificial	27.8 ± 1.6c	60.0	1 : 1	65.2 ± 4.9b	7.0 ± 1.0b	395 ± 67b	78.0 ± 14.1a
From artificial to natural	23.4 ± 0.9b	82.5	1 : 1.1	86.3 ± 6.0a	12.7 ± 2.0a	570 ± 150a	59.5 ± 13.5a
<i>P. sagitta</i>							
Natural	22.3 ± 1.0a	85.0	1 : 1	63.4 ± 6.9b	11.8 ± 2.0a	910 ± 222a	88.6 ± 33.4a
Artificial	30.4 ± 1.7c	62.5	1 : 0.7	46.3 ± 4.8c	5.3 ± 1.2b	294 ± 81b	79.8 ± 13.4a
From artificial to natural	23.3 ± 1.2b	87.5	1 : 1	66.9 ± 6.2a	11.5 ± 2.3a	931 ± 300a	100.5 ± 36.2a

\* Means ± SD; means within a column and within a species followed by the same letter are not significantly different ( $P > 0.05$ ;  $t$ -test).

Zanuncio et al. (1991a) observed a mean fecundity of 213.4 eggs per female and a longevity of one month for the Neotropical species, *Podisus connexivus* Bergroth. Differences in fecundity may be partly related to applied rearing methods and to the geographic origin of the studied strains. On the other hand, rearing history of the strains in question is believed to be a more important factor. Mackauer (1976) and Leppla & Ashley (1989) pointed out that insects adapted to the laboratory are characterized by a greater fecundity. In most above-mentioned studies on *Podisus* spp., strains were used which had recently been introduced to the laboratory, or which had been reared under a laboratory regime for only a few generations. Likewise, body weight of *P. maculiventris* females in our laboratory cultures was higher than 80 mg, whereas females collected in the field were reported to have a body weight of only about 50 mg (Morris, 1963; Evans, 1982; Aldrich, 1986). Greater body size and fecundity may thus indicate that our strains of *P. maculiventris* and *P. sagitta* were well-adapted to laboratory conditions.

On the other hand, fertility of *P. maculiventris* eggs observed in our study (ca. 50%) was markedly lower than that reported in the literature, ranging from 78% (Warren & Wallis, 1971) to 94% (Mukerji & LeRoux, 1965). Egg hatch recorded for *P. sagitta* (ca. 65%) was comparable with that found for the Brazilian species *P. connexivus* (76%; Zanuncio et al., 1991a).

Best results were obtained when using larvae of *G. mellonella* and *S. exigua* as prey. However, the nutritional value of individual *G. mellonella* larvae proved to be greater than that of *S. exigua* larvae. For example, when presented with larvae of similar weight (ca. 50 mg), fourth-instar nymphs of *P. sagitta* required  $6.0 \pm 0.5$  (mean  $\pm$  SD) early fifth instars of *S. exigua* versus  $3.2 \pm 0.3$  seventh instars of *G. mellonella* to reach adulthood. This may, in part, be attributed to the higher water content of beet armyworm larvae; these contained ca. 20% more water in comparison with wax moth larvae (Table 2). Larvae of *G. mellonella* contained considerably more protein and fat than those of *S. exigua*. Larvae of the cotton leafworm, *S. littoralis*, and of the cabbage moth, *M. brassicae*, were more aggressive and therefore less preferred prey. Larvae of the Colorado potato beetle, *L. decemlineata*, also proved to be suboptimal food for *Podisus*. Developmental rates of nymphs fed third and fourth instars of *L. decemlineata* were lower and obtained individuals were

smaller compared with nymphs fed on caterpillars (Drummond et al., 1984; De Clercq, 1993). Nevertheless, less optimal prey species were also sporadically supplied to cultures of both pentatomids. Providing variation in the kind of prey supplied is believed to enhance fitness and minimize inadvertent selection during laboratory propagation of the predators (Mackauer, 1976; van Lenteren, 1991).

Table 2. Nutritional composition of the meat-based artificial diet, and of last-instar larvae of *G.mellonella* and *S.exigua*

Diet	Components (g per 100 g fresh weight)				
	Protein	Fat	Water	Ash	Carbohydrate
Artificial	18.9	10.2	66.8	1.5	2.6
<i>G. mellonella</i> larvae*	15.7	17.8	62.8	1.3	2.4
<i>S. exigua</i> larvae†	9.0	2.2	84.8	1.2	2.8

\* Fed on artificial diet; † Fed on castor bean leaves.

In nymphal, as well as adult cultures, too high population densities provoked cannibalism. Under such conditions, adult bugs were sometimes also observed to feed on their own eggs. In addition, crowding in adult cultures of *Podisus* has also been reported to result in decreased egg production (Mukerji & LeRoux, 1965).

Despite of the fact that predators in laboratory cultures were confronted with constantly favourable climatic conditions and high prey levels, adaptability of our strains was not lost throughout the rearing programme. For example, laboratory-adapted *P.sagitta* adults showed appropriate adaptations to overcome periods of low food levels (De Clercq & Degheele, 1992c). Further, insects from our *P.maculiventris* and *P.sagitta* strains, which had been reared in an entirely artificial environment (plastic containers, paper towelling, artificial moisture sources) for up to ten years, were able to utilize fresh plant material for moisture and supplementary nutrients (De Clercq & Degheele, 1992d). Still, it may be recommendable to periodically expose these predators to plants during a rearing programme. In this respect, Ruberson et al. (1986) found that adding plant material to a normal diet of animal prey enhanced survival and shortened nymphal development and the preoviposition period in *P.maculiventris*.

#### *Artificial diet*

The gross nutritional composition of the modified meat-diet was compared with that of last instars of *G.mellonella* and *S.exigua* (Table 2). On average, the meat-based artificial diet contained 19 g protein, 10 g fat, and 67 g water per 100 g fresh weight. The composition of the meat mixture was thus more similar to that of wax moth larvae than to that of beet armyworm larvae. Cohen (1985) believed that a meat protein would better reflect the nutritional needs of a predatory insect than would plant or dairy products. He also considered the high fat and cholesterol content of the beef diet an important factor; in addition, he reported that the K/Na ratio was 2.7:1, which is a ratio similar to that in several phytophagous insects that are potential prey for predatory bugs.

The artificial diet was sufficient to support successive generations of both pentatomids. Over a period of ca. four years, more than 20 consecutive generations have been obtained through communal cultures fed exclusively on the meat diet. Data presented in Table 1 were recorded in the seventh generation on artificial diet. There were no obvious changes in developmental and reproductive rates throughout the subsequent generations on this diet. In comparison with rearing on live caterpillars, nymphal development was prolonged with four and eight days for *P.maculiventris* and *P.sagitta*, respectively. Survival in the nymphal stage was ca. 20% lower. Cannibalism was the most important mortality factor in cultures on artificial diet. However, it could be greatly reduced by avoiding crowded rearing conditions and by keeping individuals of the same age class together. There was a slightly higher proportion of male progeny when adults had developed on artificial larvae. Further, adult weights were decreased with about 25%. Fecundity of *P.maculiventris* and *P.sagitta* females reared on the meat diet was reduced to 1/2 and 1/3, respectively, of that of the insect-fed predators. However, fertility of eggs collected from cultures reared on artificial diet was comparable with that of cultures fed on live prey (50.8 and 61.3% for *P.maculiventris* and *P.sagitta*, respectively). Adult longevity was also not affected by diet. Throughout this study, bugs of both species raised on artificial diet exhibited normal activity patterns (e.g., mating, searching, flying).

The results obtained with this diet were superior to those reported for the artificial media tested by Adidharma (1986) for the development of *P.sagitta*.

This author used chemically defined, liquid media, probably based on the misconception that these bugs can only utilize liquid food. Our results are closer to those presented by Cohen (1985, 1992), who used meat-based diets for rearing *G.punctipes*. Other (semi-) artificial diets for *Podisus* spp. were reported by Khlistovskii et al. (1985), Lyashova et al. (1985) and Zanuncio et al. (1991b), but they all contained insects as components.

After up to 15 generations of feeding on artificial larvae, *P.maculiventris* and *P.sagitta* readily attacked and killed live prey larvae. The ability to subdue mobile and sometimes heavily struggling prey larvae, in particular those of *G.mellonella*, was not lost through prolonged rearing on inanimate artificial food. When nymphs, fed in previous generations on artificial diet, were provided with *G.mellonella* larvae from the second instar on, developmental times and adult weights were comparable with those of control bugs continually fed on live prey; data for development on live prey after different generations on artificial diet did not differ significantly ( $P>0.05$ ) and were therefore pooled (Table 1). Resulting females reproduced at the same rate as those of the control and had a similar total fecundity. Moreover, rearing for over 15 generations on artificial diet apparently did not affect predation potential of the pentatomids (Table 3).

Table 3. Predation on fourth-instar larvae of *S. exigua* by fourth-instar nymphs of *P. maculiventris* and *P. sagitta* (during the total instar), and on fifth-instar larvae of the prey by female adults of the predators (during 24 h), continuously reared on natural diet (live prey) and after prolonged rearing on artificial diet.

Previous diet	No. of prey larvae killed*			
	<i>P. maculiventris</i>		<i>P. sagitta</i>	
	Nymph (N <sub>4</sub> )	Female adult	Nymph (N <sub>4</sub> )	Female adult
Natural	9.5 ± 2.3	5.3 ± 1.3	8.5 ± 2.6	5.4 ± 1.4
Artificial	10.5 ± 1.6	5.1 ± 1.3	8.0 ± 2.1	5.7 ± 1.5

\* Means±SD; means within a column were not significantly different ( $P>0.05$ ; *t*-test).

In a petri dish arena, fourth instars of *P.maculiventris* and *P.sagitta* attacked a mean of ten and eight fourth-instar caterpillars of *S.exigua*, respectively,



before moulting (i.e., after 4-5 days). Female adults of both stinkbugs had killed 5-6 fifth-instar beet armyworm larvae after 24 hours, independently of previously experienced rearing conditions. These results suggest that prolonged rearing on artificial diet had no lasting detrimental effects on the quality of the predators.

### Conclusions

The predatory stinkbugs *P. maculiventris* and *P. sagitta* were easy to rear on natural hosts in the laboratory. The life cycle of both pentatomids at 23°C is relatively short, development from egg to adult taking about one month, and survival in the nymphal stage is good; females start producing eggs within one week after moulting and fecundity and rate of oviposition are high. Moreover, the rearing techniques are simple. Throughout a period of four and 11 years of laboratory rearing, *P. maculiventris* and *P. sagitta* maintained a good viability and predation capacity.

The meat-based artificial diet may be considered as a potential alternative food for the production of predatory stinkbugs of the genus *Podisus*. Although results on the meat diet were inferior to those obtained with live prey, the nutritional value of this diet was sufficient to support successive generations in the laboratory. The production of this diet is simple and inexpensive. The materials are readily available and require no complex processing. Automation of the preparation techniques may increase the practical value of the presented diet for mass-rearing purposes. Further dietary adjustments to improve the nutritional quality of the meat diet should be based on a better knowledge of the trophic physiology of these predators.

Although results of the laboratory experiments suggest that *P. maculiventris* and *P. sagitta* were of satisfactory quality after prolonged laboratory rearing, additional studies are needed to examine their performance in the field. Dispersal, i.e., walking and flight ability, prey finding and prey acceptance, functional responses, and adaptability of laboratory-reared predators should be assessed under field conditions.

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### Comparative Study of *Phytoseiulus persimilis* Strains: Initial Results

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#### Abstract

The biological activity of three *Phytoseiulus persimilis* (Acarina Phytoseiidae) strains of differing origin was investigated in the laboratory. The SI strain was field-collected in Sicily (I), the NE was mass-reared by Bunting BC Co. (GB) and the CE was field-collected in Cesena (I) and mass-reared by Biolab (I). Newly emerged pairs were isolated at  $25\pm 1^{\circ}\text{C}$  and  $80\pm 5\%$  RH and fed on *Tetranychus urticae* (Acarina Tetranychidae): SI showed significantly higher longevity (SI= $14.9\pm 7.1$ ; NE= $8.3\pm 6.0$ ; CE= $8.9\pm 5.4$  days; means $\pm$ SD), oviposition period (SI= $11.1\pm 4.9$ ; NE= $5.7\pm 4.6$ ; CE= $6.4\pm 4.9$  days) and total number of eggs laid (SI= $48.0\pm 24.8$ ; NE= $22.3\pm 24.0$ ; CE= $25.1\pm 21.7$ ); no significant difference was recorded in the pre-oviposition period or in the percentage of egg-laying females. The differences between strains appear to be attributable mainly to the greater SI longevity and longer oviposition period, as well as to its greater number of eggs laid per day, with respect to NE. A curvilinear correlation, a useful factor in quality-control tests, was found between the number of eggs laid over the first six days of life (X), and (Y) total oviposition ( $Y=8.86\cdot\exp(0.07\cdot X)$ ;  $r=0.76$ ).

#### Introduction

The predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acarina Phytoseiidae) is the most widely used beneficial agent for biological control of the red spider mite *Tetranychus urticae* Koch (Acarina Tetranychidae) in protected crops. Small-scale applications were started in 1968 and in 1990 mass-reared *P. persimilis* was used in about 7,000 ha of protected crops world-wide (van Lenteren *et al.*, 1992).

*P. persimilis* was first reported in Algeria by Athias-Henriot (1957), followed in Germany by Dosse (1958), who found this predatory mite (described as *P. riegeli*) on a consignment of orchids received from Chile and later distributed the progeny to interested workers in several European countries (Hussey, 1985). Lombardini (1959) was the first in Italy to report *P. persimilis*, which he found in Sicily on citrus leaves; he described it as *Amblyseius tardi* but Kennett and Caltagirone (1968) showed the synonymy with *P. persimilis*. Other reports of wild populations followed: in Sicily (Ragusa, 1965, 1974, 1977 and 1986; Liotta *et al.*, 1977; Vacante and Nucifora, 1985, 1986, 1987a and 1987b), at Fondi in central Italy (McMurtry, 1977) and at Cesena near the Adriatic seaboard on strawberry (Celli *et al.*, 1988). *P. persimilis* has also been reported in such other Mediterranean areas as southern France (Rambier, 1972), Greece (Swirsky and Ragusa, 1976 and 1977), Spain (Ferragut *et al.*, 1983; Garcia Mari *et al.*, 1986 and 1987) and, as an imported beneficial, in Lebanon (Dosse, 1967) and Israel (Swirsky and Amitai, 1968).

Several authors have conducted comparative studies on Phytoseiidae. Croft (1971) reported significant differences in the critical photophase prior to diapause in four *Typhlodromus occidentalis* Nesbitt strains of diverse geographic origin. McMurtry *et al.* (1976), investigating two *Amblyseius potentillae* (Garman) strains of diverse geographic origin, found differences induced by photoperiod in reproductive diapause and by relative humidity in hatching. More specifically, Hassan (1982), König and Hassan (1986), Mori and Gotoh (1986) and Fournier *et al.* (1987a and 1987b; 1988) compared various strains of *P. persimilis* in relation to pesticide resistance; Moraes and McMurtry (1985) report differing oviposition rates in *P. persimilis* strains of diverse geographic origin and Perring and Lackey (1989) found that two *P. persimilis* strains of diverse geographic origin had differences in development time and mortality of juvenile stages in response to varying thermo-hygrometric regimes.

The first application in Italy of *P. persimilis* as a biocontrol agent took place in Sicily using local material (Nucifora *et al.*, 1983; Vacante and Nucifora, 1987b), and the first releases of *P. persimilis* reared in a northern European biofactory date to 1985 in northern Italy (Celli *et al.*, 1987).

The present publication reports and discusses the initial findings of a comparative study designed to determine which of three *P.persimilis* strains is most suitable for inoculative seasonal release in a Mediterranean environment. It also discusses the development of quality control techniques for *P.persimilis* populations reared in biofactories.

#### Materials and Methods

The laboratory trials were conducted from August to October 1992 on three *P.persimilis* strains of various origin: the SI was collected in July 1992 on strawberry plants at a farm in Siracusa, Sicily, that had never employed releases of biofactory-reared predatory mites (Amore, pers.comm.); the NE was supplied in August 1992 by the Bunting BC Co. (Colchester, GB) biofactory, though originally derived from a rearing at the Institute of Horticultural Research (ex-Glasshouse Crops Research Institute) of Littlehampton (GB) in the early 1980's, selected for OP-resistance and supplemented with material field-collected in California (Stinson, pers.comm.); and the CE, collected at Cesena (northern Adriatic seaboard area) in March 1991 and since reared at the Biolab-Centrale Ortofrutticola (Cesena, Italy) biofactory (Benuzzi, pers.comm.).

The rearing of each of the three *P.persimilis* strains, 500-1000 individuals per strain, was conducted in a separate climate chamber at 20-24°C and 80±10% RH; the rearing unit was a plastic basket in a plastic tray containing bean leaves infested by *T.urticae*; water in the tray prevented the mites from escaping and kept the leaves fresh (Osakabe *et al.*, 1988).

The tests were run in a climate chamber at 25±1°C and 80±5% RH on 30 pairs of newly emerged adults per strain, collected during mating at a uniform rearing age. Each pair was isolated in a polymethyl methacrylate cylindrical cage (4.0 diameter x 4.0 height x 0.2-cm thickness) featuring a cap fitted with a stainless steel mesh disk (2.5-cm diameter, 201 mesh, 36% air permeability). Lying on an approximately 0.6-cm layer of agar gel at the bottom of each cage was a disk of bean leaf, the under blade face-upwards, infested *ad libitum* by *T.urticae*. The cage was set upside-down on a perforated basket so that the leaf disk was on top and the cap mesh on the bottom. The male was kept only the first three days in the

cage; the number of eggs laid per day was recorded throughout the lifetime of each female. The SI, NE and CE strains were initially reared in the laboratory for four, one and eight generations, respectively.

#### Statistical Analysis

Analysis of variance followed by Tuckey's test was applied to all parameters. The percentage of fertile females in the three strains was compared by the Chi-square ( $\chi^2$ ) test; the orthogonal comparison was employed to evaluate the field-collected SI against the biofactory-reared CE and NE strains.

#### Results and Discussion

The various biological parameters recorded, their strain comparisons and the findings are shown in table 1.

Table 1. Biological parameters (means $\pm$ SD) of the three *Phytoseiulus persimilis* strains; different letters indicate significant differences (ANOVA followed by Tuckey's test;  $\chi^2$  test for percent data).

Strain	SI	NE	CE
N	30	30	30
Egg-laying females (%)	100 <sup>a</sup>	93.3 <sup>a</sup>	93.3 <sup>a</sup>
Longevity (days)	14.9 $\pm$ 7.1 <sup>a</sup>	8.3 $\pm$ 6.0 <sup>b</sup>	8.9 $\pm$ 5.4 <sup>b</sup>
Pre-oviposition (days)	1.1 $\pm$ 0.4 <sup>a</sup>	1.1 $\pm$ 0.6 <sup>a</sup>	1.1 $\pm$ 0.6 <sup>a</sup>
Oviposition (days)	11.1 $\pm$ 4.9 <sup>a</sup>	5.7 $\pm$ 4.6 <sup>b</sup>	6.4 $\pm$ 4.9 <sup>b</sup>
Total eggs laid/female	48.0 $\pm$ 24.8 <sup>a</sup>	22.3 $\pm$ 24.0 <sup>b</sup>	25.1 $\pm$ 21.7 <sup>b</sup>
Eggs/day of life	3.3 $\pm$ 1.0 <sup>a</sup>	2.2 $\pm$ 1.2 <sup>b</sup>	2.7 $\pm$ 1.3 <sup>ab</sup>
Eggs/day of oviposition	4.2 $\pm$ 0.8 <sup>a</sup>	3.2 $\pm$ 1.3 <sup>b</sup>	3.8 $\pm$ 1.4 <sup>ab</sup>

#### Longevity

While the SI strain showed (Table 1) a significantly higher longevity, NE and CE did not differ significantly for this parameter. The orthogonal comparison indicates that SI differs very significantly from NE and CE taken together ( $P < 0.01$ ). Figure 1 shows the survival rate; at day 15 of female adult survival was 53.3% for SI, 13.3% for NE and 23.3% for CE.



