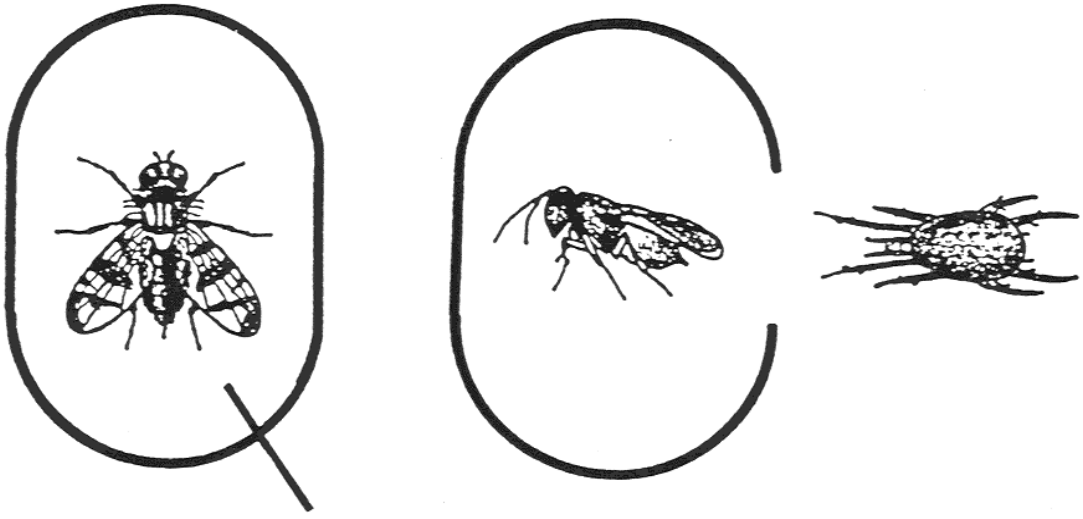
INTERNATIONAL ORGANIZATION FOR BIOLOGICAL CONTROL OF NOXIOUS ANIMALS AND PLANTS

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



REPORT

Sixth workshop
of the IOBC Global working group
"Quality Control of mass-reared Arthropods"
Horsholm, Denmark,
November 9 - 12,1992

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Introduction

The workshop was held at the facilities of Chr. Hansen's Biosystems in Horsholm, Denmark. Invitations were sent to all biological control producers in Europe and North America. A total of 26 persons (20 production managers and 6 scientists) from 12 countries, including USA and Canada, attended.

Why this workshop?

The workshop was a follow-up of the meeting held in Wageningen (NL) in March 1991. It was organized for two reasons:

1. A few producers had the feeling after the Wageningen meeting that more time should be spent for discussions of urgent problems instead of scientific presentations. Therefore, no formal scientific presentations were made at this meeting.
2. The guidelines for product control, drafted at the Wageningen meeting, needed to be reviewed and improved as soon as possible. They were tested by several producers during one year and it would have been a waste of time and energy to review them only after two years at the planned Rimini meeting in September 1993.

Objectives of the workshop

1. Review and improve the guidelines of product control as drafted at the Wageningen meeting.

2. Draft product control methods for organisms which were not included at the last meeting.

3. Discuss product control methods with regard to resources of the producers, distributors and users of beneficials, i.e. what are the costs, who pays for it, who invests how much in QC?

4. What quality information should be on the labels and what are the implications of a quality guarantee?

Definition and need for Quality Control

Quality control in insect mass production was defined as:

"production control" - monitoring the performance of all rearing operations, procedures, equipment, and environments.
"process control" - monitoring of unfinished product quality such as egg hatch, larval and pupal weight, pupation percentage.
"product control" - monitoring the final product quality such as quantity of organisms, sex ratio, emergence rate, longevity, hatching ratio, fecundity, adult size, adult mortality, storage ability, flight activity, etc..

Because of the proprietory nature of commercial mass production rearing systems only product quality control was discussed at this meeting.