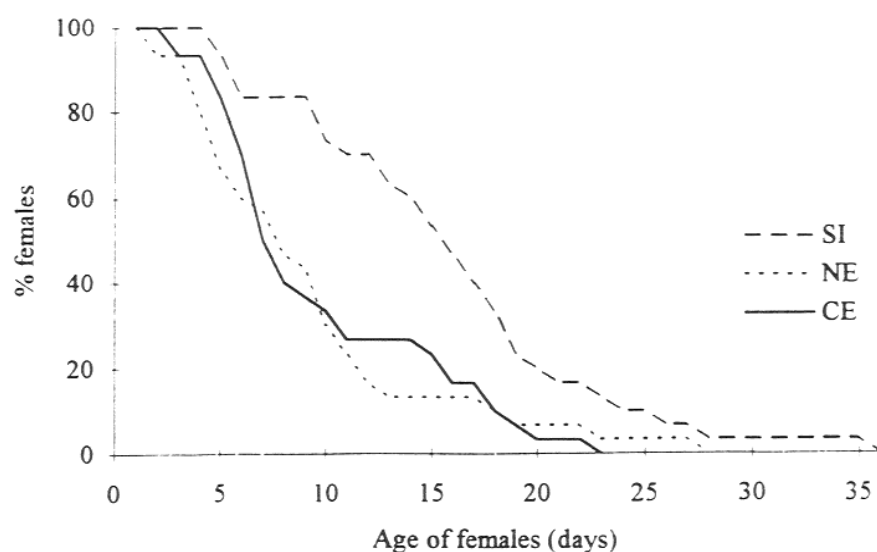


Figure 1. Survival rate of the three *Phytoseiulus persimilis* strains tested.

#### *Pre-oviposition*

No significant differences were found among the tested strains.

#### *Oviposition*

The Chi-square test indicates no difference for the tested strains in the percentage of egg-laying females. The SI strain showed (Table 1) a significantly longer oviposition period; one SI female laid eggs for up to 19 days compared with a peak at 16 days for both NE and CE. The SI strain showed a significantly higher total number of eggs laid per female, NE and CE did not differ significantly. The orthogonal comparison indicates that SI differs very significantly from NE and CE taken together ( $P < 0.01$ ). The peak number of total eggs laid by one female was 95 for SI, 81 for NE and 78 for CE. SI registered a significantly higher daily oviposition than NE, whereas CE did not differ significantly from the other two strains, whether throughout adult lifespan or for the oviposition period alone (Table 1). Figure 2 indicates the daily average number of eggs laid per live female and figure 3 shows the daily total oviposition of 30 females per tested strain. The SI oviposition trend is always higher than that of the other two strains.

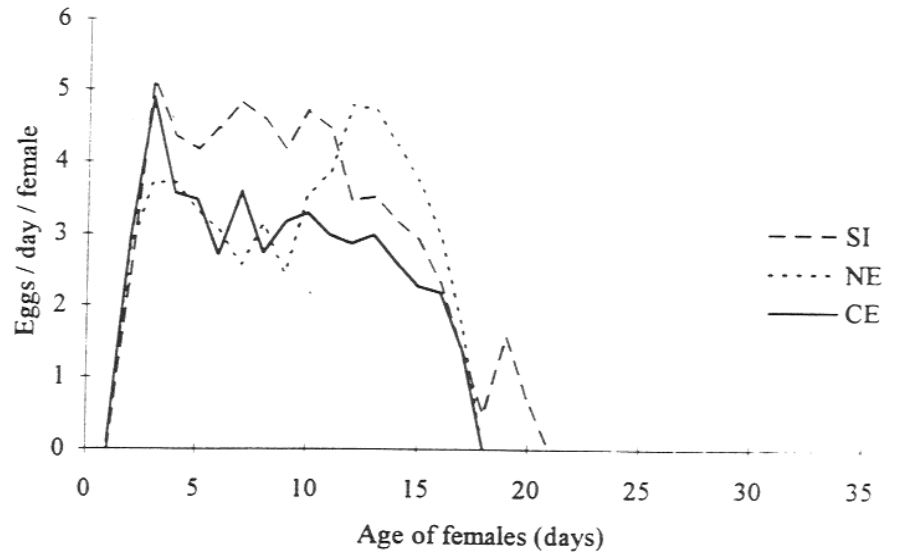


Figure 2. Average daily oviposition of live females of the three *Phytoseiulus persimilis* strains tested.

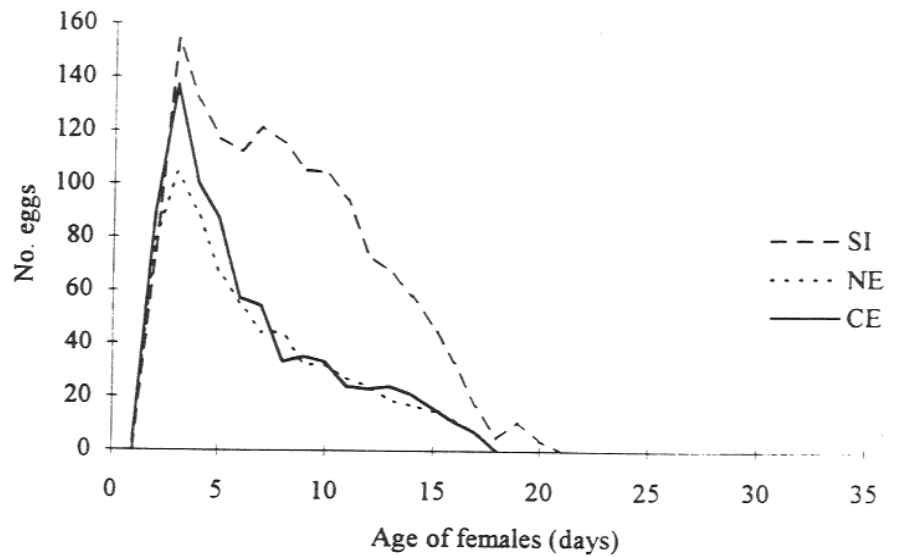


Figure 3. Daily total oviposition of 30 females of the three *Phytoseiulus persimilis* strains tested.



### Quality Control Test

A curvilinear correlation was found (Fig. 4) between the number of eggs laid over the first six days of adult life and total oviposition for the three tested strains, taken together and separately. This correlation indicates the viability of counting the number of eggs laid only during the initial period of the adult life (1 day of pre-oviposition + 5 days of oviposition) saving time and work in quality control tests, as suggested by van Lenteren (1993).

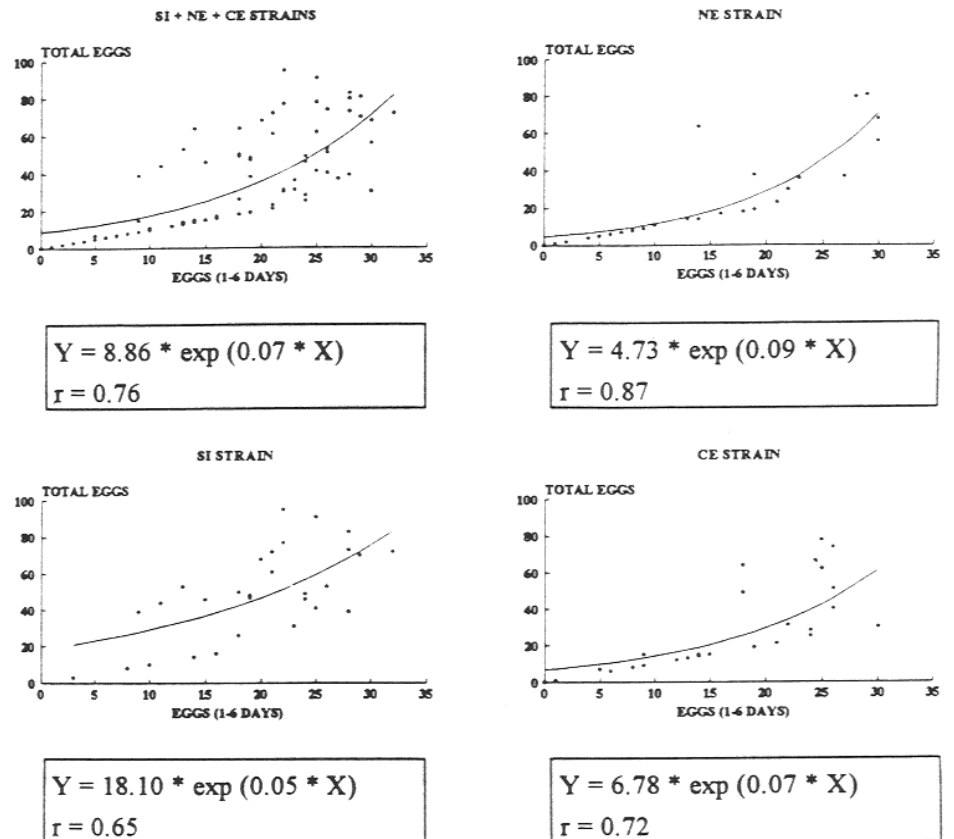


Figure 4. Correlation between the number of eggs laid over the first six days of adult life and total oviposition in the three *Phytoseiulus persimilis* strains (taken together and separately).

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### **Genetic Characterization of *Phytoseiulus persimilis* Strains**

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#### **Abstract**

An allozymic survey on cellulose acetate membranes of five Italian and North-European strains of *Phytoseiulus persimilis* showed no significant genetic differentiation among them. The analysis of single specimens at the *Pgi-2* locus also showed that diploid females and haploid males have the same allelic frequencies both within the same strain and between different strains, the only exception being the Cesena one. Different rearing population sizes do not seem to affect polymorphism, at least for a limited period (about 18 generations).

#### **Introduction**

The study of the genetic structure and polymorphism of animal taxa can be suitably approached by gene-enzyme system analysis, either for taxonomic/systematic determinations to the genus level or for more practical applications, such as control of the genetic structure and its modifications in lab-reared strains of pests or beneficials, using allozyme coding genes as markers. Specifically, we faced the problem of quality control of beneficial mites. The purposes of our study were: 1. The allozymic characterization of five *Phytoseiulus persimilis* Athias-Henriot strains (North-European, Cesena and Sicilian strains), 2. The testing of their genetic differentiation, and 3. The maintenance of possible genetic variability under artificial breeding conditions.

### Materials and Methods

A preliminary analysis of the Cesena strain was carried out on cellulose acetate membranes to check the expression of the following enzymes: adenylate kinase (ADK, E.C.2.7.4.3); esterase (EST, E.C. 3.1.1.1.); fumarase (FH, E.C. 4.2.1.2); glucose 6-phosphate dehydrogenase (G6PDH, E.C. 1.1.1.49); glutamate-oxalacetate transaminase (GOT, E.C. 2.6.1.1);  $\alpha$ -glycerophosphate dehydrogenase ( $\alpha$ -GPDH, E.C. 1.1.1.08); hydroxybutirate dehydrogenase (HBDH, E.C. 1.1.1.30); hexokinase (HK, E.C. 2.7.1.1); isocitrate dehydrogenase (IDH, E.C. 1.1.1.42); lactate dehydrogenase (LDH, E.C. 1.1.1.27); malate dehydrogenase (MDH, E.C. 1.1.1.37); 6-phosphogluconate dehydrogenase (6PGDH, E.C. 1.1.1.44); phosphoglucomutase (PGM, E.C. 2.7.5.1.); phosphoglucose isomerase (PGI, E.C. 5.3.1.9) and xanthine dehydrogenase (XDH, E.C. 1.2.1.37).

Batches of ten to 20 animals crushed with a glass rod in 10  $\mu$ l of extracting buffer (Tris-Cl 0.1M, pH 7.5).  $\alpha$ -GPDH, HBDH, LDH and XDH gave no readable patterns at any of the concentrations tested; ADK, FH, GOT and HK bands were obtained only from samples of at least 20 animals per 10  $\mu$ l. Observed allozymic patterns of seven zymograms could be related to the expression of a single locus; on the other hand, ADK, GOT and PGI clearly showed two enzyme systems and MDH three enzyme systems; a total number of 16 loci were therefore scored.

In a second step a total of 30-60 animals were analyzed, as samples pooled according to the predetermined optimal concentration, for the North-European (NE), Cesena (CE), and Sicilian (SI) strains reared at the Istituto di Entomologia "G. Grandi" of the University of Bologna (Ent). Additional samples of the Sicilian field-collected strain (SIN) and of the one mass reared at Biolab (BIO; also derived from a Sicilian population) were analyzed (Table 1).

In order to get a good estimate of the population structure, detect polymorphic loci and follow their allele inheritance, particular efforts were made to obtain electrophoretic phenotypes from single specimens of both sexes. Readable patterns were obtained for the *Pgi* locus according to the following procedure: each phytoseid mite was homogenized in a drop of 2 or 1  $\mu$ l (for females and males, respectively) of extracting buffer on a precooled glass. Using a p20 Pipetman disposable tip, each sample was then transferred to the marked origin of the

cellulose acetate membrane. This analysis was carried out for the four strains found to be polymorphic (Table 1) and on SIN and BIO strains after nearly 18 generations of rearing, at the Ent and Biolab, respectively. The main variable between the two rearing conditions consisted of very different population sizes (about  $10^3$  in Ent and at least  $10^5$  in Biolab). The cellulose acetate electrophoretic procedure and staining recipes followed Grafton-Cardwell et al. (1988) and Mantovani and Scali (1991).

Table 1. Number of females (F) and males (M) scored at the *Pgi-2* locus for each genotypic class; n = sample size. Strains were North-European (NE), Cesena (CE), Sicilian field collected (SIN), and Sicilian strain from Biolab (BIO). On SIN and BIO, the analysis was also performed after 18 generations (SIN<sup>18</sup> and BIO<sup>18</sup>).

STRAIN	GENOTYPIC CLASSES			
	90/90	90/100	100/100	n
	F,M	F *	F,M	F,M
NE	11,10	32	19,23	62,33
CE	46,30	18	0,4	64,34
SIN	15,10	17	28,20	60,30
SIN <sup>18</sup>	18,12	35	42,18	95,30
BIO	16,12	23	22,19	61,31
BIO <sup>18</sup>	13,12	42	35,18	90,30

\* No males were scored in this genotypic class

## Results

The allozymic survey of the three strains reared at Ent showed the absence of diagnostic loci. Moreover, all loci were monomorphic for the same allele, the only exceptions being the *6Pgdh* and *Pgi-2*. In particular, the *PGI-2* patterns in pooled samples of the NE and CE strains were interpreted as due to the sum of homo- and heterozygous individuals for two alleles (*Pgi-2*<sup>90</sup> and *Pgi-2*<sup>100</sup>), while SI was homozygous for the faster allele (*Pgi-2*<sup>100</sup>). We therefore decided to singly analyze additional specimens for this enzyme, either of the NE or the CE strains,

to ascertain the correctness of our interpretation, establish allelic frequencies, and verify whether the Sicilian strain (either field collected or Biolab reared) was really homozygous at the *Pgi-2* locus. Actually, its homomorphic genetic structure could be due to rearing conditions leading to bottlenecks or differential survival of some genotypes.

First of all, the real occurrence of homo- and heterozygous females and of invariably homozygous males for either of the two alleles in all the analyzed strains was clearly established (Table 1). Interestingly, no significant differences were detected between the allelic frequencies of females and males of the same strain (Table 2). In all strains, female allelic frequencies matched the Hardy-Weinberg equilibrium, the only exception being found for the field-collected Sicilian strain. It showed a significant deficiency of heterozygotes. Furthermore, the Cesena strain was significantly different in its allelic frequencies from the other strains, because it showed the *Pgi-2*<sup>90</sup> allele as the most common. Analysis carried out on samples of SIN and BIO strains after 18 generations showed no significant differences in comparisons with previous results.

#### Discussion

No alternative alleles were found at any locus among the analyzed strains of *P. persimilis*; the absence of diagnostic loci thus prevent their distinction on a simple zymotype basis. Also the polymorphic *Pgi-2* locus showed no differences in the allelic frequencies among strains, with the exception of the CE, where the most common allele of NE and SI strains (*Pgi-2*<sup>100</sup>) became the rarest. The analysis carried out on samples of the SIN and BIO strains after 18 generations indicated that, at least for a limited time (4 months), the different population sizes in Ent and Biolab did not affect genetic polymorphism. The monomorphism found in SI strain, reared in Ent for a longer period, should to be verified with further analysis after a considerable number of generations. Finally, we point out that the absence of heterozygous males is apparently due to the haploid condition determined by their pseudo-arrhenotoky (Schulten, 1985). This peculiar mechanism excludes the paternal chromosome set within 48 hours after from normal fertilization and therefore only one allele is expressed in adult tissues.

Table 2. Allelic frequencies at the *Pgi-2* locus in females (F), males (M), females plus males (F+M).  $\chi^2$  F, tests for the Hardy-Weinberg equilibrium in females;  $\chi^2$  F:M, tests for allelic frequencies between the two sexes, in each strain;  $\chi^2$  F+M/STRAINS, tests for allelic frequencies NE, CE, SIN and BIO strains;  $\chi^2$  G<sup>0</sup>:G<sup>18</sup>, tests for allelic frequencies of the first generation vs those after 18 generations.

Strains were North-European (NE), Cesena (CE), Sicilian field collected (SIN), Sicilian strain from Biolab (BIO), SIN after 18 generations (SIN<sup>18</sup>), and BIO after 18 generations (BIO<sup>18</sup>).

STRAIN	F	M	$\chi^2$ F	$\chi^2$ F:M	F + M	$\chi^2$ F + M/STRAINS	$\chi^2$ G <sup>0</sup> :G <sup>18</sup>
NE							
90	0.435	0.452	NS	NS	0.408	NS	
100	0.565	0.548			0.592		
CE							
90	0.882	0.859	NS	NS	0.864	P<0.001	
100	0.118	0.141			0.136		
SIN							
90	0.390	0.333	P<0.001	NS	0.380	NS	
100	0.610	0.667			0.620		
							NS
SIN <sup>18</sup>							
90	0.374	0.400	0.01<P<0.05	NS	0.380		
100	0.626	0.600			0.620		
BIO							
90	0.451	0.387	NS	NS	0.440	NS	
100	0.549	0.613			0.560		
							NS
BIO <sup>18</sup>							
90	0.378	0.400	NS	NS	0.380	NS	
100	0.622	0.600			0.620		

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### Parasitization and Predation of *Diglyphus isaea*

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#### Abstract

*Diglyphus isaea* is widely released in European greenhouses for the biological control of leafminer pests (Diptera Agromyzidae). In the laboratory (25±1°C; RH=75±10%; photoperiod L:D=16:8; light intensity 300 lx), with hosts supplied *ad libitum*, third instar larvae of *Liriomyza trifolii* (Burgess), *D. isaea* showed a high parasitization (350.1±40.9 eggs laid per female) and predation (884.6±82.7 larvae per female) potential (means±SE). Female longevity was 31.9±2.5 days and parasitoids continued host-killing even after oviposition, to death. Body length appeared not to be correlated to total fecundity, while longevity appeared to be correlated to predation. Host-feeding improved the maturation of oocytes vs. females fed only on honey. The highest number of mature oocytes was recorded one day after emergence (in honey-fed females) or after the end of host-feeding (in the parasitizing females). Host-feeding enabled old females to mature new oocytes and no significant differences were found in oocyte availability in old vs. young females, confirming that even old females can parasitize. The dissection of female abdomens after contact with hosts may be useful as quality control test of parasitization capacity.

#### Introduction

*Diglyphus isaea* (Walker) (Hymenoptera Eulophidae) is a palearctic species mass-reared by a few biofactories in Europe (Celli et al., 1991) and widely used in greenhouses for biological control of the leafminer pests *Liriomyza trifolii*

(Burgess) and *L. huidobrensis* (Blanchard) introduced into Europe from America some years ago (Arzone, 1979; Süss, 1991), as well as the palearctic *L. bryoniae* (Kaltenbach) (Diptera Agromyzidae). In the life cycle, as described by Minkenberg and Van Lenteren (1986), the adult female paralyzes the host larva before oviposition and usually lays one egg (exceptionally 2-5) near or on it (*D. isaea* is facultatively gregarious); the parasitoid larvae are ectophagous and pupate inside the mines. Adult females can feed hosts, and host-killing without oviposition can reach very high levels.

The present study was designed to investigate the potential for parasitization and host-killing (predation) of *D. isaea* and to verify oosorption in this synovigenic species and use this characteristic for quality-control tests by counting mature oocytes in parasitizing and non-parasitizing females.

### Materials and methods

The tested strain of *D. isaea* was originally collected in Sicily and has been mass reared at the Biolab facilities (Italy) since 1988. Two experiments were set up in the laboratory.

#### *Experiment 1. Parasitization and Predation Potential*

This trial was carried out in a climate chamber at  $26 \pm 1^\circ\text{C}$ ,  $75 \pm 10\%$  RH, 16:8=L:D photoperiod, and 1,300lx light intensity. Because adults are attracted to direct lighting, the experimental cages were covered with cardboard to bring the diffuse illumination inside them to about 300lx. The cages were built with transparent plastic and measured 35 x 25 x 20cm. Twenty newly emerged (less than 2 h old) pairs of parasitoids were isolated, one pair per cage, and supplied with water (moist cotton) and honey. Hosts were supplied daily *ad libitum* with 2-4 bean leaves infested on average by 120 third-instar larvae of *L. trifolii*. The petioles of the infested leaves were immersed in water in a plastic vial. Female mortality was checked daily and dead males were replaced. After exposure to parasitoid activity, the mines of the leaves were opened under a stereomicroscope. The number of parasitized larvae and the number of eggs laid nearby, the number of larvae killed without oviposition (predation) and the number of larvae and pupae still alive were



recorded daily. Dead *D.isaea* females were measured to determine an eventual relationship between body length and total oviposition. Two infested leaves were put daily into an identical cage under the same conditions but without parasitoids as a control of natural mortality of host larvae.

#### *Experiment 2. Oosorption*

This trial was carried out in a climate chamber with an L:D=16:8 photoperiod and corresponding thermoperiod of  $27\pm1^{\circ}\text{C}$  and  $20\pm1^{\circ}\text{C}$ ;  $80\pm10\%$  RH. Direct lighting was not employed and light intensity was ca. 300 lx. Throughout the experiment parasitoids were fed on honey. Three groups of ca. 500 parasitoids (males+females) were established. Group 1 acted as a control and was fed only on honey; the other two groups were exposed to the hosts for seven days; group 2 on emergence day and group 3 on day 42 after emergence (table 1). Groups 2 and 3 were put into separately clear plastic cages (60 x 35 x 40cm) with French bean plants infested by third instar larvae of *L.trifolii*. About 300 new larvae were added daily for six days; on the seventh day, live females were isolated in glass petri dishes (10 cm diameter) and kept in contact for one day with a bean leaf fixed on moist filter paper and infested by at least 20 larvae. The females were then isolated in individual glass tubes (10 x 1.5 cm diameter) and each bean leaf exposed to the parasitoids on day 7 was examined for parasitizing females. Only females that preyed and laid eggs were used to evaluate oosorption. At varying intervals from emergence (Group 1) or from contact with hosts (Groups 2 and 3), 12 females per interval were killed by freezing and their abdomens dissected under a stereomicroscope to count mature oocytes (tab.2).

Table 1. Oosorption in *Diglyphus isaea* tested after contact with third instar larvae of *Liriomyza trifolii*.

Group	Contact with host larvae (7 days in all)
1 (Control)	no
2 (Young females)	1 day after emergence
3 (Old females)	42 days after emergence

Table 2. The number of mature oocytes in *Diglyphus isaea* was investigated at varying intervals from emergence (Group 1) or after contact with hosts (Groups 2 and 3).

Group	Interval (days)
1 (Control)	0, 1, 2, 4, 7, 14, 21, 28, 35, 42, 49
2 (Young females)	0, 1, 2, 4, 7, 14, 21, 28, 35, 42, 49
3 (Old females)	0, 1, 2, 4, 7, 14

## Results and discussion

### *Experiment 1. Parasitization and Predation Potential*

The 23,098 control larvae of *L.trifolii* in the infested leaves not exposed to *D.isaea* showed very low natural mortality (0.8%) and were therefore disregarded in the evaluation of parasitoid activity. A total of 78,233 *L.trifolii* larvae were exposed to the 20 pairs of *D.isaea* and 31.6% were killed by parasitoids (parasitization + predation). Females never killed all the larvae exposed, thereby confirming that the hosts supplied were truly *ad libitum*. The females generally began predation and parasitization the second day after emergence, although some had started the first day (Fig.1). Female longevity was  $31.9 \pm 2.5$  days (mean $\pm$ SE) (Fig.2; table 3).

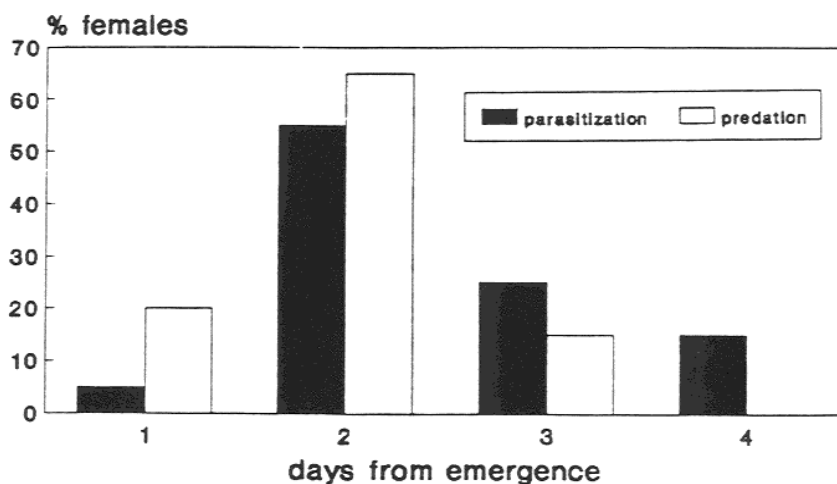


Fig.1. Beginning of activity of *Diglyphus isaea* females

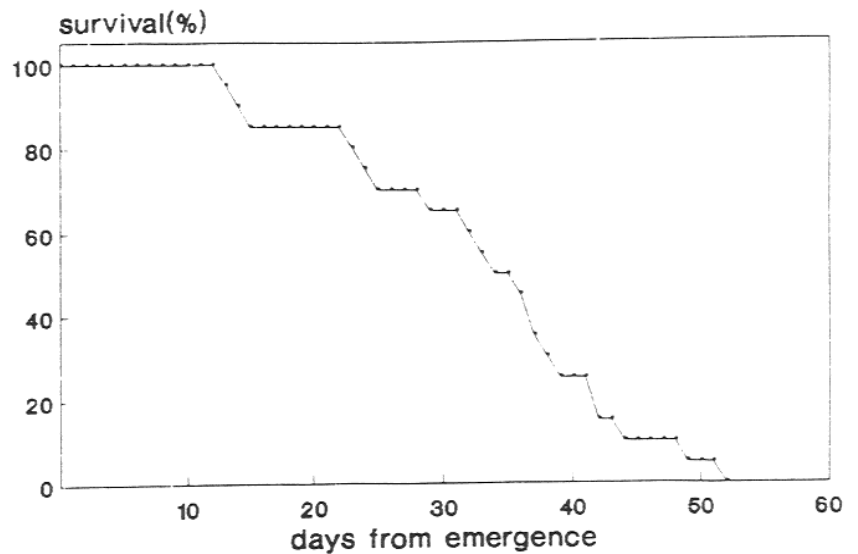


Fig.2. Survival of *Diglyphus isaea* females

Females laid only one egg per larva, but 2.3% of parasitized larvae had two eggs nearby and 0.08% had three eggs. These data confirm that superparasitism is occasional, probably promoted by overcrowding and/or a shortage of hosts.

Daily trends of mean parasitization and predation are shown in figure 3. Oviposition rose to about 20 eggs by day 5 after emergence and started to decline around day 15. About 80% of the total eggs were laid by day 22; no eggs were laid after day 35. Predation rose to about 30 killed larvae by day 4 and exceeded 50 larvae by day 10, 11 and 12 (a female registered a peak predation of 110 larvae in 24 h). Thereafter the average predation started to decline (80% of larvae were preyed upon by day 28) but, unlike parasitization, it continued throughout the life of females.

Table 3 summarizes the activity of *D.isaea*. Mean parasitization per female was about 350 *L.trifolii* larvae, a single female registered 751 eggs laid. Mean predation per female was more than 850 *L.trifolii* larvae and the same female that laid the most eggs registered the highest number of preyed larvae (n=1,333). The

total amount of killed larvae (parasitization+predation) averaged 1,234.7 per female with a mean predation:parasitization ratio of 2.76:1.

Table 3. Activity of isolated females of *Diglyphus isaea* supplied daily with third-instar larvae of *Liriomyza trifolii* throughout imaginal life (mean±SE)

No. Pairs	Longevity (days)	Oviposition period (days)	Total larvae parasitized/ female	Total larvae preyed/ female	Parasitization + Predation/ female	Parasitization : Predation ratio
20	31.9 ± 2.5	22.9 ± 2.0	350.1 ± 40.9	884.6 ± 82.7	1234.7 ± 117.0	2.76 ± 0.26

The body length of adult females was not correlated to total oviposition, total predation or longevity, which is in agreement with Minkenberg's data (1989) for pupal length. While a linear correlation was not found between longevity and total oviposition ( $r=0.44$ ;  $P=0.05217$ ), it appeared to exist between longevity and total predation (Fig.4). This is explained by the behaviour of females, which continued to kill larvae after the end of oviposition to death.

#### Experiment 2. Oosorption

Figure 5 shows the mean number of mature oocytes found in the *D.isaea* females of the three groups. *D.isaea* is a synovigenic species; no mature oocytes were found in newly emerged females. Host-feeding very much improved the maturation of oocytes in these females vs. females fed on honey. The highest number of mature oocytes was always recorded one day after emergence (in group 1) or one day after the end of contact with hosts (in groups 1 and 2). Thereafter, oosorption quickly reduced the number of mature oocytes per female.

In group 1, oosorption was completed between days 7 and 14 after emergence, and in group 2 between days 35 and 42 from the end of host-feeding. No significant differences were recorded in oocyte maturation and resorption of old females (group 3), vs. young females (group 2), so long as data were available for both groups (up to day 14 after host-feeding) (Kruskal-Wallis test). Host-feeding

thus induced new oocyte maturation, thereby providing opportunities for parasitization even by long-stored females.

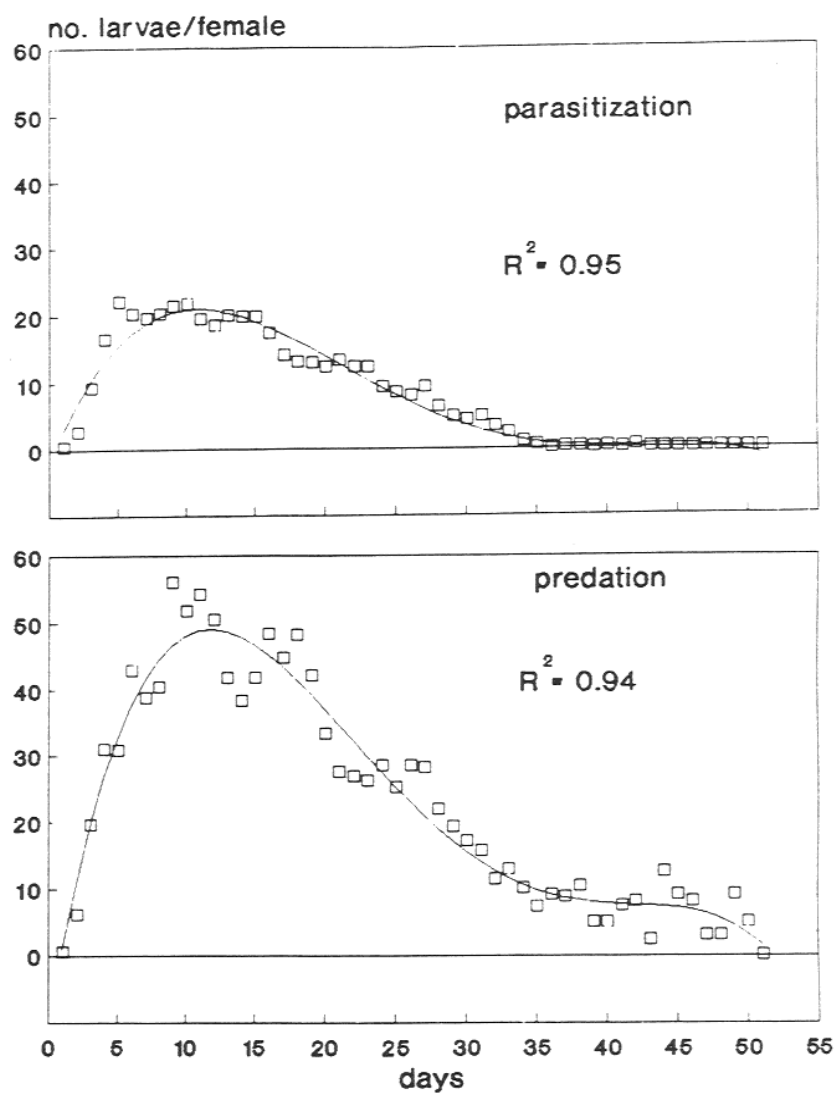


Fig.3. Daily trend in parasitization and predation of *Diglyphus isaea*

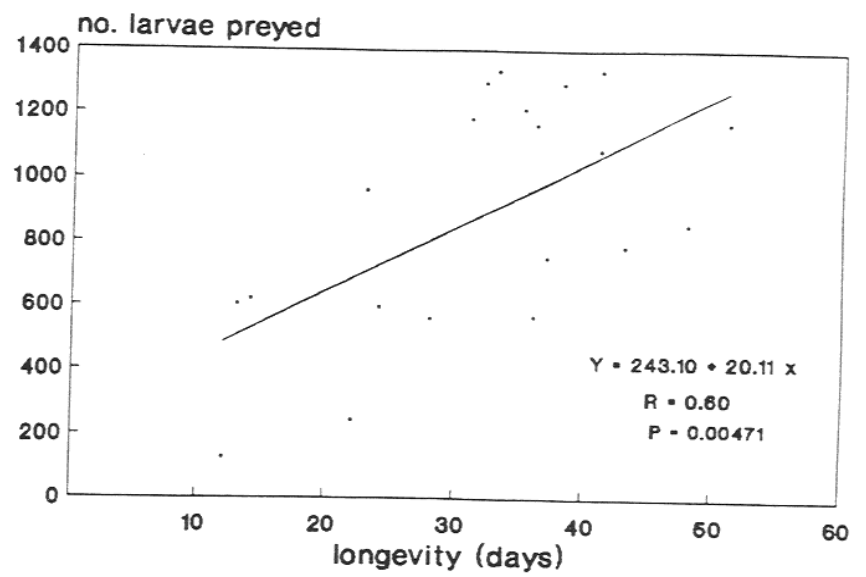


Fig.4. Correlation between longevity and total predation in *Diglyphus isaea*

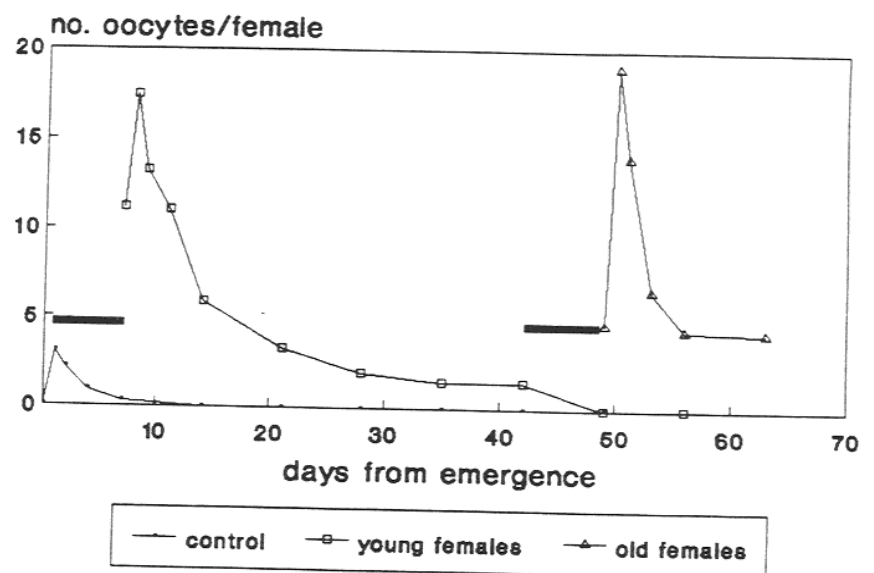


Fig.5. Oocyte maturation and resorption of *Diglyphus isaea* females

### Conclusions

*D. isaea* showed a high potential for parasitization and predation. By supplying hosts *ad libitum*, predation was higher than parasitization and appeared not to be explainable simply by host-feeding. The recorded longevity of parasitizing females was about one month on average, and parasitoids continued host-killing even after the end of oviposition, up to death. Body length appeared not to be correlated with total fecundity, while longevity appeared to be correlated with predation and not oviposition.

In this synovigenic species, maturation of oocytes was improved in host-fed females vs. females fed only on honey. The highest amount of mature oocytes was always recorded one day after emergence in honey-fed females and after the end of host-feeding in the parasitizing females. Host-feeding allowed old females to mature new oocytes and no significant differences were found in oocyte availability in them vs. young females, confirming that even old females are able to parasitize. The dissection of female abdomens after contact with hosts may be useful as a quality control test of parasitization capacity.

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### Cold Storage of *Diglyphus isaea*

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#### Abstract

The cold storage of *Diglyphus isaea* adults is one of the main problems in mass production. Female mortality rate at 4°C and 10°C and their parasitization potential after storage at the same temperatures were tested vs. control regime (20-27°C). The stored 4°C and 10°C regimes resulted in a significantly lower mortality than the control, and the former temperature registered a better score than the latter; male mortality was always higher than the female rate. A weekly 2-hour period at 22°C with light was not effective in improving survival in females stored at 4°C and 10°C. The 4°C regime proved to be best for storage, recording the lowest mortality rate. The number of progeny and mature oocytes per female of the individuals stored at 4°C and 10°C showed a significant decrease over time, the decline in biological parameters being evident after 60 days, oviposition decreased faster at 10°C than 4°C. A correlation also existed between the number of eggs laid and the number of mature oocytes per female.

#### Introduction

*Diglyphus isaea* (Walker) (Hymenoptera Eulophidae) is widely used in Europe for biological control of the exotic leafminer pests, *Liriomyza trifolii* (Burgess) and *L. huidobrensis* (Blanchard) (Diptera Agromyzidae), as well as the palearctic *L. bryoniae* (Kaltenbach). The parasitoid is mass reared by a few biofactories (Celli *et al.*, 1991) and one of the main problems in mass production is adult storage before marketing. Steinberg *et al.* (1991), who have studied the effect of storage

conditions on adults of *D. isaea* at three constant temperatures (15°, 20° and 26°C), found that the storage of honey-fed *D. isaea* adults at 20°C seems the most appropriate regime.

#### Methods and Materials

The tested strain of *D. isaea* was originally collected in Sicily and has been mass reared at the Biolab facilities (Italy) since 1988. Two experiments were set up to investigate the performances of the cold-stored parasitoids.