J. Van Lenteren discussed the increased demand control products. There is a very slow development of new pesticides. In 1960 there were 20 new pesticides and in 1990 only 1 new pesticide introduced. Of approximately 300 active ingredients presently available for insect and mite control 120-180 are on black lists for re-evaluation. In future the number of insecticides and acaricides will decrease. Therefore there, is great need for alternatives to pesticides and biological control is one of the alternatives. Reliability must be an absolute condition for biocontrol products to succed. Having quality control standards is to the benefit of commercial producers as it is proof to the grower and to the international bio-control community of a high quality product. Poor quality of natural enemies results in negative advertisement for biological control. Problems with quality will also lead plant protection agencies to demand quality standards and tests. In developing biocontrol standards, however, it is important to distinguish between the "need to know" and "nice to know" and to be practical and not idealistic. Good standards should be able to separate the good products from the bad. Regulatory authorities want the producers to set the guidelines for standards. The reliability and visibility of biological control would be improved considerably if standards for acceptable quality could be developed for all marketed products. The biocontrol industry has expanded very rapidly. In Europe 40-60,000 ha now use biological control in a variety of crops including vegetables, maize, orchards, vineyards. Microbial biocontrol agent sales in Europe increased from US$ 35 million to 120 million from 1985-1991. In the same period, macrobial (insect and mite predators and parasitoids) sales increased form US$ 10 to 40 million. Bumble bees used for pollination account for US$ 10 million in 1991 (Table 1).

Worldwide there are 50 commercial beneficial production companies employing over 3000 people and two large European companies with more than 450 employees together. The majority of biocontrols are produced by 10 large production companies. Macrobial mass production involving parasites and predators is a difficult process that requires considerable work by public and private research scientists and producers in selecting and rearing a natural enemy that will do the job. The starting culture must have a good genetic composition, be reared on the natural host and tested for efficacy on the commercial crop.

Table 1. Sales of pesticides and biocontrol agents (incl. bumble bees for pollination) in Europe in million US$ (end-user values)

Microbials	1985	35
	(Bacillus)	31
	1991	120
	(Bacillus)	110
	1985	10
Macrobials (insects, mites, nematods)	1991	40
	(Bumble Bees)	10
Total pesticides	1991	24000

A proposal for EC Quality Control programme funding

A proposal for European Community funding for "Designing and implementing quality control of beneficial insects: towards more reliable biological pest control" will bis submitted by J. van Lenteren. This will be a three years programme with a planned start date of March, 1993 and has 5 objectives:

1. Develop realistic, simple and reliable quality control methods at research laboratories for the 10 natural enemies which are widely used in biological pest control in europe today.
2. Test simplified quality control methods under commercial conditions.
3. Evaluate outcomes of different quality control tests and improve the methods for practical use.
4. Implementation of quality control methods at mass production companies in Europe and develop proposals for EC standards.
5. Design training material for short courses for those who will work on quality control at production companies.

Programmes to develop these objectives will be assigned to research laboratories and private companies and workshops held each year in Europe to coordinate the work. The costs of the project are to be shared between the E.C., 8 private companies and 8 research institutes within the following countries: Denmark, France, Germany, Hungary, Israel, Italy, The Netherlands, Portugal, Switzerland, United Kingdom, and Spain.

International regulation of biocontrol Product Quality

A general discussion was conducted on the present and future status of legislation for mass-reared beneficials in Europe and North America. The results of the discussion are summarized in table 2. Different countries have varying import permit requirements of live organisms but most do not restrict entry or distribution of the commonly mass-reared

Table 2. Present status of requirements for importation and registration of mass-reared beneficials used for biocontrol.

COUNTRY	IMPORT REGISTRATION required	EFFICACY DATA for registration required
FINLAND	X	
NORWAY	X	
SWEDEN	X	
DENMARK	X	
GERMANY	X	
THE NETHERLANDS	X	(X)
BELGIUM *		
UNITED KINGDOM	X	
FRANCE	X	X
SPAIN	X	
ITALY *	X	
SWITZERLAND	X	X
AUSTRIA	X	X
HUNGARY	X	X
CANADA	X	
USA	X	
ISRAEL	X	
JAPAN	X	X

* Only reporting the name/list of organisms
( ) might be required in future

beneficials within the country. France, Austria, Switzerland, Hungary and Japan require efficacy data before registration. England, the United States and Canada have restrictions on entry and within country distribution of some non-indigenous organisms such as *Orius*.

The future situation will be more complex as a number of countries and states are currently examining the status or the need to further regulate the importation, sale, distribution and quality control of biocontrol agents. The development of quality control guidelines by the IOBC global working group will allow input from biocontrol researchers and commercial producers and should help to develop more uniform standards in this area.

Canada and the United States regulatory authorities have recently requested input from biocontrol researchers and producers on the question of regulation. Agriculture Canada has recently formed a working group to provide guidelines for the regulation of indigenous beneficial insects, mites and nematodes used in augmentative and inundative releases. In North America, commercial biocontrol producers in California have formed the Association of Natural Bio-Control Producers (ANBP) and are currently working on quality assurance guidelines for producer, distributer and applicator. Association membership to the ANBP is open to producers and distributers outside of the United States. The objective of this association is to represent producers and to develop a producer quality certification and monitoring program toward continued improvement of product and service. J. Van Lenteren suggested that a similar European association of producers be established.

Quality Control issues

A brainstorming of the group was conducted in order to recognize and evaluate relevant topics of Quality Control. A number of issues were submitted by members of the group and these were priorized and discussed in order of interest. (Table 3.) There was not time to deal with all questions but group concensus was reached on some items.

Table 3. Relevant issues for Product Control submitted and priorized by the group

Issue	Priority
Who pays when product does not reach accepted standard?	2
Who checks quality and who pays random inspection (at distributor level)?	1
Who standardizes qualitiy tests?	4
How do you know if poor results are due to poor quality? Accountability? How far does responsibility for quality go for? producer, distributor, endurser? Who judges / decides?	9
Expiry date on label or package?	6
Shipping conditions, who checks?	8
Partial fulfillment of limits/standards, Consequences?	5
Is compensation by quantity for poor quality allowed, legal?	5
Standardized label requirements, information for enduser?	7
International quality labels, who certifies?	3
Contaminants - pathogenes, other insects, plants	9

1. Who checks quality control and pays for random inspection at the distributer level? There was considerable discussion over how this could be done but most agreed that it would be best if international IOBC guidelines could be agreed upon by researchers and producers and then independent government authorities would be able to check quality control as necessary using the same standard worldwide.

   In Europe, plant protection services (government bodies) or research institutes could conduct quality tests. They have the advantages of being close to the producers, distributers and users, they have some experience and could start immediately. Non-government organizations (e.g. IOBC, EPPO, FAO) do not have any structures available for testing purposes.

   Participants from North America opposed to the idea of government regulation of quality checks and proposed a discussion of this issue at a meeting organized by the Association of Natural Biocontrol Producers (ANBP) on January 31, 1993.

   It was agreed so far to prepare final versions of guidelines soon after the Rimini meeting 1993 and present them to EPPO and/or EC and/or FAO for approval.

2. A quality standard is not a guarantee for efficacy in the field. Some degree of efficacy may be determined on a laboratory basis, but conditions in the greenhouse and field are too different for drawing final conclusions based on laboratory data. Methods must yet be developed for valid field efficacy testing. A future goal will be to relate quantatively the results of the quality control tests conducted under laboratory conditions to the actual performance of the beneficial in the field.

3. The idea of an international IOBC quality control label indicating that the product conformed to IOBC quality control testing standard was discussed. This would require approval by the IOBC and raised the question of who would do the certification or product testing and would this be on a regional, national or international level? Periodical checks of random samples would require a well structured organization between testing laboratories and IOBC. The financial consequences should then be discussed.

4. An expiry date on the package was agreed to be necessary to prevent poor quality products from being sold by distributers or used by consumers. This could be a packing date or an expiry date and would relate to instructions on shipping, storage or use provided by the producer.

   It was agreed that development and approval of standardized Product Control guidelines should be organized by the IOBC working group. Internationally approved guidelines will be needed in future. New guidelines will be developed during workshops or during the year and submitted to the convener of the IOBC working group who will distribute them to competent working group members for reviews and comments.

5. A discussion was conducted whether poor quality could be compensated by quantity. It was agreed that quality standards set by the guidelines are the lower limits which must

A number of other longer term quality control issues were mentioned but not discussed due to time constraints. These included: the use of unnatural hosts, pathogenicity, contaminants such as other insects and plants, standardized label requirements and instructions for consumers.

Subgroup working session

The workshop was divided into working groups: Trichogramma and Greenhouse Beneficials. Within these groups discussion was focused on reviewing and improving the 1991 quality control guidelines. Trial had been conducted by various producers to reassess the technical and economic feasibility of the original tests. These were discussed within each group and changes and additions made where necessary.