##### *Experiment 1. Mortality*

Newly emerged parasitoids (maximum 48 h old) were placed in groups of 55 (both males and females) in plastic vials 3.5 cm diameter and 9.0 cm long, each vial plugged by a robber stopper with about 1 cm<sup>2</sup> of steel mesh for ventilation. The adult artificial diet of honey, sucrose and water was solidified with agar gel and pushed down through the steel mesh. The parasitoids were fed as soon as they were put into the vials and every 14 days throughout storage period. The parasitoids were kept for the first 24 hours after collection at 22°C + light for feeding and mating; the mortality rate during this period was recorded but not used in calculations. Thereafter, the parasitoids were stored in climatic cells at the five regimes listed in table 1 (40 vials per regime).

Table 1. Storage regimes of *Diglyphus isaea*.

REGIME	THERMOPERIOD	PHOTOPERIOD	RH
4°C	constant	darkness	70 ± 10%
4°C*	constant 22°C 2 h/week	darkness light 2h/week	70 ± 10%
10°C	constant	darkness	70 ± 10%
10°C*	constant 22°C 2h/week	darkness light 2h/week	70 ± 10%
Control	27 °C light 20 °C darkness	L:D = 16:8	80 ± 10%

The mortality of males and females was recorded every 14 days in four vials per regime; each check was made on four vials so as to calculate the mortality rate in a non-cumulative way. The experiment was completed after 140 days.

#### *Experiment 2. Females Activity*

Groups of parasitoids were placed in plastic vials, fed on the same artificial diet and stored at 4°C and 10°C as in experiment 1 (table 1). Twenty-five live females in each regime were removed away from the climatic cell 14, 31, 59, 87, and 119 days from the beginning of storage to evaluate their activity. Each female was isolated in a glass petri dish (10 cm diameter), provided with honey droplets placed on the inside dish cover, and then put back in climatic cell (25±1°C; 80±10% RH; L:D=16:8 L:D photoperiod; 30±10 lx light intensity). Every day for seven days, each female was supplied with a French bean leaf (cv. Blue lake) infested by at least ten third-instar larvae of *L.trifolii*. The infested leaves were fixed to moist filter papers to prevent drying. All the leaves were removed from the petri dish and examined by stereomicroscope to count: 1. Eggs laid by the parasitoid, 2. Larvae of *L. trifolii* paralyzed or killed without oviposition, and 3. Hosts still alive (larvae and pupae). After the 7-day exposure to the host, each *D.isaea* female was placed in a glass vial with honey for 24 hours and then killed by freezing. The number of mature oocytes contained in the abdomen of each female was counted under a stereomicroscope after Nicoli and Pitrelli (same volume).

#### *Statistical analysis*

In experiment 1, a three-factorial analysis of variance was used to compare mortality rates (data tranformed to  $\arcsin \sqrt{x}$ ). The factors studied were: 1. Regimes (4°, 10°, 4°\*, 10°C\*and the control), 2. Sex, 3. Days of storage (14, 28, 42, 56, 70, 84, 98, 112, 126 and 140 days). The mortality trend of both sexes vs. storage days was analyzed by the curvilinear relations: i)  $Y = \alpha - \beta \exp(-\gamma x)$ ; ii)  $Y = \alpha \exp(\beta x)$  (Snedecor and Cochran, 1980).

In experiment 2, the linear correlation of the parameters investigated vs. storage

days, and the relation between total progeny of each female vs. the number of mature oocytes were analyzed. The parallelism test was used to compare the slopes of lines. A comparison of the parameters investigated between 4°C and 10°C was carried out by t Test.

## Results and Discussion

### *Experiment 1: Mortality*

All the factors considered and all the relative interactions were significant (table 2).

Table 2. Three-factor analysis of variance (ANOVA) of mortality (%).

SOURCE OF VARIATION	d.f. effect	F	P
REGIMES (R)	4	413.32	< 0.001
SEX (S)	1	708.36	< 0.001
DAYS OF STORAGE (D)	9	261.55	< 0.001
R*S	4	7.39	< 0.001
R*D	36	5.59	< 0.001
S*D	9	6.15	< 0.001
R*S*D	36	5.02	< 0.001

Statistical analysis showed that an increase of the storage period induced an increase in mortality at all regimes. Mortality trends (%) for the various regimes, as in figures 1a and 1b, describe the mortality vs. days and indicate high coefficients of correlation ( $r$  ranged between 0.88 and 0.98). Mortality was always higher for males than females at all the regimes. Differences in mortality were found between regimes. male and female mortality was affected by temperature. Both 4°C and 4°C\* regimes appeared to be more suitable than 10°C and 10°C\*, all of them inducing a lower mortality vs. control (20-27°C). The weekly two hours periods at 22°C + light were not effective in improving survival and no significant differences were recorded in mortality between 4°C and 4°C\* regimes, or between 10°C and

10°C\* regimes. The only differences were found after 70 days of storage, when males showed a higher mortality at 10°C\* vs. 10°C. Differences also occurred at 112 days, when females showed a higher mortality at 4°C vs. 4°C\* (Tukey HSD test,  $P < 0.05$ ).

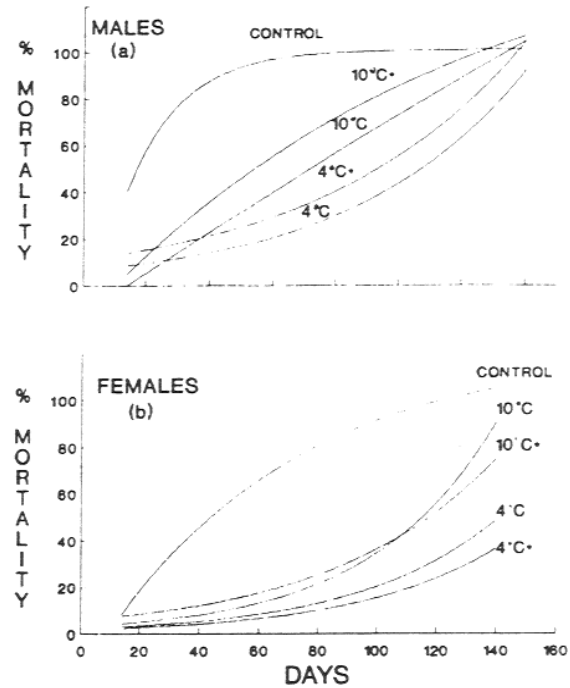


Fig.1. Mortality trend (%) of the regimes for males (a) and females (b) of *D. isaea*.

#### Experiment 2. Female Activity

The pre-oviposition period was not affected by cold storage, i.e., no differences between 4°C and 10°C were recorded. The progeny per female was significantly affected by storage period in both regimes. The parallelism test showed that oviposition decreased faster at 10°C; the difference for this parameter was

significant only at 119 days storage (Student t-test) (Fig. 2).

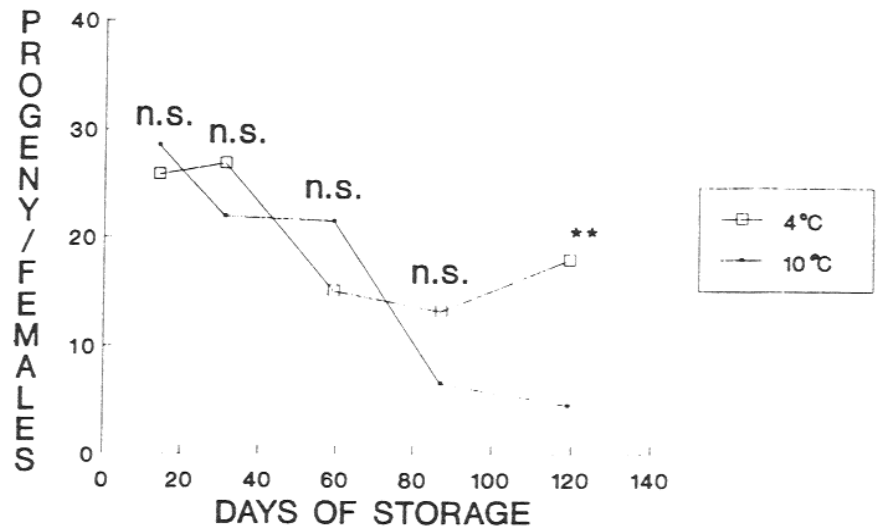


Fig.2. Comparison of progeny per female between 4°C and 10°C.

The percentage of parasitizing females was affected by storage period only at 10°C, when it showed a significant decrease (Fig. 3). The number of mature oocytes per female showed a significant decrease for both regimes, although no difference was registered between the two regimes either in the slope of the lines or in the average number of oocytes per female (Fig. 4). Given the data for all the females, figure 5 shows the correlation between the number of eggs laid and the number of mature oocytes per female. The oocyte count may be a less time-consuming technique of quality control than counting the eggs laid in the mines over seven days.

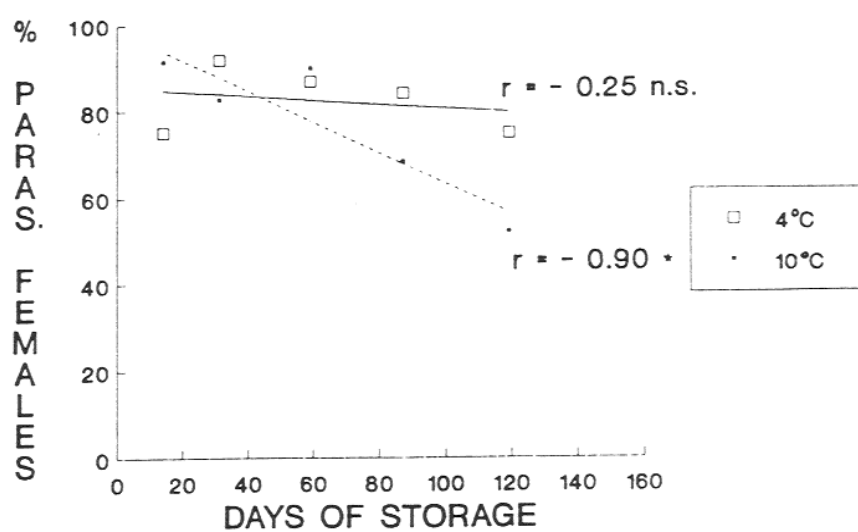


Fig. 3. Comparison of the parasitizing females between 4°C and 10°C.

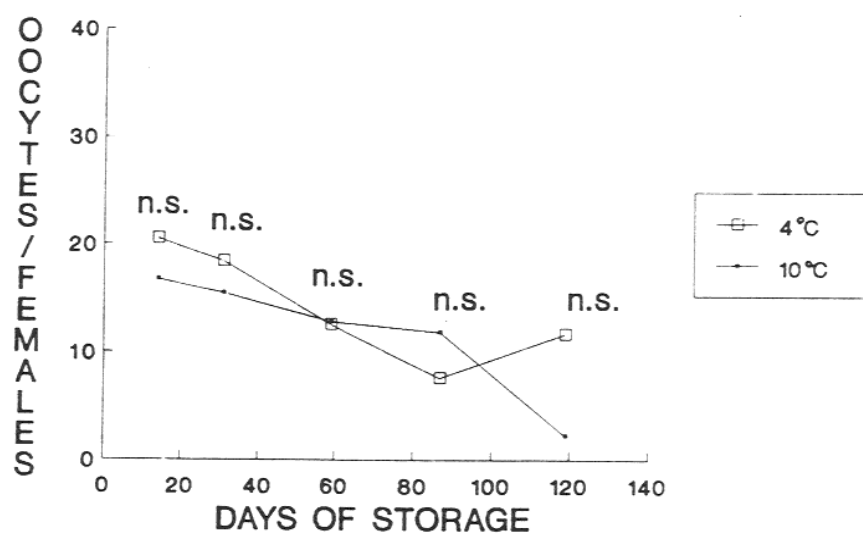


Fig. 4. Comparison of number of oocytes per female between 4°C and 10°C.

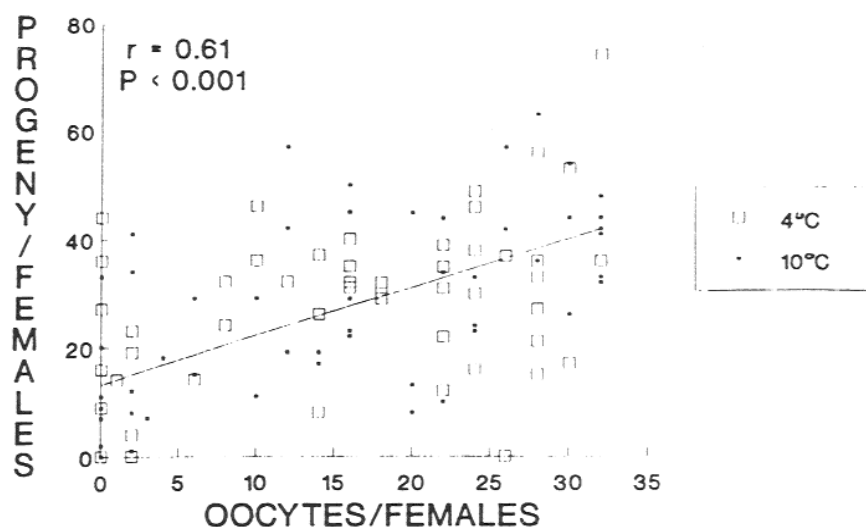


Figure 5. Correlation between the number of eggs laid per female and the number of mature oocytes.

#### Acknowledgements

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**Aspects of Product Control in *Phytoseiulus persimilis*, *Aphidius colemani* and *Diglyphus isaea***

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**Abstract**

Different product control criteria were tested and evaluated for three commercially available natural enemies. Fecundity test of the predatory mite, *Phytoseiulus persimilis*, showed that total fecundity for a 5-day period is more indicative of egg-laying capacity than daily oviposition. Hence it was recommended to change the relevant guideline to: "...Fecundity:  $\geq 10$  eggs/female for 5 days after egg-laying starts...". Initial design and preliminary results of a flight test conducted with the aphid parasitoid *Aphidius colemani*, led to the following suggested guideline: "Flight activity: response of emerging wasps  $\geq 90\%$ ; n=10, fifty mummies per replicate." For the leafminer parasitoid *Diglyphus isaea*, no relationship was found between parasitoid size and its fecundity and longevity. Therefore, it was suggested to omit adult size from the list of product control guidelines for *D. isaea*.

**Introduction**

In the last two workshops of the global IOBC working group "Quality Control of Mass Reared Arthropods", guidelines for product control of several commercially available natural enemies were designed (van Lenteren and Steinberg 1991; van Lenteren and Bigler 1993). Upon finalizing those guidelines and officially accepting them, a few more tests had to be carried out. Here we report on different product control criteria which were tested and evaluated for three natural enemies: the predatory mite *Phytoseiulus persimilis* Athias-Henriot, the aphid parasitoid

*Aphidius colemani* Viereck and the leafminer parasitoid *Diglyphus isaea* (Walker). Emphasis was put on prompt methods which will yield simple and straightforward results.

#### A. Fecundity Test of *Phytoseiulus persimilis*

One of the crucial parameters a producer needs to know about mass-reared predatory mites, is an estimate of their egg-laying capacity (as well as adult longevity). The guideline for adult fecundity suggests a seasonal test which should result in: "... $\geq 2$  eggs/female/day for 5 days after egg-laying starts;  $n=20$ ..." (van Lenteren and Steinberg 1991; van Lenteren and Bigler 1993). Preliminary observations showed that daily oviposition often drops to less than two eggs due to physiological state of the female predator, lack of prey and/or any other technical constraint arising while conducting the test. On the other hand, daily oviposition may sometimes be two or three times higher than the suggested standard (S. Steinberg, unpublished data). A question is therefore raised: should we measure daily fecundity over a 5-day period, or total fecundity for the same period. This issue is addressed in the test reported herein.

#### Materials and Methods

##### *The Bioassay*

The fecundity test used a slight modification of the method developed at Koppert Biosystems (W. Ravensberg, pers. comm.). Leaf discs of brown beans (*Phaseolus vulgaris*), 3.5-cm diameter, were infested by ample amounts of the two-spotted spider mite (*Tetranychus urticae* Koch, all developmental stages). The discs were placed on Agar, their infested lower side facing upward, in Corning<sup>®</sup> polystyrene 6-well plates with a foamed polyethylene insulated lid (Fig. 1). One adult female of *P. persimilis* was put on each leaf disc. Three predators were examined simultaneously in each plate: they were held in one row of three wells for the first 24 hours, then transferred to freshly infested discs in the second row of three wells for another 24 hours and subsequently removed to a new plate. After removing the predator, the number of eggs laid on the old leaf discs was counted. This procedure lasted for five days after oviposition started. All together, a maximum

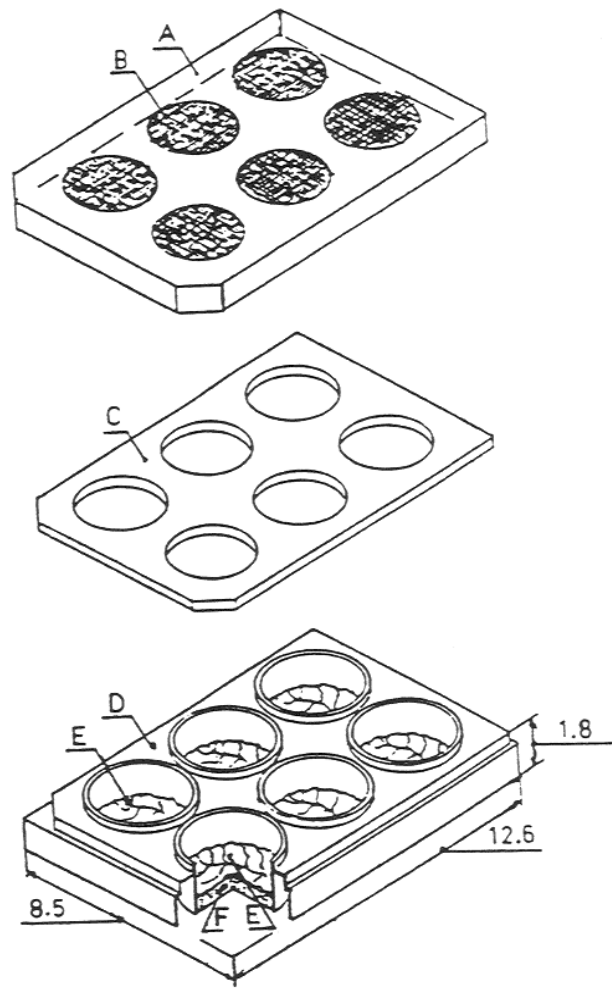


Figure 1. Bioassay for testing fecundity of *Phytoseiulus persimilis*. A. Polystyrene lid, B. Fine mesh netting, C. Foamed polyethylene insulation, D. Polystyrene 6-well plate, E. Leaf disc (3.5-cm diameter), and F. Agar layer (0.5-1 cm thick). Parts A, C and D are tied firmly together by rubber bands. Dimensions are given in centimetres.

of nine female predators (=9 replicates separated on 3 different plates) were tested at the same time.

The experiment was carried out during May and June, 1993. It took place in an environmental chamber at  $25\pm 1^{\circ}\text{C}$ ,  $60\pm 10\%$  RH and 16L:8D light regime. Thirty two adult females of *P. persimilis* were taken at random from material collected at the greenhouse production unit. The bioassay plates were placed up side down, i.e., the infested leaf discs facing downward, so as to simulate the true orientation of the predators in the field. Daily egg counts of each individual test were registered, and mean and standard error for daily oviposition were calculated.