GUIDELINES FOR PRODUCT CONTROL OF NATURAL ENEMIES

Summarized by J.C. van Lenteren (greenhouse beneficials) and F. Bigler (Trichogramma)

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/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 meeting the comments were discussed and tests were adapted after evaluation of last years test results. Several new guidelines were drafted that will be tested during the coming year. **Producers will inform Dr. F. Bigler before 1-1-93** which guidelines they will test. 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 next full meeting of the IOBC global working group on "Quality Control of Mass Reared Arthropods" in Italy (September 1993) it is expected that most of the tests will be officially accepted and 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.

For all natural enemies:
When rearing procedures are changed all elements of a set of guidelines should be carefully tested for untreated and treated natural enemies.
Ad expiry date on container or packaging material.

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

Test conditions:	Temperature: 22°C; RH: 60-90%; Light regime: 16L:8D.
Quantity & Emergence rate:	>= the number of adults specified on the label which will emerge during 2 weeks[4]; a weekly or batch-wise test[1]
Sex-ratio:	>= 98% females; n=500; a weekly or batch-wise test
Adult size:	Head width >= 0.28 mm; n=20 females; an annual test[2]
Fecundity:	> 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).[3]
Flight activity:	Test to be developed; an annual test[5]

Comments:

1 Earlier we included a 75% emergence threshold because with the batch-wise tests we do not measure size. In that case % emergence is a simple indicator of quality. Suggestion: mention the number and the % of parasites which should emerge. Example: 100 adult parasites should emerge from this card during two weeks; percentage emergence should be >= 75% based on checking 500 black pupae.

2 Discussion about possible relationship between (host) pupal size and size of emerging adult *Encarsia* learned that there is no clear relationship and that measurement of pupal size is not sufficient as an indicator of adult size. Much more basic work needs to be done before we can possibly use pupal size instead of head width of *Encarsia*.

3 Fecundity test is done differently in France: before the above guideline can be changed first a good correlative experiment should be done by C. Fleurynck

4 The emergence period may be shortened to 10 days in future

5 Short-distance flight test based on Enkegaard's design will be tested by several producers (see annex 1). Entomology Wageningen will develop a long-distance emergence test.

- Annual tests to be performed in August/September/October when the population is at its lowest number

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

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

Test conditions:	Temperature: 22 (20-25)°C; RH: 70±%; Light regime: 16L:8D.
Quantity:	number of live predators as specified on the container; a weekly or batch-wise test.
Sex-ratio:	> 45% females; a weekly test; n=500
Longevity[1,2]:	minimum 5 days, reached by at least 80% of the females in the sample; n=20; a seasonal test.
Fecundity[1,2]:	>2 eggs/female/day for 5 days after egglaying starts; n=20; a seasonal test.

Comments:

1 Question raised at Denmark meeting: should we measure daily fecundity over 5 day period, or total fecundity for the same period. More data needed before final choice can be made. Data will be sampled during the coming year.

2 The longevity and fecundity tests will be conducted simultaneously on brown beans (*Phaseolus vulgaris*) infested by an ample amount and all developmental stages of the two-spotted spider mite *Tetranychus urticae*.

Coordinators: S. Steinberg, J. Dale

Diglyphus isaea (Walker) (Hymenoptera: Eulophidae)

Test conditions:	Temperature: 22°C; RH: 60+/-5%; Light regime: 16L:8D.
Quantity:	>= number of live adults specified at the label; a weekly or batch-wise test
Adult mortality:	<= 5%, based on 3 containers sampled and n= 500 or more; a weekly or batch-wise test
Sex-ratio:	>= 45% females; n=500; conducted once every 4 weeks
Adult size:	no relationship found, but data limited. Contact Nicoli and study Cambridge paper to suggest a future guideline
Fecundity:	>= 50 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 need to test flight activity as it is a trait tested for during the normal rearing procedure - during the following year Duclos will test whether oviposition of a small group of wasps will give the same results as with the individually tested wasps
Coordinators:	*C. Fleurynck & G. Nicoli*

Dacnusa sibirica Telenga (Hymenoptera: Braconidae)

Test conditions:	Temperature: 22°C; RH: 60+/-5%; Light regime: 16L:8D.
Quantity:	>= number of live adults specified at the label; a weekly or batch-wise test
Adult mortality:	<= 5%, based on 3 containers sampled and n= 500 or more; a weekly or batch-wise test
Sex-ratio:	>= 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 (additional study of data necessary; will be collected by coordinators
Fecundity:	>= 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. trifoliii* is not available: *L. bryoniae* or *Chromatomya syngenesia*).
Comments:	No need to test flight activity as it is a trait tested for during the normal rearing procedure
Coordinators:	*R. Greatrex, J. Dale*

***Aphidius*[1] spp. (Hymenoptera: Braconidae)**

Test conditions:	Temperature: 22°C; RH: 60+/-5%; Light regime: 16L:8D.
Quantity:	When shipped as adults >= the number of live adults as specified on the package. When shipped as mummies: >= 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:	<= 5 %, based on 3 containers sampled and n= 500 or more; a weekly or batch-wise test
Emergence rate:	70%; 50% emergence within 5 days; n=500; conducted once every 4 weeks
Sex-ratio:	> 45% females; a weekly test; n=500
Adult size:	relevant ??
Fecundity:	> 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 petridish. Procedure for *Aphidius colemani* (or *A. matricariae*) targeted at *Aphis gossypii*: each female wasp is offered 20 *A. gossypii* on cucumber leaves in a petridish. To be tested 4 times per year. Several methods will be studied and compared next year[2].
Flight activity:	as mummies can easily be damaged during the harvesting process, a flight test will have to be developed[3]

Comments:

1 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.

2 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 (30cm, 12 cmØ), 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 ± 7 days prior to the experiment. Wasps or plants are changed every day. Another test will be developed with leaf discs in petridishes (J. van Schelt). Cucumber leaf discs are put on an agar layer. 25 *A. gossypii* nymps are put on the leaf, 25 such petridishes 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.

3 S. Steinberg will develop a flight test for *Aphidius*

Coordinators: J van Schelt & S. Steinberg

***Aphidoletes aphidimyza* (Diptera: Cecidomyiidae)**

Test conditions:	Temperature: 22°C; RH: 80%; Light regime: 16L:8D. Weekly emergence test at 25°C.
Quantity:	number of adult insects as specified on the label; a weekly or batch-wise test
Emergence rate:	70% emergence within 7 days; a weekly or batch-wise test; n=500
Sex-ratio:	>= 45% females; a weekly or batch-wise test; n=500
Adult size:	relevant ??
Fecundity:	> 40 eggs/female within 4 days; n=25; a monthly test; Procedure: allow females to oviposit individually on *M. persicae* on sweetpepper leaves (for details see Van Schelt, Wageningen proceedings pp 90-95).

longer distance flight test will have to be developed

Comments:

Coordinators: *A.T. Gillespie & J. Douma*

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

Test conditions: Temperature: 25°C; RH: 70-90%; 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 females in the sample; n=20; a seasonal test.

Hatching rate: > 65% within 5 days; n=200; eggs must be isolated to prevent cannibalism after emergence; a weekly or batch-wise test.

Predator quality: > 65% of newly hatched larvae has to develop to 2nd 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 petridish (30 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.

When shipped in 2nd larval stage

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

Composition: >= 65% of 2nd instar larvae has to develop to 3*rd* instar larvae within 5 days; to be conducted once a year or when the rearing system is changed. Procedure: offer individual, freshly emerged 2*nd* instar larvae at least 100 prey items on a leaf on agar in a petridish (30 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.

Comments: a test to evaluate the searching capacity of 1st and 2nd instar larvae of *Chrysopa* should be developed.

Coordinators: M. Benuzzi & Californian producer

***Orius* spp. (Hemiptera: Anthocoridae)**

Test conditions: Temperature: 22°C; RH: 70+/-5%; Light regime: 16L:8D.

Quantity: The number of live adults as specified on the container. Counting method: 3 samples from each of 3 containers, assessed by weight, sample size 30 insects per container. A weekly or batch-wise test.
Adult/nymph ratio:to be specified by the producer on the label; a weekly or batchwise test done similarly with the quantity test; n=300

Sex-ratio: >= 45% females; a weekly or batch-wise test

Adult size: Will be studied in relation to fecundity

Fecundity: > 20 eggs/female during a 10-day oviposition period - start after pre-oviposition period of 5 days; n=30 pairs individually kept as described in annex 2. A monthly test. Tests for groups and individual insects will be compared during the coming year and discussed in Italy, Sept 1993.

Comments: - new test, will be adopted next year
- the species (composition) must been mentioned on the container

Coordinators: W.J. Dale & G. Manzaroli

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

Test conditions: Temperature: 22°C; RH: 70±5%; Light regime: 16L:8D.