### Results and Discussion

Fecundity of *P. persimilis* exceeded by far the suggested criterion, i.e., higher than two eggs per female per day throughout the test period. Moreover, daily oviposition in days two and three, almost doubled the requested standard (Fig. 2). None of the individuals examined died within the test period. Eleven predators laid more than two eggs per day for five consecutive days. The rest oviposited less than two eggs per day in one to four days of the test. Figure 3 demonstrates that even ovipositing less than two eggs per day in up to three days, the predator still yielded a total higher than ten eggs per five days, which is considered the minimum standard fecundity for the whole test period. Total fecundity for the 5-day period seems therefore to be more indicative of the egg laying capacity of *P. persimilis* than daily oviposition. Hence we recommend to adjust the relevant guideline as follows: "...Fecundity:  $\geq 10$  eggs/female for 5 days after egg-laying starts...".

Test duration is measured as 1.5 hours per day with nine replicates for trained personnel. This includes handling and maintenance of the bioassay. However, some methodological considerations should be taken into account. 1. Picking individual female predators for the test should be done consistently by staff familiar with the morphological differences between females and males, as well as between young and gravid females of *P. persimilis*. 2. Also the "history" of each individual tested is unknown, e.g., nutrition, age, number of matings. This may cause variable results which should be compensated by an adequate sample size, i.e., a minimum of 20 individuals (see guidelines, van Lenteren and Steinberg

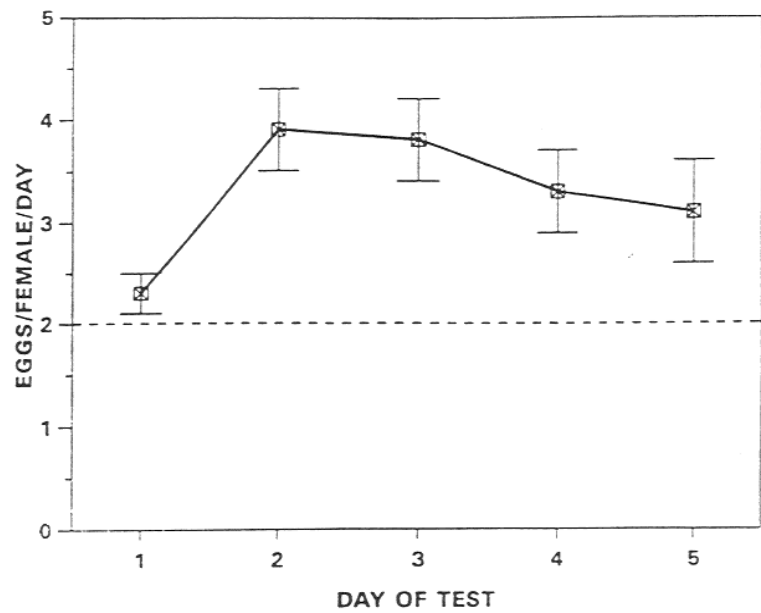


Figure 2. Fecundity test of *Phytoseiulus persimilis*. Mean daily oviposition for five days after egg laying starts. Bars represent standard error (n=32). Dashed line corresponds to the suggested fecundity guideline.

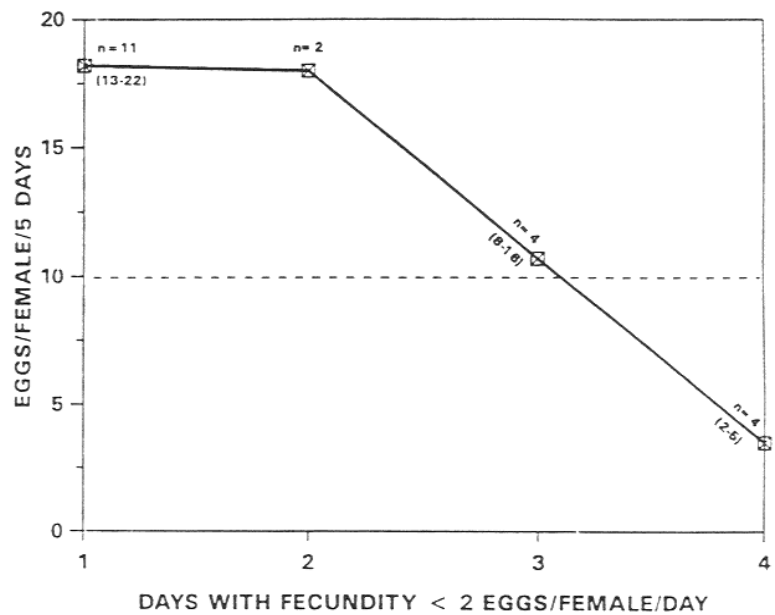


Figure 3. Fecundity test of *Phytoseiulus persimilis*. Comparison of daily oviposition and total fecundity over the five day period. Dashed line corresponds to the suggested guideline for total fecundity over five consecutive days. Range of total fecundity is given in parenthesis.

1991; van Lenteren and Bigler 1993). In the present study,  $n=32$  kept variability relatively low (Fig. 2).

#### **B. Flight Test of *Aphidius colemani***

As an endoparasitoid which mummifies its host, *A. colemani* is often processed and applied commercially as pupae within the aphid mummies. Any mechanical process involved in production and packaging of "clean mummies" might affect flight propensity of the emerging wasps. The ability to fly is one of the basic traits the parasitoid uses for host habitat location (Hagvar and Hofsvang 1991). It is therefore essential to establish a test which will examine short range flight ability of freshly-emerged *A. colemani*. Initial design and preliminary results of such a test are reported herein.

#### **Materials and Methods**

##### *The Bioassay*

The flight test was conducted in a 36 x 40 x 55-cm ventilated cage (Fig. 4). A 12 x 15-cm yellow sticky trap was placed in the middle of the cage at a height of ca. 35 cm above its floor. The sticky trap was held vertically on a wooden stick which had a base dipped in water. Thus only adult parasitoids which were able to fly following emergence could reach the sticky trap either directly or through climbing on the wooden stick. Fifty mummies of the cotton aphid, *Aphis gossypii* Glover, parasitised by *A. colemani* on a cucumber leaf, were held in a separate petri dish at the corner of the cage. Prior to the test, mummies were inspected to ensure that no parasitoid emergence took place. After 12-14 days, counts were made of the wasps caught on the sticky trap, in the water and of those lying dead on the cage's floor. Those counts were compared with the number of emergence holes in the mummies. No distinction was made between males and females as their motivation to fly was assumed to be the same.

The bioassay was conducted during April and June 1993 in an environmental chamber at  $25 \pm 1^\circ\text{C}$ ,  $60 \pm 10\%$  RH and 16L:8D light regime. Twelve replicates (=12 cages with 50 mummies each) were tested. Data of all replicates was pooled and expressed in terms of percentage of adult wasps found on each site in the cage.

### Results and Discussion

About 90.5% of the emerging parasitoids were found on the sticky trap, 7.9% in the water at the base of the trap and 1.6% were found dead on the cage floor. The high proportion trapped on the sticky plate indicates that the majority of adults were able to fly. However, this bioassay could not trace whether the wasps found in the water and on the floor did fly initially. In ten of the 12 replicates, the wasp count at the different sites in the cage was identical to the number of emergence holes in the mummies, i.e. all emergents were found. In two replicates there was ca. 5% difference, apparently due to individuals which either escaped from the cage or died in sites other than those checked. The body and wings of the wasps were found unharmed. This, however, may not be the case in processed mummies where harming the parasitoid pupae and therefore yielding defective adults is more likely to happen. The time invested in the bioassay is 30 minutes per replicate. It includes pre-test counting and assessment of the mummies, setting the flight cage and counting the results. The suggested product control criterion is: "Flight activity: response of emerging wasps  $\geq 90\%$ ;  $n=10$ , fifty mummies per replicate."

Testing flight propensity, which is a post-emergence trait, should not be mixed with evaluating emergence rate. The latter is a product control criterion by itself (see van Lenteren and Steinberg 1991; van Lenteren and Bigler 1993), which might be affected not only by the mechanical processing of the mummies but also by the age of the parasitoid pupae at the time of processing.

Finally, it should be emphasized that the current test examines short range flight ability of *A. colemani* and not long range foraging behaviour of the wasp. On one hand, the dimensions of the bioassay arena enable the parasitoid to perform a complete flight activity but, on the other hand, neither the size of the flight cage nor the host related chemical and visual cues which are not present, let the parasitoid express his capabilities with regard to long range host habitat location.

### C. Body Size vs. Fecundity and Longevity in *Diglyphus isaea*

*D. isaea* is an idiobiontic species, i.e., its host development ceases after oviposition. Thus the host stage at oviposition represents a set food package (Askew and Shaw 1986) which can be easily measured. Host size might in turn influence the body

size of the adult wasp. Fitness of parasitoids, defined by their survivorship and number of offspring, is usually positively correlated to the size of the parasitoid (e.g., Charnov *et al.* 1981). *D. isaea* is supplied commercially as an adult. Variability in female and male size is often observed in mass production. From the product control stand point, it is therefore essential to study the relationships between female size and its fecundity and longevity. The results of such a study, which includes the development of a basic design of a fecundity test, are presented herein.

## Materials and Methods

### *Real Fecundity*

*D. isaea* is described as a synovigenic species which will emerge with less than a full complement of eggs (Minkenberg 1990). Therefore potential fecundity, i.e., count of mature eggs in the female wasp upon emergence (Croft and Copland 1993), may not reflect actual or real fecundity of the parasitoid (Leather 1988). Thus, real fecundity, the actual number of eggs produced, was recorded throughout the life span of *D. isaea* in the experimental system.

### *The Bioassay*

The individual oviposition unit in which a 48-hours-old mated female of *D. isaea* was exposed to larvae of *Liriomyza trifolii* (Burgess), consisted of a single brown bean plant bearing two cotyledons each infested by a minimum of 30 second to third instar leafminer larvae. The plant was held within a ventilated transparent plastic jar inverted over a PVC cylinder (Fig. 5), thus creating enough space (ca. 30-cm high) for the plant and parasitoid to stay together. Each day the female parasitoid was given a freshly infested plant using the following procedure: first the wasp is attracted to the top of the plastic jar (a light source is held above the latter), then the jar containing the parasitoid is removed from the top of the plant and closed, this is followed by lifting the PVC cylinder upwards. The old plant is then replaced by a new one; a slit in the wooden base holds each plant in place. Ultimately, the parasitoid is returned to the cage in an inverse order. This procedure was repeated until the wasp died "naturally" in the experimental system.



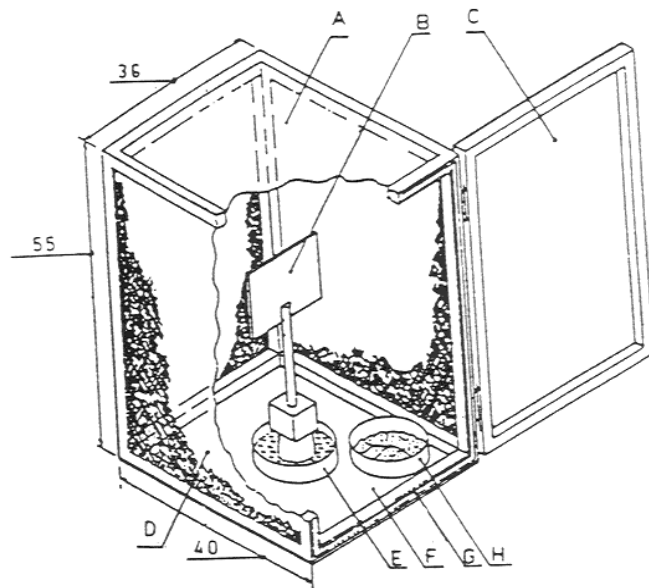


Figure 4. Cage for short range flight test of *Aphidius colemani*. A. Top (transparent prexy-glass), B. Yellow sticky trap (12X15 cm), C. Door (transparent prexy-glass), D. Wall (fine mesh netting), E. Petri dish filled with water, F. Floor (white PVC), G. Rubber insulation for the door, and H. Cucumber leaf with 50 mummies of the cotton aphid. Cage dimensions are given in centimetres.

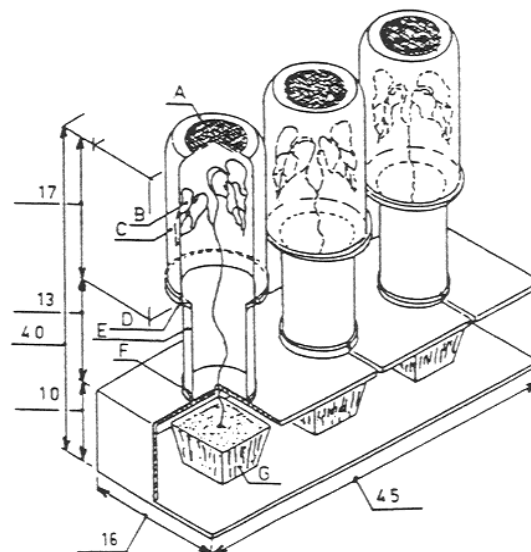


Figure 5. Fecundity test of *Diglyphus isaea*. Three individual oviposition units placed on a wooden base. A. Fine mesh netting, B. Leafminer infested brown bean cotyledon, C. Inverted plastic jar, D. Separable joint, E. PVC white cylinder, F. Separable joint, and G. Pot filled with plant substrate. Dimensions are given in centimetres.

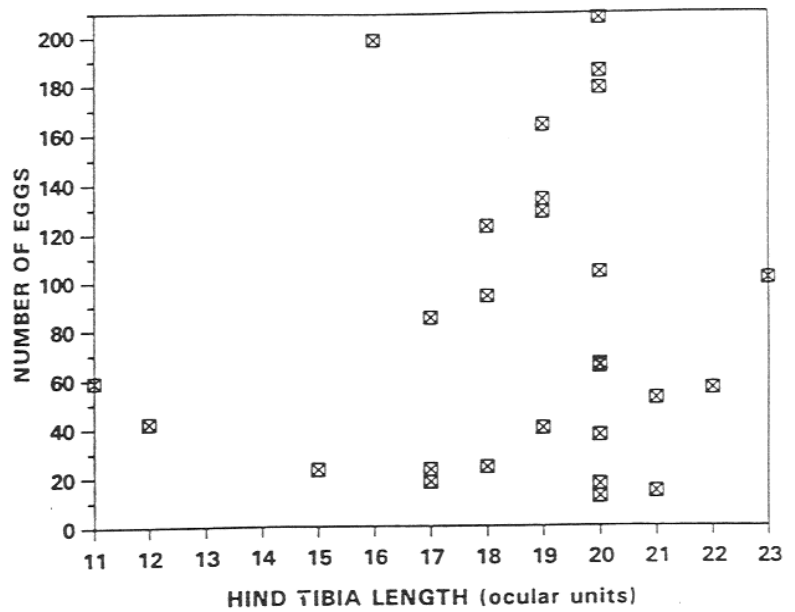


Figure 6. A scatter diagram showing the relationship between body size (hind tibia length) and female fecundity (eggs oviposited per individual) in *Diglyphus isaea*. To express hind tibia length in mm, multiply the ocular units by 25.6. Results from the linear regression analysis are:  $y = 29.97 + 2.88x$ ;  $r^2 = 0.016$ ,  $P > 0.05$ ,  $df = 25$ .

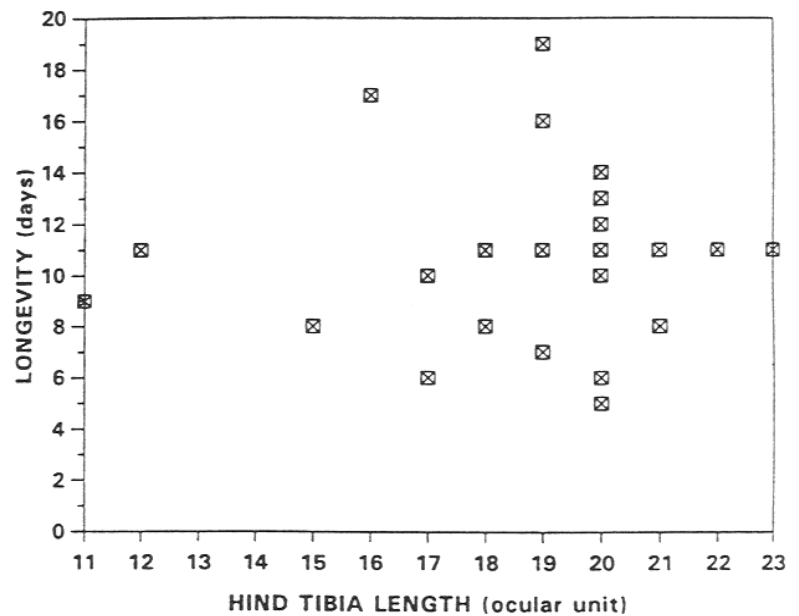


Figure 7. A scatter diagram showing the relationship between body size (hind tibia length) and female longevity in *Diglyphus isaea*. To express hind tibia length in mm, multiply the ocular units by 25.6. Results from the linear regression analysis are:  $y = 9.29 + 0.06x$ ;  $r^2 = 0.002$ ,  $P > 0.05$ ,  $df = 25$ .

The bioassay was conducted between February and July 1993 in an environmental chamber at  $25\pm 1^{\circ}\text{C}$ ,  $60\pm 10\%$  RH and 16L:8D light regime. Twenty seven female wasps were examined altogether. No access to carbohydrate food (e.g., honey solution) was provided to the parasitoids. The plants removed daily from the oviposition units were kept under the same conditions for another 24-48 hours, at the end of which mines were dissected under a stereoscopic microscope and *D. isaea* eggs and young larvae were counted. The size of the female parasitoid was recorded as hind tibia length, measured by a micrometric ocular after the parasitoid died. Adult size was regressed against its total fecundity and longevity.

### Results and Discussion

The scatter diagrams on figures 6 and 7 show no relationship between parasitoid size and its fecundity and longevity. Similarly, Minkenberg (1989, 1990) found that increase in size of *D. isaea* did not result in increased fecundity and survivorship. Apparently, host feeding by females of *D. isaea*, which is essential for continuous egg production throughout the life span of the parasitoid, affects fecundity far more than the size of the wasp. This hypothesis is supported by the positive relationship found between fecundity and longevity of the parasitoid (results from linear regression analysis are:  $y = -62.8 + 14.1x$ ;  $r^2=0.65$ ,  $P<0.001$ ,  $df=25$ ). Conversely, Heinz and Parrella (1990) reported that adult longevity and female fecundity of *Diglyphus begini* (Ashmead) were positively correlated with the parasitoid body size. The same relationships were demonstrated by Croft and Copland (1993) for *Dacnusa sibirica* Telenga, an endoparasitoid of agromyzid flies. In light of the aforementioned results, we recommend to omit adult size from the list of product control criteria for *D. isaea*. As to the fecundity/longevity bioassay, the time invested in it is 1.5 hours per day with three replicates which includes preparation of the infested plants, handling the wasps and recording the results. This design may be used as a basic bioassay for any fecundity or longevity related treatments of *D. isaea*.

### Acknowledgements

Yehuda Zuntz is thanked for drawing the bioassays' set-ups.

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*persicae* (Sulzer) as well as *Aphis gossypii* Glover equally well (van Schelt, 1993).

In the proceedings of the Vth Workshop of the IOBC Global Working Group "Quality Control of Mass Reared Arthropods" *A. colemani* is not mentioned and tests were done with *A. matricariae* on *M. persicae* (van Lenteren et al., 1991). During the VIth Workshop a distinction was made between the two species. *A. colemani* has to be tested on *A. gossypii* and *A. matricariae* on *M. persicae* (van Lenteren, 1993). The proposed fecundity test received some criticism, because the full potential of the female parasite was not assessed. Tests were done in petri dishes and only 25 aphids per female were offered. In fact this was considered more of a host acceptance test than a fecundity test. Therefore, new research was initiated to improve the fecundity test. Two directions were chosen: 1. Trying to assess the total fecundity with the petri dish method, and 2. Development of a test with the use of complete plants.

#### Material and methods

All experiments were conducted in an environmental room at 22°C, 60±5% RH, and 16L:8D photoperiod, unless stated otherwise.