Quantity[1]: When delivered in container: the number of live predators as specified on the container, excluding eggs. When delivered as slow release system: the number of live predators as specified on the label, excluding eggs when sold, and the number of predators released per week during a specified number of weeks. Set-up for testing quantity is given in annex 3. Three samples from different packages should be tested per batch. A weekly or batch-wise test.

Sex-ratio: -

Adult size: -

Fecundity: Follow the procedure as given in annex 4. Some fecundity tests will be done according to this method with different types of food (flour mites, *Ephestia* eggs)

Comments: [1] two types of counting methods will be compared: a dry and a wet method
- new test, will be adopted next year
- no test for other *Amblyseius* spp. were developed as they are applied on a very limited area; if tests can be based on this design

Coordinators: J. Douma + ????

***Aphelinus abdominalis* author? (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: >= 45% female, n= 250, batch-wise or weekly

Adult size: very variable, relationship size/longevity to be tested

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

Flight activity: not necessary ??

Comments:

Coordinators: C. Fleurynck & H. Haardt

Trichogramma brassicae (=T. maidis) and T. minutum

Test conditions	Temperature: 23 ± 2°C, RH : 70 ± 10 %, light regime: 16 L : 8 D
Rearing hosts:	Ephestia kuehniella, Sitotroga cerealella
Species identification:	the species specified on the label [1]
Parasitism:	number of black eggs of 100 host eggs of at least 5 release units (cards, capsules) or of 500 host eggs of bulk material [2], weekly or batch-wise test
Emergence rate:	〉 85 % (independent of storage techniques), check of 5 x 100 black eggs, [3] weekly or batch-wise test
Sex-ratio:	〉 50 % females, 100 adults assessed on 5 release units each or 5 x 100 adults of bulk material, weekly or batch-wise test [4]
Number of females:	% parasitism x % emergence x % females [5]
Fecundity and longevity [6]	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* are UV irradiated and provided at the 1st day. Fresh eggs of *S. cerealella* are provided at day 1 and 3. The total number of black eggs and the number of living adults is recorded after 7 days. [7] 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. One fresh egg-mass of natural hosts, *Ostrinia nubilalis* and *Choristoneura fumiferana* respectively, (〈 24 hrs old) are added for 24 hrs. [8] Honey and water are provided as described above. After separation of the egg-masses from the 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. The test should be performed twice to 4 times a year depending on the rearing system (number of generations on factitious hosts).

Coordinators: F. Bigler, G. Eden

Comments:

1) Identification of species for checks is a problem and needs help by taxonomists. In future, enzyme or DNA techniques might be used.

2) The total number of parasitized eggs of bulk material is calculated by weighing a small amount (e.g. 5 x 0.01 g), counting the number of black eggs from these samples and then calculating the total number.

3) % emergence is considered as a simple, rapid indicator of quality. The emergence periode and pattern depend on the mixture of developmental stages released together and should be specified on the label.

4) It is important to check only after the full emergence periode

5) The minimal quantity of females should be specified on the label. As an alternative to the calculation of the number of females by taking in account % parasitism, % emergence and sex-ratio, the number of adults could be counted at the end of their emergence and the sex-ratio be assessed on 5 x 100 adults

6) Standardized conditioning of adults (age, food, water, handling etc.) is crucial for minimizing variation (see procedures described by Cerutti and Bigler, Wageningen proceedings, 1991)

7) In the first draft of guideline (Wageningen proceedings 1991) we proposed a 3 days fecundity because of a well established correlation between 3 days and total fecundity (Pintureau et al. 1981). For practical purposes we propose now a 7 day fecundity (manipulation of test sets in weekly intervals). Comparisons of 3 and 7 days fecundity will be performed for *E. kuehnilla* by F. Bigler, G. Eden and F. Kabiri, for *S. cerealella* by B. Wührer and the data will be presented at the Rimini meeting in Sept. 93.

8) It was discussed whether the 24 hours parasitization periode should be reduced. Experiments will be carried out by F. Bigler, G. Eden and F. Kabiri with 0.5, 1, 2, 4, 8 and 24 hours periodes. The data will be presented at the Rimini meeting in Sept. 93

ANNEX

Annex 1: short-distance flight test for parasites and predators

Annex 2: fecundity and mortality test for *Orius* spp.