##### *Petri Dish Method*

The bioassay tray consisted of a round plastic "petri dish-type" tray with a lid which can be closed tightly (31-mm height; 77-mm diameter, Bock, Art.Nr.41113). In the lid a gauze was incorporated for air exchange. In the dish 1 cm of water agar (1%) is poured and cooled to 30°C. Just before it solidifies, a punched cucumber leaf disc is put upside down on the agar. It is very important to use a fresh leaf with maximal turgor, else the life span of the leaf is insufficient to last for the test period (11 days). Around 15 adult aphids (*A. gossypii*) are put on the leaf with a fine brush. The petri dishes are placed upside down to simulate a more natural situation for the aphids and to prevent the leaf being fouled by honeydew. After two days the adult aphids are removed. In this way between 100 and 150 young aphids per petri dish can be used for testing.

The parasite *A. colemani* was used in these tests. The material was reared using *A. gossypii* as a host and cucumber as host plant. After mummification the material

was processed to produce loose pupae. A few hundred pupae were placed in an emergence box of hard transparent plastic (12-cm height, 11-cm diameter). Honey was provided as food. Adults up to 12 hours old were used. The release and transfer of female parasites was carried out in a cold room (8°C). Transfer of a parasite to another petri dish was accomplished by placing a small glass vial over the parasite. After the female had walked in, the vial was gently tapped above a fresh petri dish. The mummies were counted after nine days. In experiment 3, mummies were left to develop in order to determine the sex ratio of the offspring.

#### *Experiments with Petri Dishes*

In experiment 1, males and females were mated by putting them together in a glass vial with honey for 24 hours before the start of the test. An average of 150 aphids per petri dish was offered; 25 females were used. Females were transferred to new petri dishes after one, two, three and 24 hours. After 48 hours the parasites were removed. Experiment 2 differed from experiment 1 only in the number of transfers. Wasps were transferred to new petri dishes after three and 24 hours. After 48 hours the parasites were removed. The relative distribution of mummies over the three periods was compared to experiment 1. Experiment 3 had the same set-up as experiment 2 except the wasps were used directly from the emergence box. In this way the time consuming pairing and mating is skipped. To test if the females mated successfully in the emergence box, the mummies were reared further and emerged wasps were sexed. The sex ratio was expressed as the number of females divided by the total. In experiment 4 the sensitivity of the test was checked by intentionally harming the parasites before checking the fecundity. Before testing, adult wasps were stored for three days at 3°C with honey.

#### *Cage Method*

Two cage types were tested. One consisted of a wooden base with a PVC frame and a cotton gauze (40 x 40 x 80 cm). The other cage type consisted of a transparent thin plastic tube (80-cm height, 15-cm diameter) which was placed on the pot. On the sides and in the top, gauze was glued. Cucumber plants were sown in peat and reared until the first five leaves were present. With both types of cages

ten female wasps (direct from the emergence box) were tested on an ample amount of *A. gossypii* ( $\pm 500$ , all stages). Parasites were left in the cage until death.

## Results

### *Petri Dish Method*

The average number of mummies per female per parasitization period is presented in figures 1-4 for the different experiments. Also the total number of mummies per female are given. From experiment 1 to 4 respectively 120, 216, 174 and 83 mummies were found. These data are all significantly different from each other. (Mann-Whitney  $P < 0.05$ ). The relative distribution over the three periods are presented in figure 5. For experiment 1 the results of the first three periods has been summated. In the first three experiments, a similar pattern was found. In experiment 4 the intentionally harmed adults produced only 22% of the total number of mummies in the first period. This was lowest of all trials though not significantly different ( $\chi^2$ ). In experiment 3, when adult wasps were taken directly from the emergence box, the sex ratio of the mummies for the three periods was 56%, 93% and 84% females respectively.

### *Cage Method*

Unfortunately no figures can be presented from the cage method, because reliable results were extremely difficult to obtain. The main reason for this was collapse of the plants which were very fragile and did not last long, especially under artificial light. An additional problem was condensation of water on the inner walls of the tube. We also suffered from intruders and escapers. In Israel the same problems were encountered, the aphids also remained very small which affects the degree of parasitization (pers. comm. S. Steinberg).

## Discussion

The petri dish method presented in this paper seems to be a reliable test for assessing the fecundity of *Aphidius* species. In contrast to the method discussed at the Vth IOBC Workshop (van Lenteren et al., 1991), there is no limitation in the number of aphids offered. Because all the aphids are of almost the same age (N1,

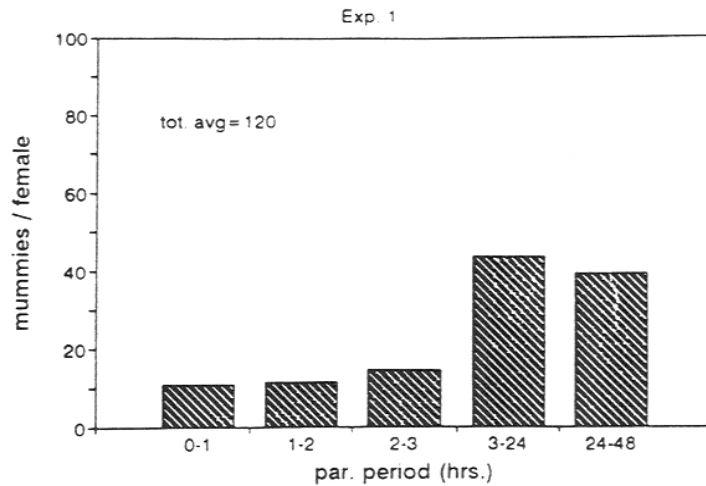


Figure 1. The number of mummified aphids per female wasp per parasitization period and the total average number of mummies per female. *A. colemani* (25) were individually tested in petri dishes on an ample amount of *A. gossypii*. Adult wasps were isolated and mated before the test.

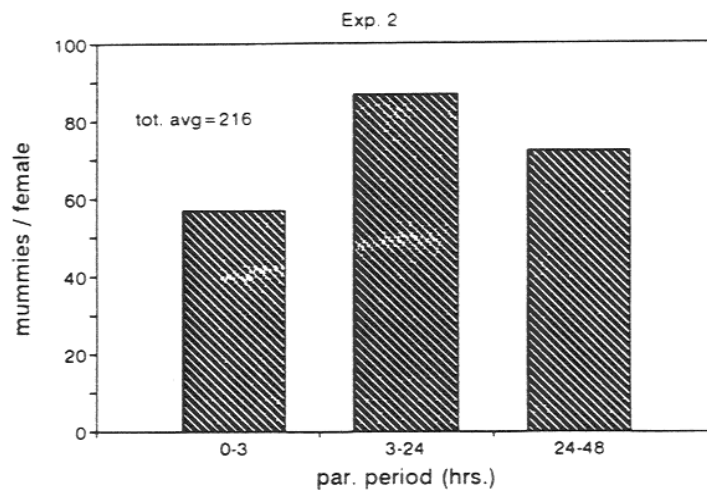


Figure 2. The number of mummified aphids per female wasp per parasitization period and the total average number of mummies per female. *A. colemani* (25) were individually tested in petri dishes on an ample amount of *A. gossypii*. Adult wasps were isolated and mated before the test.



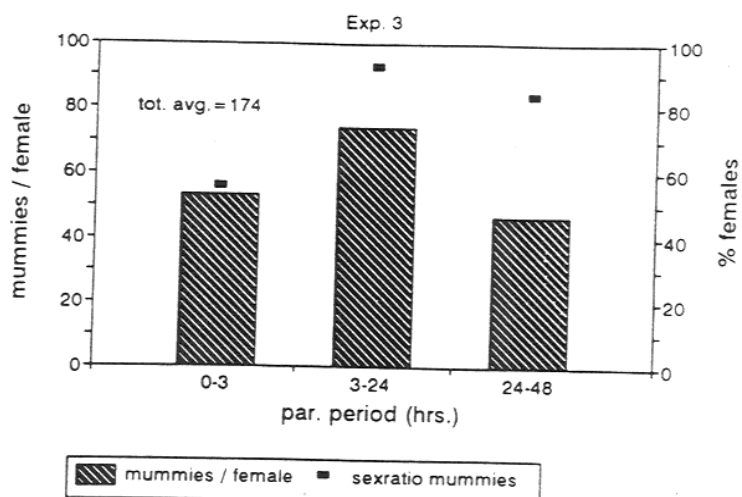


Figure 3. The number of mummified aphids per female wasp per parasitization period and the total average number of mummies per female. *A. colemani* were individually tested in petri dishes on an ample amount of *A. gossypii*. Wasps were taken directly from the emergence box. The mummies were reared further to determine the sex ratio.

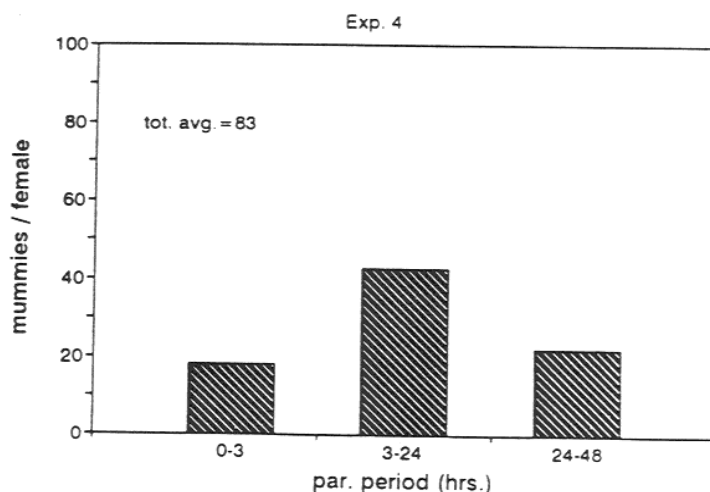


Figure 4. The number of mummified aphids per female wasp per parasitization period and the total average number of mummies per female. *A. colemani* (25) were individually tested in petri dishes on an ample amount of *A. gossypii*. Wasps were taken directly from the emergence box and stored three days at 3°C prior to the experiment.

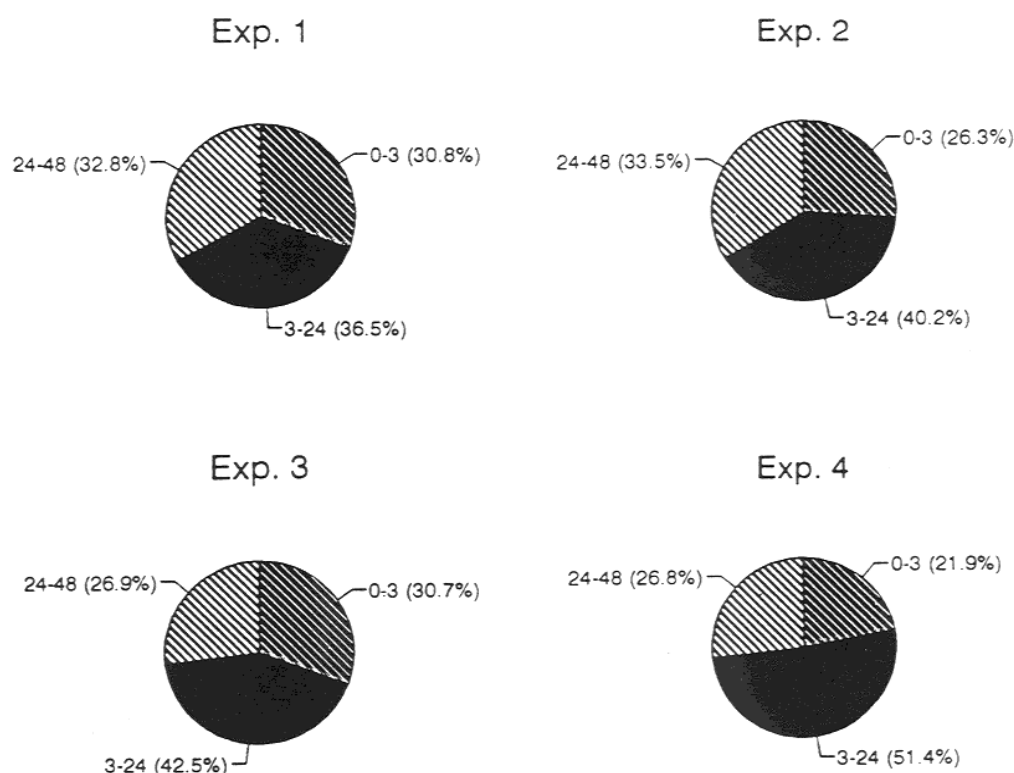


Figure 5. The relative distribution of the mummies over the three periods. The first three periods of experiment 1 were summated.

N2), this method has several advantages. No young aphids have to be transferred at the start of the experiment and, because the aphids are very young, they will mummify without producing offspring.

Experiment 1 was designed to find out the optimal number of necessary transfers. *A. colemani* is preovigenic and starts egg-laying immediately, so as a precaution petri dishes were changed after one, two, three and 24 hours. After 48 hours the parasites were removed. It is obvious that the number of transfers in the beginning of this experiment was too high. With two transfers (experiment 2) there is always an ample amount of aphids available for parasitization. Optimal transfer time is at three and 24 hours after start of the experiment. In this way the total

amount of mummies is divided in almost three equal parts. Two days of testing seems enough because the average female life span is about 4-5 days and most eggs are laid during the first two days (van Steenis, 1992).

The results from experiment 3 suggest that it is not necessary to isolate pairs for mating before the trial. When parasites were used directly from the emergence box, the total egg laying capacity was only slightly lower than that obtained in experiment 2, when wasps were paired for mating. Because the sex ratio was strongly female biased, it was concluded that mating was not a problem. The total number of mummies produced per female in the first experiment was much lower than in experiment 2 and 3. This can be explained by the quality of the leaves used. They were picked during the daytime, when the sun was shining, and did not have their maximal turgor. The parasitized aphids of some petri dishes had to be transferred because the leaf started yellowing before they mummified. Because of this, the potential number of mummies may not have been realized. In later experiments, leaves were always picked in the morning, as early as possible. The total average of 216 and 175 mummies in experiments 2 and 3 agrees with the results found by van Steenis (1992). He tested for nine days and counted the number of eggs laid by immediate dissection of the aphids. In total, an average of 388 eggs laid per female were found, but a small number of aphids received two eggs and 28% died during development. Experiment 4 showed that this test also can assess a lower quality of the insect. Because the percentage of mummies formed during the first three hours was the lowest of all experiments, this may be an indication that eggs are absorbed during storage of adults. The influence that storage of mummies has on the fecundity of emerging wasps still must be tested. Moreover, the decision on an acceptable limit for the fecundity of *Aphidius* as a criterion in product control should be discussed further.

The method in which whole plants are used seems not very practical. Maybe it is possible to improve the quality of the plant by placing it in the greenhouse. But it is not easy to have a stable climate there and to fulfil the test conditions as proposed during the VIth IOBC Workshop. Reductions in space and costs strongly favour the petri dish method.

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### **Evaluating Competitiveness of Sterile *Ceratitis capitata* from the Hawaii Sterile Fruit Fly Rearing Facility**

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#### **Introduction**

At the USDA-APHIS Hawaii Sterile Fruit Fly Rearing Facility in Waimanalo, we routinely measure the quality of our mass-produced insects using a series of evaluations based on the RAPID tests plus assessments of parameters such as larval development time, volume of pupae per tray of diet, and dosimetry readings from our irradiators. The data from these evaluations are essential for determining if our production procedures are adequate, but they tell us very little about how our flies will interact with wild females in target populations. Because of this, additional tests are run periodically to determine if our flies are competitive and compatible with wild flies. These include release-recapture tests and evaluations of courtship and mating compatibility.

#### **Release-Recapture Tests**

Dispersal and responsiveness to trimedlure are tested at least annually in small-scale release-recapture tests. Two d before eclosion, pupae are dusted with Day-Glo powder and, if appropriate, irradiated using the current sterilization protocol, now 150 Gy under hypoxia. Recently emerged flies are released into the field at the center of an array of trimedlure-baited bucket traps. Typically, we release flies from wild and laboratory strains simultaneously and then check the traps daily for at least two weeks. Up to now, we have used a 4 x 4 grid of traps with a 50-m inter-trap spacing.

Overall, we normally capture approximately a quarter of the flies we release into the grid, or about half of the males we release. The proportion of laboratory-reared flies recaptured has consistently been slightly higher than that of wild flies, typically collected as larvae in peaches from the Kula region on the island of Maui. The exception to this was our most recent (1993) test, when recapture of laboratory-reared, irradiated flies and wild flies was essentially equal. The vast majority (>90%) of the flies are usually captured within three days (laboratory-reared) or five days (wild). The difference between colonized and wild flies is not unexpected; the latter develop more slowly and do not show peak responsiveness to trimedlure until ca. three days after eclosion.

The trapping grid we've been using was originally designed, in part, to provide supporting data for some of our efforts to develop and evaluate trapping systems for Mediterranean fruit flies. We have found that it is too small and captures flies too quickly to provide accurate estimates of either dispersal or survival in the field. We are currently redesigning our trapping protocol to correct these problems.

#### **Analysis of Courtship Behavior**

We are trying to develop quantitative analysis of courtship behavior as a tool for predicting mating compatibility of different strains of Mediterranean fruit fly. Courtships are videotaped in either a laboratory or field-cage study. Then, a time code is written on the tape; the code labels each frame of the videotape with a specific numeric address. To analyze a courtship sequence, each frame is viewed separately, and the behavior of the flies in that frame is entered onto a separate line on a spreadsheet. Analysis of the spreadsheet allows us to develop ethograms and compute frequencies of rhythmically repeated behaviors, percent of time spent in different behaviors, and other quantitative measures of behavior. In initial tests, differences between laboratory and wild flies -for example, in the pulse-train frequency of courting males- have become apparent, but we do not yet have sufficient data to tell if such differences are related to mate choice.

#### **Field-Cage Mating Tests**

Field-cage mating tests have been our main tool for evaluating the mating

compatibility of sterile and wild-type flies. Guava trees (2.0 to 2.4-m tall) are enclosed in 3-m diameter cylindrical mesh cages. Typically, fifty unmated male flies from each of two strains of Mediterranean fruit fly are released into a cage shortly before sunrise. About 15 to 20 min. later, 50 unmated females from each strain are released. Most of these tests involve one strain that is marked with Day-Glo powder (usually sterile flies, 5-7 days post-eclosion) and an unmarked strain (wild-type flies collected as larvae in fruit; 2 weeks old). Throughout the day, all mating pairs are captured in separate plastic vials, and the time and location of the mating are noted on the vial. The mated pairs are then returned to the laboratory where their marks, if any, are identified and the widths of their heads are measured.

We started running field-cage tests to complement an ARS pilot program in which sterile flies from the Waimanalo facility were being released over the coffee-growing region of the island of Kauai. Initial tests were designed to evaluate effects of post-production handling and release procedures. Treatments included, among others, sterile flies that were straight from the factory, flies that had been packaged and shipped to Kauai as pupae, and flies that were released from aircraft and collected from the field. Flies from all treatments were tested with wild-type flies which were collected as larvae in, with one exception, coffee cherries. In our early tests, through the winter of 1991-1992, we noted a moderate tendency for the flies to mate assortatively; i.e., sterile females selected sterile males as mates almost 70% of the time, but wild females chose sterile males only 40-45% of the time. Thus, the sterile flies appeared to be somewhat less than 100% competitive but were sufficiently compatible to be useful in a sterile insect program. Also, overall participation of sterile flies in mating appeared to decline moderately in aerially released flies.

While these tests were being run, though, the Kauai program started to go downhill. Initially, percent hatch of medfly eggs from the core of the release area, eventually ca. 75 km<sup>2</sup>, indicated that the sterile flies were doing a reasonably good job of mating with wild females. Then, sterility started to stay in the 5 to 15% range even when overflooding ratios were 50 to 1 or higher. During the peak coffee season in 1992, medfly populations in the test area exploded despite continued releases of sterile insects. Results of the field-cage tests provide a clue as



to what may have happened. In tests run after the winter of 1992-1993, wild females from Kauai seldom selected sterile males as mates; an average of approximately one of the typical 40-60 matings per cage involved a wild female and sterile male. This suggested that the continued pressure from SIT releases on Kauai selected for wild females that somehow avoid sterile males when choosing a mate. We are in the process of running tests to determine if evolved resistance to our sterile insects was the cause of the poor performance of Waimanalo flies in Kauai. If resistance appears to be the cause, the emphasis of our testing will shift toward identifying the mechanism of resistance so that such problems can be minimized or avoided in the future.

### **Evaluation of Insect Quality at the Hawaii Sterile Fruit Fly Rearing Facility: Competitiveness and Compatibility with Wild Flies**

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#### **Introduction**

At Waimanalo, we routinely conduct a series of standardized laboratory evaluations to ensure that we are efficiently producing healthy, viable Mediterranean fruit flies, *Ceratitis capitata* (Wied.). In addition, a second category of evaluations are periodically conducted to determine if our mass-reared sterile flies are competitive with wild flies in target populations. Current tests include assessments of dispersal, survival and mating compatibility.

#### **Release-Recapture Studies**

Small-scale release-recapture tests are run at least annually to compare dispersal, longevity, and responses to trimedlure among laboratory and wild strains of Mediterranean fruit flies. Pupae are dusted with Day-Glo powder to mark the flies. Recently emerged flies from lab and wild strains (ca 1000 per strain) are released simultaneously from a single point at the center of a grid of traps. Traps are left in place and serviced daily for at least two weeks following the release. The traps are constructed from 1-L plastic buckets with six 1.2-cm diameter holes spaced evenly around each bucket at ca. 3 cm from the top; the traps contain controlled-release devices for trimedlure (2 g of lure) and naled. For the past two years, we have used a 4 x 4 grid of traps with 50 m between adjacent traps. This plot design

represents a compromise because the studies were also designed to support our efforts to develop and evaluate trapping systems for Mediterranean fruit flies. Future tests may be redesigned to provide more precise information the relative dispersal and longevity of flies from various strains or treatments.

In these tests, we have recovered 20-30% of the flies we have released (i.e., 40-60% of released males). Recovery of laboratory-reared flies has typically been about 5% higher than recovery of wild-type. Wild flies are collected as larvae in fruit (peaches from Kula, Maui, or coffee from various islands), which is held in the laboratory until larvae are mature. Recovery of sterile (irradiated at 150 Gy) flies was similar to that of unirradiated flies in 1992 but was lower (i.e., similar to that of wild flies) in 1993. Flies used in 1993 tests were of substantially lower quality (as determined by the RAPID tests) than those used in 1992, and we suspect that the radiation may have affected the weaker 1993 flies to a greater degree. Capture of laboratory flies has typically peaked at 24-48 hours after eclosion; capture of wild flies has peaked at 48-72 hours.

#### **Quantitative Analysis of Courtship Behavior**

Quantitative analysis of courtship behavior is being examined as a possible tool for predicting mating compatibility of laboratory-reared Mediterranean fruit flies. Courtship encounters are videotaped, and a time code is than written on the tape. The code labels each frame (30 frames per second) with a specific numeric address and allows us to follow and record the timing of events accurately. Individual frames of each courtship are than viewed in sequence, and the behaviors exhibited by the flies in each frame are entered onto a separate line of a spreadsheet. The resulting data are used to generate ethograms, compute frequencies of rhythmically repeated behaviors and estimate the percent age of time spent in different behaviors. We hope to be able to relate these quantitative representations of courtship to acceptance or rejection of potential suitors as mates. To date, we have noted that laboratory and wild males appear to differ in the frequency of rhythmic shifts in the amplitude of wing fanning.

### Field-Cage Mating Tests

Field-cage mating tests have been our main tool for evaluating the mating compatibility of sterile and wild-type flies. Guava trees (2.0 to 2.4-m tall) are enclosed in 3-m diameter cylindrical mesh cages. Typically, fifty unmated male flies from each of two strains of Mediterranean fruit fly are released into a cage shortly before sunrise. About 15 to 20 minutes later, 50 unmated females from each strain are released. Most of these tests involve one strain that is marked with Day-Glo powder (usually sterile flies, 5-7 days post eclosion) and an unmarked strain (wild-type flies collected as larvae in fruit; ca. 2 weeks old). Throughout the day, all mating pairs are captured in separate plastic vials, and the time and location of the mating are noted on the vial. The mated pairs are then returned to the laboratory where their marks are identified, and the widths of their heads are measured.

These tests were initiated to complement a pilot-scale sterile insect release study that was being run by USDA-ARS researchers in the coffee-growing region of the island of Kauai. The procedures were evaluated as well as the compatibility of sterile and wild flies. The treatments we examined included sterile flies that were straight from the factory, flies that had been subjected to various packing and shipping regimens, and flies that were released from aircraft and collected from the field. In our early tests (through the winter of 1991-1992), we noted a moderate tendency for the flies to mate assortatively; i.e., sterile females selected sterile males as mates almost 70% of the time, but wild females chose sterile males only 40-45% of the time. Thus, the sterile flies appeared to be somewhat less than 100% competitive but were sufficiently compatible to be useful in a sterile insect program. As these tests continued, the Kauai program started to 'go downhill'. ARS's initial estimates of sterility (egg hatch) indicated that the sterile flies were doing a reasonably good job of mating with wild females. After an extended period of releases, though, sterility began to remain low despite high over flooding ratios, and Mediterranean fruit flies populations in the test area appeared unaffected by the sterile flies. Results of the field-cage tests provide a clue as to what may have happened. In tests conducted since the winter of 1992, Kauai females have been very hesitant to mate with sterile males; the number of such matings as averaged ca. one out of the typical 40-65 matings of 100 pairs of flies per cage. In contrast,

wild females from other islands have shown relatively little preference for wild or sterile males. The most likely explanation is that the continued pressure from SIT releases on Kauai selected for wild females that somehow avoid sterile males when choosing a mate. Further tests are being run to give us a conclusive test of this hypothesis. If the results hold, we will start looking for the mechanism for this apparent resistance. Resistance to SIT in this case would affect the way we evaluate the quality of our flies and perhaps the way that the technique is potentially used in action programs. Specifically, use of moderate to low overflooding ratios to manage relatively large populations would be viewed as problematic.

In addition to our efforts, other USDA researchers are helping APHIS evaluate the competitiveness of its sterile insects. For Mediterranean fruit fly, two examples are Bob Heath at Gainesville, Florida, who is looking at pheromone production by wild and sterile flies, and Eric Jang at Hilo, Hawaii, who is investigating the relative ability of sterile males to cause wild females to switch from seeking mates to seeking hosts for oviposition.

## **Quality Control of the Screwworm Fly Production Plant, Chiapa de Corzo, Mexico**

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### **Introduction**

The Screwworm Production Plant at Chiapa de Corzo, Mexico, produces approximately 250 million pupae per week. These screwworm, *Cochliomyia hominivorax* (Coquerel), pupae are irradiated with Cesium 137 and dispersed as sexually sterile flies at specific locations in Central America to eliminate reproduction within native populations of the pest. Quality control tests are performed to assure the success of screwworm eradication using the sterile insect technique.

The quality control of mass-reared screwworms involves three categories of testing: 1. production control- ingredients used in the larval diet and environmental conditions, including irradiation, 2. processes control- pupal weight, fly emergence and sex ratio, and 3. product control- flight agility, sterility, sexual aggressiveness, and longevity. These routine tests are performed to assure that the mass-reared insects meet quality control standards and are competitive with native screwworm flies (Table 1).

Dietary ingredients, including the spray-dried egg powder and blood, milk derivative, gelling agent, bacterial inhibitor, and water, are tested before being used in the larval medium. Calcium chloride is added to decompose waste products.

Horse meat provides a nutritional substrate for first instar larvae and flies are fed a mixture of horse meat, honey, and rice hulls. Sawdust is used as a pupation substrate. These materials must meet or exceed established specifications or they are rejected.

Table 1. Weekly results of quality control main parameters, August 1993.

QUALITY CONTROL STRAIN : CR-91

PARAMETERS	FROM : JULY 25 TO: JULY 31	FROM : AUGUST 01 TO: AUG 07	FROM : AUGUST 08 TO: AUG 14	FROM : AUGUST 15 TO: AUG 21	FROM : AUGUST 22 TO: AUG 28
EMERGENCE TIME (HRS./MIN)	16:29	17:03	18:07	20:18	17:13
EMERGENCE (%)	86.02	86.26	86.25	86.95	86.10
M A L E (%)	51.29	51.49	51.28	51.05	52.61
MALFORMATIONS (%)	00.70	00.46	00.93	00.47	00.47
FLIGHT AGILITY (%)	90.78	91.34	90.25	90.78	90.69
MORTALITY (%)	00.59	00.44	00.32	00.30	00.37
SEXUAL (%) AGGRESSIVITY	81.44	79.52	71.70	80.24	79.30
LONGEVITY W/O FOOD DAYS/HRS.	04.03	03.13	04.07	04.16	04.13
LONGEVITY W/ FOOD DAYS/HRS.	12.06	13.22	11.12	10.20	11.10
*PUPAL WEIGHT (MGS.)	40.68	39.50	39.93	39.97	39.56

\* AUGUST 29 TO SEPTEMBER 04/93.

39.99 mgs

Process control monitoring begins with pupal weight, an excellent indicator of larval growth. Changes in pupal weight may also indicate mechanical or environmental malfunctions in rearing processes. Time and period of fly emergence

are monitored relative to temperature and humidity. Records are kept on emergence during the first 72 hours, the period during which all viable pupae yield flies. Remaining pupae are examined and classified as dried, empty, immature, or decomposed to ascertain possible causes of non-emergence. The time of emergence is also important for predicting the length of time that the pupae will remain viable during long-distance shipment. Finally, sex ratios are recorded daily for each batch of irradiated pupae by randomly sampling 500 pupae and holding them for emergence. Physical condition, such as eye color, size, wing malformations, and other defects, is also noted.

Product control tests include flight agility to determine the motility and competitiveness of reared versus native flies. This parameter is measured by placing 1600 pupae in a dispersal box containing gelled honey diet and counting the number of flies that escape within 48 hours after initial emergence. Flies remaining in the box are examined to identify the causes for their lack of flight. Sexual aggressiveness tests measure courtship behavior of mass-reared males and their ability to copulate with mass-reared females. Females are dissected 120 hours after the sexes are combined to determine the presence of spermatozooids. Longevity of flies held with and without food and water is tested by recording the time at which 50% mortality occurs under specific conditions of temperature and relative humidity. This indicates the potential duration of survival in the field under a wide range of conditions. Longevity is a sensitive indicator of product quality.

Quality control monitoring and testing is conducted to assure the production of screwworm flies that are competitive with those in the native populations targeted for eradication.





## CONCLUSIONS

## Rearing Systems

**Patrick J. Gomes**

United States Department of Agriculture, APHIS International Services, 6505 Belcrest Road, Hyattsville, Maryland 20782, USA

The group responsible for rearing systems identified the broad-based needs of the participants and others interested in consistent mass production of high quality arthropods. These interests included a wide range of organisms reared around the globe for agricultural and human health purposes. While many general needs were identified, specific actions and follow-up rest primarily with the workshop participants. Each participant has a vested interest in advancing the development and full implementation of total quality rearing systems. Mass rearing programs of specific interest to the participants in this session included:<sup>1</sup>

ORGANISM	COUNTRY
Codling Moth	New Zealand, Canada
Parasites (Medfly & <i>Anastrepha</i> spp.)	Mexico, Costa Rica
Medfly	Guatemala, USA, Chile, Peru, Argentina (2 facilities), Australia
Melon fly	Japan
Queensland fruit fly	Australia
<i>Trichogramma</i>	Mexico (20 facilities), Switzerland, Canada, UK, many others
Pink Bollworm	U.S.A., Pakistan
Screwworm	Mexico, Panama
Tsetse fly	Africa
Sugarcane Borer	Cuba
Sheep Blowfly	Australia
Old World Screwworm	Malaysia
Sweetpotato Weevil	Japan
Onion fly	Europe

<sup>1</sup> Most biological control specialists attended the other concurrent sessions, so their interest in mass reared natural enemies is indicated in the proceedings of those sessions.

The Rearing Systems session identified needs and actions that will lead to significant improvements in the mass rearing of both natural enemies and

pestiferous species. These crosscutting issues are divided into the following categories:

#### *Data Acquisition, Analysis and Exchange*

##### **Need:**

To improve the acquisition, analysis and sharing of quality control data.

##### **Statement:**

The statistical basis for sampling should be examined, defining database requirements and the format for sharing data. Standardization will help producer and customer determine product quality.

##### **Actions:**

United States Department of Agriculture (USDA) and International Atomic Energy Agency (IAEA) will compare computerized methods of recording data at fruit fly facilities. This will allow a comparison of insect quality for strains that are produced at different locations but evaluated using standardized QC methods. Software will be exchanged with others, i.e. New Zealand codling moth.

#### *Communication, Coordination and Review*

##### **Need:**

To increase communication and coordination among mass rearing facilities, public and private, and to periodically evaluate their efficiency in meeting the consumer's need for a consistent supply of high quality organisms for control purposes.

##### **Statement:**

Explore ways to establish or expand networks for the purpose of exchanging information, ideas and innovations. Conduct periodic reviews of rearing operations that will benefit producer and consumer alike.

##### **Actions:**

1. Association of Natural Biocontrol Producers (ANBP) will exchange their membership list with USDA and other cooperating institutions, indicating the ones with rearing facilities.
2. USDA/APHIS will conduct a workshop of sterile insect technique (SIT) facility directors and production chiefs in early 1994.
3. Cross-utilization and a greater degree of interaction is recommended among facilities, both government and private.
4. A quarantine facilities workshop will be sponsored by the USDA/National Biological Control Institute (NBCI).
5. Rearing methods and procedures for mass production of fruit fly parasitoids will be compiled by USDA/ARS, the University of Hawaii and Mexico to support their practical use and application, especially as they pertain to large scale control and eradication programs.

### *Technology Transfer and Training*

#### Need:

To facilitate the transfer of technology, training and education as it pertains to improving the quality of mass-reared arthropods.

#### Statement:

Mass rearing systems for beneficial organisms and SIT should be documented for the benefit of facility staffs. New or improved technologies, products, equipment or methods serve to benefit the entire mass rearing community and should be shared to the extent possible. Workforce development can be enhanced through exchange of key personnel.

#### Actions:

1. International Organization for Biological Control (IOBC) could take a leading role in encouraging universities to adopt curricula aimed at the production and use of mass-reared arthropods for both biological control and SIT.
2. New Zealand and Canadian codling moth facilities will explore the possibility of exchange.
3. IAEA will work closely with USDA/APHIS to develop and pilot test a temperature sensitive lethal (TSL), males only strain of medfly.
4. Mass rearing and quality assessment methods for parasitoids of medfly and other fruit flies will be compiled and documented by USDA/APHIS, the University of Hawaii and Secretariat of Agricultural and Water Resources (SARH), Mexico.

### *Education, Information and Promotion*

#### Need:

To educate and inform others of the value of using quality mass-reared arthropods to address agricultural, environmental and human health concerns.

#### Statement:

This is an ongoing requirement for both privately and publically-funded mass rearing facilities.

#### Actions:

1. USDA/NBCI currently is working with Walt Disney Studios to produce a video promoting biological control.
2. IOBC will continue its education efforts.

### *Quality Management Approaches*

#### Need:

To adopt quality management for all areas of arthropod mass rearing.

#### Statement:

Management should empower employees by delegating authority, responsibility and accountability. Creativity and innovation to improve

production, process or product controls should be rewarded. Worker safety and health are key parts of mass rearing systems.

Actions:

1. Introduce new management concepts beginning with managers - USDA.
2. Incorporate as a key topic at USDA, SIT workshop in 1994.
3. Develop for natural enemies - ANBP.

*Research and Development*

Need:

To continue research and development aimed at improving the production of mass-reared arthropods.

Statement:

The process for identifying needs and priorities requires input and support from the producer and user.

Actions:

1. Production Control.

- Establish diet testing standards - USDA, IAEA and New Zealand.
- Refine irradiation procedures to increase insect competitiveness in SIT Programs - USDA and IAEA.
- Examine worker safety and health associated with rearing - USDA and New Zealand.
- Develop disposal or recycling of spent diet and disposal of other waste products - USDA.
- Develop *in vivo* and *in vitro* production of beneficial organisms - work being done outside of this IOBC working group.

2. Product Control.

- Assess mating compatibility - USDA and IAEA for a temperature sensitive lethal strain of medfly.
- Improve methods of field evaluation - USDA and IAEA for TSL strain of medfly.
- Determine nutritional requirements of organisms - USDA for fruit flies.
- Assess learning cues for parasitoids reared on alternate hosts - USDA/ARS and the University of Hawaii.
- Evaluate chilling sterile flies to anaesthetize - USDA.

GROUP MEMBERS: Rick Frey; Pat Gomes, Chairman; Jorge Hendrichs; Norm Leppla; Dale Maki; Hilda Montoya; and David Rogers.

## Predators

### Carol Waddington

Tri-Cal Biosystems, 400 Casserly Road, Watsonville, CA 95076, USA

The 1993 meeting on quality control of predators resolved a number of questions that had emerged from previous sessions and identified goals for the future. In a relatively short time, we have made considerable progress in establishing workable guidelines for the rearing of predators in insectaries.

A few questions remain regarding the standardization of methodology used in evaluating quality such as counting methods, appropriate food sources, and temperature ranges. Thanks to some new data, the frequency of tests has been modified to reflect the true relationship with assessing quality. The evaluation of fecundity has now been resolved in favor of total rather than daily measurements. Research has shown that we need to establish criteria for each species of predator, and these responsibilities for further investigation have been assigned. We also determined that some of the criteria need to be revised to a stricter standard.

As our knowledge of quality becomes more sophisticated, we may need to establish standards for sample sizes and confidence intervals that give meaningful results. Also, flight activity and dispersal for many species have not been documented, and this may be important for determining quality in the field. Another topic for the future is the development of a preliminary list of criteria for quality control of new natural enemies to be presented at the San Diego meeting in 1995. The two predator species selected are *Hypoaspis miles* (Acarina: Laelapidae) with D. Elliott as coordinator, and *Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae) with A. Hale as coordinator.





## Parasitoids

**Mary F. Purcell**

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### *Parasitoid species*

Revision of guidelines for quality control testing were discussed for six commercially used parasitoid species:

- *Encarsia formosa*
- *Trichogramma brassicae*
- *Diglyphus isaea*
- *Dacnusa sibirica*
- *Aphidius* spp.
- *Aphelinus abdominalis*

Coordinators from industry and research institutions were identified to perform the necessary testing.

Needs for future testing were discussed primarily for four parasitoid species:

Species	Need
<i>Encarsia formosa</i>	- Correlate head size and host pupal size - Flight tests
<i>Trichogramma brassicae</i>	- Develop guidelines for each species or strain - DNA mapping for species identification
<i>Aphidius</i> spp.	- Flight tests
<i>Aphelinus abdominalis</i>	- Unspecified

### *Quality Control Guidelines*

Quality control guidelines (with similar format as the above natural enemy species) will be developed for new species and made available at the next QC meeting: *Aphytis melinus*, *Diachasmimorpha longicaudata*, *Eretmocerus californicus*, *Leptomastix dactylopii*, *Metaphycus helvolus*, other *Trichogramma* species.