Annex 3: Determination of quantity of *Amblyseius*; wet method (J. Douma, Koppert Biosystems)

1. Empty the contents of a sachet of *Amblyseius* in a cylinder with cc indication and measure the contents in cc
2. Mix the contents thoroughly with a spoon
3. Put 4 cc. of the raw material in a cylinder and gently shake and tap the cylinder so that the material settles
4. Empty the contents into a set of two sieves. The upper sieve size is 0.315mm and the lower sieve size is 0.045 mm.
5. Run cold water through the sieves for a few minutes. The predatory mites, storage mites and fine particles pass through the upper sieve; the coarse material from the upper sieve can be discarded.
6. Trickle hot water over the lower sieve so that all mites are disabled but not completely destroyed.
7. Gently shake the sieve in a shallow dish with a little water and detergent, so that the contents are evenly distributed.
8. Place the sieve on top of a graph paper which is covered in plastic and has a specified area for counting. Count the predatory mites.
9. The total number of predatory mites in one sachet is:
   $$\frac{\text{total predatory mites per 4 cc}}{4} \times \text{the contents of the sachet in cc} =$$

Annex 4: Fecundity test for *Amblyseius*

Annex 5: Fecundity test for *Encarsia*: copy from Wageningen Proceedings p. 80-89

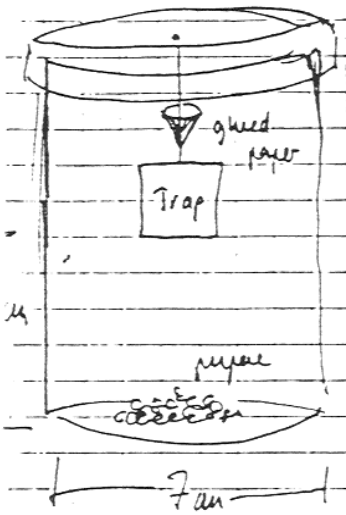
Annex 6: Fecundity test for *Aphidoletes*: copy from Wageningen Proceedings p. 90-95

Annex 1
Flight test par/pred.

Flight test

Containers : transparent (plastic) containers, 10 cm in height, 7 cm in diameter, covered with lids.

Trap : 2 × 2 cm yellow cardboard smeared with insecttrapping glue.



Suspended from the lid by string on which a glued piece of paper/plastic is placed to prevent adults walking to the trap.

The trap suspended so the bottom of the trap is 5 cm above the container floor.

Samples : Samples of pupae is placed on the container floor - e.g 100 pupae per container. 5-10 replicates.

Observationperiod - until no further emergence takes placed, i.e. 7-10 days

Note : The size of the cardboard may be to small to allow easy counting of the 70-90 adults expected to be caught after 7-10 days. Replacement of traps (or lids + traps) during

Annex 2

A method for quality control of Orius sp.

Fecundity
The number of eggs laid per female during the first 10 days after the beginning of the test.

Mortality
The rate of dead females 10 days after the beginning of the test.

From a bottle of Orius of which the adult rate is at least 50% take 30 males and 30 females. Place them pair by pair in a small bottle. In the bottle also put a piece of French bean, and some irradiated *Ephestia kuehniella* or *Sitotroga cerealella* eggs. Store the bottles in a climate room at 25°C, 80% RH and 16 L.
Each two or three days the fecundity has to be checked. The last control will take place 10 days after the start of the test.
At the control the piece of bean has to be taken away and replaced for a new, fresh one. The remaining *Ephestia* or *Sitotroga* eggs are removed, and fresh eggs are added.
The number of eggs on each piece of bean is counted.

$$\text{Fecundity} = \frac{\text{total number of eggs / female}}{30} > 20$$

$$\text{Mortality} = \frac{\text{number of dead females on day 10}}{30} * 100\% < 20\%$$

Frequency:
Monthly or seasonally

Notes:
- The quality of the bean is very important. On old and dry beans the number of eggs is lower then on fresh beans
- The moment of control is also important: As the egglaying mainly takes place in the evening, changing of the beans has to be done in the morning to avoid disturbance.
- As the pre oviposition period is 3 - 5 days at 25°C, test period must be at least 14 days.
- Standard deviation of the fecundity is almost as big as the average, so the number of 30 females is the absolute minimum.