#### *Future Tests*

Tests that should be conducted in the future to improve quality of parasitoids:

- Behavioral tests (i. e., oviposition, searching, entrainment to host).
- Performance of parasitoids in the field.
- Relationship between laboratory and field performance (i.e., what parameters are important, fecundity, longevity, adult size?).
- Taxonomic identification, including DNA mapping techniques.
- Delivery systems for distributing parasitoids uniformly in the field.
- Cold storage techniques and impact on parasitoid performance (i.e., fecundity and longevity).
- Flight tests.
- Reduction of superparasitism in the laboratory rearing (i.e., *D.longicaudata*).
- Environmental impact (i.e., non-target species if the parasitoid is not host specific)

## Quality Control Guidelines for Natural Enemies

Report of the 3rd meeting on guidelines for natural enemies applied in greenhouses

J.C. van Lenteren<sup>1</sup>, F. Bigler<sup>2</sup> and C. Waddington<sup>3</sup>

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### Introduction

The guidelines listed herein 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. These measures should preferably be carried out by the producer **after all handling procedures just before shipment**. The user, farmer or grower, should only perform a simple quality test, e.g. percent emergence or number of live adults. Some tests are to be carried out frequently by the producer, i.e., on a daily or weekly basis. Others will be done less frequently, i.e., on an annual or seasonal basis, or when the rearing procedure will be changed. This is specified in the tests. For each test two coordinators are 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 which are essential for the completion of the quality control guidelines.

Most of the tests were drafted at the Wageningen 1991 meeting. All participants of that meeting and mass production companies were asked to comment on these drafts. During the Horsholm and Rimini meetings the comments were discussed and tests were adapted after evaluation of last years test results. Several new guidelines will be drafted and tested during the coming year. Further appointments have been made about additional tests and experiments for improving guidelines. These tests and experiments are specified under the comment section at each natural enemy below. During the meeting of the IOBC global working group on "Quality Control of Mass Reared Arthropods" at Rimini, Italy (September 1993) most of the tests were accepted and will now function as standard 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.

**Actions agreed upon at Rimini meeting, September 1993**

1. For each of the tests, the coordinators will work out more detailed guidelines and send these to M. Benuzzi before 1 January 1994. M. Benuzzi will send a set of complete guidelines to all producers. In 1994 the producers will apply the tests and report the results at the next meeting of the EC working group. A reminder concerning these activities will be circulated in March 1994 by J.C. van Lenteren.
2. Data sheets will be drafted before the next meeting by G. Burgio and S. Maini on *Ostrinia nubilalis*, J.C. van Lenteren on greenhouse whitefly and *Encarsia formosa*, C. Meierrose on Lepidoptera, and S. Steinberg on *Phytoseiulus persimilis*.
3. K. Alcock will circulate ISO Standard Instructions on Quality Control for the industry
4. Taxonomy and identification of pest/natural enemy species/strains is problematic; possibilities for using molecular biological techniques will be checked in Antibes (Wajnberg) and Wageningen (van Lenteren).
5. New quality control tests to be developed:

Species	Researcher
<i>Aphytis melinus</i>	J. Blehm, Ventura, USA
<i>Cryptolaemus montrouzieri</i>	Nature's Alternatives, USA & Biol. Control Industries, Israel
<i>Diachasmimorpha longicaudata</i>	USDA/ARS Purcell, Sivinsky
<i>Eretmocerus californicus</i>	Bunting UK
<i>Leptomastix dactylopii</i>	Biolab Cesena, Italy & Biol. Control Industries, Israel
<i>Metaphycus helvolus</i>	Fillmore Insect., Ventura, USA
<i>Trichogramma</i> spp.	Inst. Biological Control, Darmstadt (D)

**For all natural enemies:**

1. When rearing procedures are changed all elements of a set of guidelines should be carefully tested for untreated and treated natural enemies.
2. The expiration date for a shipment should be given on the container or packaging material.

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

Test conditions: Temperature: 22±2°C; RH: 60-90%; Light regime: 16L:8D.

Quantity & Emergence rate:  $\geq$  the number of adults specified on the label which will emerge during 2 weeks;  $n=1000$ , based on 3 subsamples; a weekly or batch-wise test.

Sex-ratio:  $\geq 98\%$  females;  $n=500$ ; conducted once every 4 weeks.

Adult size: Head width  $\geq 0.28$  mm;  $n=20$  females; an annual test<sup>1</sup>.

Fecundity:  $\geq 7$  eggs/female/day for days 2, 3 and 4 after emergence of the adult,  $n=15$ ; an annual test. Procedure is described by Ravensberg in Wageningen proceedings 1991 (80-89).<sup>2</sup>

Flight activity: Test to be developed; an annual test<sup>3</sup>.

Comments:

<sup>1</sup> Available information does not show a relationship between host pupal size and size of emerging adult *Encarsia*, so measurement of pupal size is not sufficient as an indicator of adult size. Koppert and Wageningen will perform new experiments to study a possible relationship.

<sup>2</sup> 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.

<sup>3</sup> A short-distance flight test will be tested by several producers based on a design which will be sent to them by January 1, 1994, this test can be combined with the emergence rate test, a weekly or batch-wise test. Entomology Wageningen will develop a long-distance emergence test. This will be an annual tests to be performed in August/September/October when the population is at its lowest number.

Coordinators: J.C. van Lenteren & W. Ravensberg

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***Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae)**

Test conditions: Temperature:  $25 \pm 2^\circ\text{C}$ ; RH:  $60 \pm 5\%$ ; Light regime: 16L:8D.

Quantity:  $\geq$  number of live adults specified on the label; a weekly or batch-wise test.

Adult mortality:  $\leq 5\%$  of the number of live adults specified on the label, based on 3 containers sampled and  $n=500$  or more; a weekly or batch-wise test.

Sex-ratio:  $\geq 45\%$  females;  $n=500$ ; conducted once every 4 weeks.

Fecundity:  $\geq 40$  eggs/female from 3rd to 7th day after emergence;  $n=30$ ; an annual test; pre-oviposition period of *Diglyphus* is 2 days; procedure: daily oviposition of a single pair of wasps on brown beans (*Phaseolus vulgaris*) infested with sufficient (minimum 30 L2 & L3 larvae) *Liriomyza trifolii*, if insufficient hosts are offered many might be killed by host feeding. Other host species can be used but should then be specified.

Comments:

- No relationship was found between pupal size and fecundity, measurement of pupa does not give an indication about the quality of the adult, Entomology

Bologna will report on this issue next year.

- No need to test flight activity as it is a trait tested for during the normal rearing procedure.

Coordinator: G. Nicoli

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***Dacnusa sibirica* Telenga (Hymenoptera: Braconidae)**

Test conditions: Temperature:  $22\pm 2^{\circ}\text{C}$ ; RH:  $60\pm 5\%$ ; Light regime: 16L:8D.

Quantity:  $\geq$  number of live adults specified on the label; a weekly or batch-wise test.

Adult mortality:  $\leq 5\%$  of the number of live adults specified at the label, based on 3 containers sampled and  $n=500$  or more; a weekly or batch-wise test.

Sex-ratio:  $\geq 45\%$  females;  $n=500$ ; conducted once every 4 weeks.

Adult size: There is a linear relationship between size and fecundity, so adult size does not need to be measured if fecundity is being assessed<sup>1</sup>.

Fecundity:  $\geq 50$  eggs/female within 5 days;  $n=10$ ; an annual test; procedure: daily oviposition of a single pair of wasps on brown beans (*Phaseolus vulgaris*) infested with sufficient *Liriomyza trifolii* and a source of carbohydrate (other hosts if *L. trifolii* is not available: *L. bryoniae* or *Chromatomya syngenesiae*).

Comments:

<sup>1</sup> Additional data necessary; will be collected by coordinators, no need to test flight activity as it is a trait tested for during the normal rearing procedure.

Coordinators: R. Greatrex & J. Dale

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***Aphidius*<sup>1</sup> spp. (Hymenoptera: Braconidae)**

Test conditions: Temperature:  $22\pm 2^{\circ}\text{C}$ ; RH:  $60\pm 5\%$ ; Light regime: 16L:8D.

Quantity: When shipped as adults  $\geq$  the number of live adults as specified on the package. When shipped as mummies:  $\geq$  the number of live adults that have to emerge from the package. A minimum of 3 containers should be counted. A weekly or batch-wise test.

Adult mortality:  $\leq 5\%$  of the number of live adults specified on the label, based on 3 containers sampled and  $n=500$  or more; a weekly or batch-wise test.

Emergence rate:  $\geq 70\%$ ;  $n=500$ ; conducted once every 4 weeks.

Sex-ratio:  $> 45\%$  females; a weekly test;  $n=500$ .

Adult size: Needs to be studied<sup>1</sup>.

Fecundity:  $\geq 65\%$  parasitism;  $n=25$ ; to be conducted 4 times per year. Procedure for *Aphidius matricariae* targeted at *Myzus persicae*: each female wasp is offered 20 *M. persicae* aphids on sweet pepper leaves in a petri dish. Procedure for *Aphidius colemani* (or *A. matricariae*) targeted at *Aphis gossypii*: each female wasp is offered 20 *A. gossypii* on cucumber leaves in a petri dish. To be tested 4 times per year. Several methods will be studied and compared next

year<sup>2</sup>.

Flight activity: As mummies can easily be damaged during the harvesting process, a flight test will have to be developed<sup>3</sup>.

Comments:

<sup>1</sup> The target pest - the aphid species name - for which this parasite should be used needs to be mentioned on the container. Also the *Aphidius* species name must be given.

<sup>2</sup> A test will be developed with whole plants (S. Steinberg). A potted cucumber plant bearing 4-5 leaves is used. The plant is kept in a transparent plastic cylinder (30 cm, 12-cm diameter), which has one ventilation hole in the top and two at the side, covered by fine mesh gauze. The plant is infested by an ample amount of aphids (*A.gossypii*). Infestation  $\pm 7$  days prior to the experiment. Wasps or plants are changed every day. Another test will be developed with leaf discs in petri dishes (J. van Schelt). Cucumber leaf discs are put on an agar layer. 25 *A.gossypii* nymphs are put on the leaf, 25 such petri dishes are prepared. Parasites are allowed to oviposit for 24 hrs. In both tests aphids will have to be dissected for parasite larval presence, or parasites are counted when they are in the mummy stage. For both tests reliability and time investment will have to be assessed.

<sup>3</sup> S. Steinberg will develop a flight test for *Aphidius* in cooperation with J.C.van Lenteren

<sup>4</sup> Hansen will study the relationship of mummy size to adult size

Coordinators: J. van Schelt & S. Steinberg

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***Aphelinus abdominalis* Dalman (Hymenoptera: Aphelinidae)**

Test conditions: Temperature: 22°C; RH: 60-80%; Light regime: 16L:8D.

Quantity: the number of live adults and/or mummies as specified on the label; a weekly or batch-wise test.

Adult mortality: <10% per package; n=250, based on sample from 3 containers; a weekly or batch-wise test.

Emergence rate: 80% within 2 weeks, n=200, weekly or batch-wise test.

Sex-ratio:  $\geq 45\%$  female, n=250, batch-wise or weekly.

Adult size: Very variable, the relationship size to longevity to be tested (to be discussed with C. Fleuryneck).

Fecundity: >60 eggs/female during 8 days, n=10. Females fed with honey. Indirect measure on whole plant by counting mummies. Plants infested with *Macrosiphum euphorbiae* (ample amount) on tomato. Annual test.

Coordinators: C. Fleuryneck & H. Haardt

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***Phytoseiulus persimilis* Athias-Henriot (Acarina: Phytoseiidae)**

Test conditions: Temperature: 22 (20-25)°C; RH: 75 $\pm$ 5%; Light regime: 16L:8D.

Quantity: Number of live predators as specified on the container; a weekly or batch-wise test.

Sex-ratio: >60% females; seasonal test or 4x/year; n=100.

Longevity<sup>1</sup>: Minimum 5 days, reached by at least 80% of the females in the sample; n=20; seasonal test or 4x/year.

Fecundity<sup>2</sup>: >10 eggs/female for 5 days, counting from the second day of egg-laying; n=20; seasonal test or 4x/year.

Comments:

- Need to determine whether there is any significant difference between tests done at 25°C or 22°C. Italian tests were done at 25°C, but greenhouse temperatures are 22°C.

<sup>1</sup> S. Steinberg data resolved the earlier question regarding measurements of fecundity in favour of total fecundity.

<sup>2</sup> University of Bologna researchers and C.Waddington will determine whether longevity standard is too high.

Coordinators: S. Steinberg & J. Dale

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***Trichogramma brassicae* (= *T.maidis*) (Hymenoptera: Trichogrammatidae)**

Test conditions: Temperature: 23±2°C; RH: 75±10%; Light regime 16L:8D.

Rearing hosts: *Ephestia kuehniella*, *Sitotroga cerealella*.

Species identification: The species specified on the label<sup>1</sup>.

Emergence rate and period: >80% (independent of storage techniques); check of 5x100 parasitized (black) eggs<sup>2</sup>; weekly or batch-wise test.

Sex-ratio: >50% females; 100 adults on 5 release units each or 5x100 adults of bulk material; weekly or batch-wise test.

Number of adults: Determined from 5 release units (card, capsule) at each release or from 5x100 parasitized (black) host eggs of bulk material, batch-wise test.

Fecundity and Longevity: 20 females (age 24 hrs) are confined individually in glass tubes; at least 200 factitious host eggs (<24 hrs) are glued with water on a small cardboard strip; a small droplet of honey and a droplet of water are added directly to the wall of the vial. Eggs of *E.kuehniella* (<24 hrs old) are UV irradiated and provided at day 1 and removed after day 7; fresh eggs of *S.cerealella* are provided at days 1, 3 and 5. The number of living adults is recorded after day 7. Egg-cards are incubated and the number of black eggs is counted not earlier than at day 10. Minimum fecundity after 7 days is 40 eggs/female; mortality after 7 days <20%; monthly test or batch-wise if batches were exposed to special treatments (e.g., storage procedures, long-range shipments).

Natural host parasitism: 20 females (24 hrs old) are confined individually in tubes; two fresh egg-masses of natural host, *Ostrinia nubilalis*, (<24 hrs old) are added for 4 hrs; honey and water are provided as described above; after



separation of the egg-masses from females they are incubated for 3 days; the number of black eggs is counted. This test is an indirect measure of the acceptance and suitability of the natural host egg. The test should be performed 2 to 4x/year depending on the rearing system (number of generation on factitious hosts).

Comments:

- <sup>1</sup> Identification of species for checks is a problem and needs help by taxonomists. In future, enzyme or DNA techniques might be used.
- <sup>2</sup> % emergence is considered as a simple, rapid indicator of quality. The emergence period and pattern depend on the mixture of developmental stages released together and should be specified on the label.
- <sup>3</sup> Standardized conditions of adults (age, food, water, handling, etc.) is crucial for minimizing variation (see procedures described by Cerutti and Bigler, Wageningen Proceedings, 1991).

Coordinators: F. Bigler & G. Eden

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*Aphidoletes aphidimyza* (Diptera: Cecidomyiidae)

Test conditions: Temperature: 22°C (20-25°C); RH: 80±5%; Light regime: 16L:8D. Weekly emergence test at 25°C.

Quantity: Number of adult insects as specified on label; weekly or batch-wise test.

Emergence rate: ≥70% emergence within 7 days: number of pupae should be known; weekly test; n=100

Sex-ratio: ≥45% females; a weekly or batch-wise test; n=500.

Adult size: Appears to be relevant to genetic fitness (L. Gilkeson's data). D. Elliot will check for next meeting.

Fecundity: Total fecundity >40 eggs/female within 4 days; n=25; monthly test;

Procedure: allow females to oviposit individually on *Myzus persicae* on sweet pepper leaves (for details see Van Schelt, Wageningen proceedings pp. 90-95).

Flight activity: Applied Bio-Nomics will evaluate and adapt the flight test for *Encarsia formosa*.

Comments:

- Have to fly anyway in rearing procedure.

Coordinators: A.T. Gillespie & J. Douma

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*Chrysoperla carnea* (Steph.) (Neuroptera: Chrysopidae)

Test conditions: Temperature: 23-27°C; RH: 70±5%; Light regime: 16L:8D.

*When shipped as eggs*

Quantity: The number of eggs as specified on the package; a weekly test.

Longevity: Minimum 5 days reached by at least 80% of the larvae in the sample;

n=20; seasonal test.

Hatching rate: >65% within 7 days; n=100; eggs must be isolated to prevent cannibalism after emergence; a weekly or batch-wise test.

Predator quality: >65% of newly hatched larvae have to develop to second instar larvae within 4 days; to be conducted once a year or when the rearing system is changed. Procedure: offer individual, freshly emerged larvae at least 50 prey items on a leaf on agar in a petri dish (12-cm diameter); n=30; three species of aphids can be used as prey items: *Aphis gossypii* on cucumber, *Macrosiphum euphorbiae* on strawberry or potato, or *Myzus persicae* on sweet pepper.<sup>1</sup>

#### *When shipped in second larval stage*

Quantity: Number of live predators as specified on the package; a weekly or batch-wise test.

Composition: ≥50% of second instar larvae has to develop to third instar larvae within 5 days; yearly test or when the rearing system is changed. Procedure: offer individual, freshly emerged second instar larvae at least 100 prey items on a leaf on agar in a petri dish (12-cm diameter); n=30; three species of aphids may be used as prey items: *Aphis gossypii* on cucumber, *Macrosiphum euphorbiae* on strawberry or potato, or *Myzus persicae* on sweet pepper.<sup>1</sup>

<sup>1</sup> Jake Blehm will evaluate whether other prey may be used as a food for these tests, and whether n=20/30 is sufficient to establish a meaningful confidence interval. Preliminary data indicate that n=100 is the minimum, and n=200-400 may be necessary.

Comments:

- A test to evaluate the searching capacity and locomotion capacity of first and second instar larvae of *Chrysoperla* should be developed.

Coordinators: M. Benuzzi & J. Blehm

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#### *Orius* spp. (*O. insidiosus*, *O. majusculus*, *O. albidipennis*, *O. laevigatus*) (Hemiptera: Anthocoridae)

Test conditions: Temperature: 22°C (20-25°C); RH: 80±5%; Light regime: 16L:8D.

Quantity: Number of live adults/nymphs as specified on the container. The species should be identified whether individual species or a mix. Counting method: 3 samples from each of 3 containers, assessed by weight, sample size 30 insects/container. A weekly or batch-wise test.

Sex-ratio: ≥45% females; test every four weeks; n=500.

Adult size: University of Bologna will check. Current data indicate a weak correlation (R=0.6) between adult size and fitness for one species (*O. laevigatus*).

Fecundity:<sup>1</sup> >20 eggs/female during a 10-day oviposition period; counting from the

second day of egg-laying; n=30 pairs individually kept. A monthly test. Find new data for each species 2x/year. Pre-oviposition period depends on temperature.

Flight activity: Needs to be tested, especially for differences in flight behaviour and dispersal of three species. Biobest will evaluate and adapt flight test for *Encarsia formosa*.

<sup>1</sup> This may be too low. Koppert, Bunting, and Biobest will evaluate per species and will also evaluate whether to use groups or individuals for fecundity studies.

Comments:

- Some disagreements on whether we need to test sex ratio monthly. Still some questions about sub-sampling.
- The species (composition) must be mentioned on the container

Coordinators: W.J. Dale & M.G. Tommasini

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***Amblyseius cucumeris* (Oudemans) (Acarina: Phytoseiidae)**

Test conditions: Temperature: 22°C (20-25°C); RH: 75±5%; Light regime: 16L:8D.

Quantity: When delivered in container: the number of live predators as specified on the container, excluding eggs. When delivered as controlled release system (CRS): the number of live predators as specified on the label, excluding eggs when sold.

Sex-ratio: An annual test. C.Waddington to check literature.

Adult size: J.Douma will pursue.

Fecundity: Total fecundity should be ≥7 eggs/female during 1 week. Count from second day of egg-laying. Test 1x/year. Data will be presented in San Diego on any variability between food sources (flour mites, *Ephestia* eggs) by Applied Bio-Nomics, Koppert, and Bunting.

Comments:

- In one test, Koppert found large variation between samples done with dry and wet method (n=20). Willem Ravensberg agreed to do more tests of both methods and get data for variability. Also, a procedure on how to select females used for these needs to be developed.

Coordinators: J. Douma

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