Koppert B.V., Johannette Klapwijk, november 1992

Annex 3: Determination of quantity of *Amblyseius*: wet method (J. Douma, Koppert Biosystems)

1. Empty the contents of a sachet of *Amblyseius* in a cylinder with cc indication and measure the contents in cc
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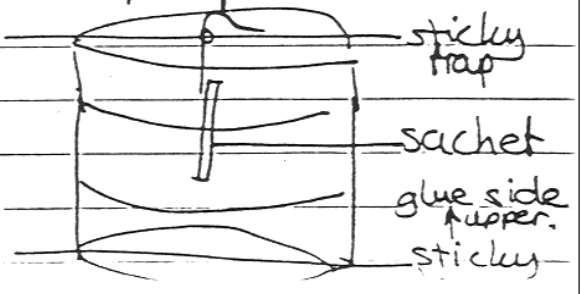
6. Trickle hot water over the lower sieve so that all mites are disabled but not completely destroyed.
7. Gently shake the sieve in a shallow dish with a little water and detergent, so that the contents are evenly distributed.
8. Place the sieve on top of a graph paper which is covered in plastic and has a specified area for counting. Count the predatory mites.
9. The total number of predatory mites in one sachet is:
   $\frac{\text{total predatory mites per 4 cc}}{4} \times \text{the contents of the sachet in cc} =$

Annex 4: Fecundity test for *Amblyseius* (will be added later)

Still
Dry method: test to be provided by J. Dale

Quantity: bottle – the number of live predators excluding eggs.
SRS – the number of live predators excluding eggs when sold
– the number of predators released in a specified number of weeks.



Annex 4

A method to measure the fecundity of *Amblyseius cucumeris*

Overmeer et al. (1982) described a method where he used a 8x15 cm tile of plastic, 5 mm thick, which is easy to examine under the dissection microscope, and a thick piece of foam plastic (3 cm) saturated with water (see Fig.). The surface of the foam plastic is equal to that of the tile. In the latter set-up 4 short strips of tissue paper, 7 cm wide, are stretched along the 4 edges of the tile, and folded over the edges in such a way that about 1.5 cm of the periphery of the tile is covered by the tissue paper and the rest of the strips hang down in the water in a tray so that the tissue paper remains wet. On the tissue paper, just above the edges of the tile, an extra barrier is added which consists of a rectangle of sticky material. This can be done with the aid of a syringe. In case a mite leaves the substrate, it can walk on the wet tissue paper barrier until it touches the sticky material. The wet tissue paper diminishes the speed of the mites considerably, and when a mite touches the rectangle of sticky material it will turn round and move back to the plastic substrate to find food and shelter.
Food is added to the arena.
It is also necessary to provide the mites some shelter. Small roof-shaped pieces of thin transparent plastic cut from sheets for overhead projection. The mites move to the undersurface of the shelter and hide there in a upside down position. Eggs are laid on or near these resting sites.

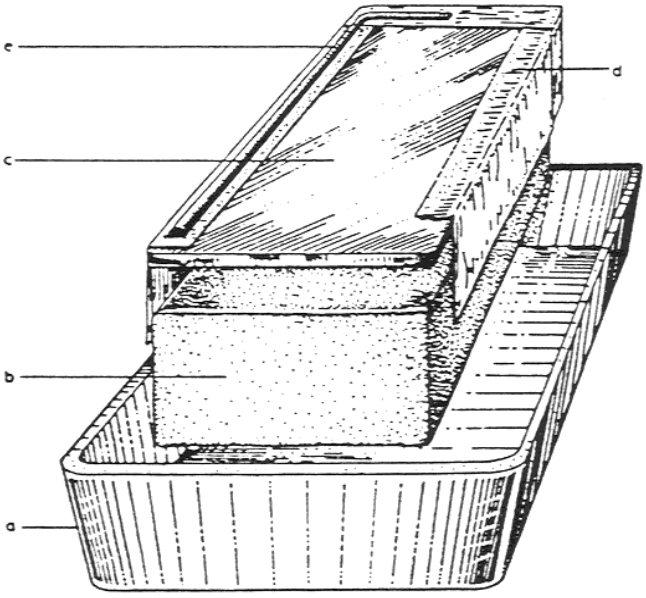


Fig. 2.1.4.1.1. Rearing arena. (a) Plastic tray with water, (b) foam plastic, (c) plastic tile, (d) tissue paper, (e) sticky material.

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Overmeer, W.P.J. 1985. Rearing and Handling. In: W. Helle & M.W. Sabelis (Eds.), Spider Mites. Their Biology, Natural Enemies and Control, Vol. 1B. Elsevier, Amsterdam: 161-170.

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