IOBC Quality Control Guidelines
for natural enemies

Updated July 2002

***Amblyseius degenerans* Berlese (Acarina: Phytoseiidae)**

Test conditions	Temperature:	22±1°C
	RH:	70±5%
	Light regime:	16L:8D

Quality control criteria

Quantity	The number of mites as specified on the label, excluding eggs
Sex-ratio	≥ 50% females; n=100; an annual test
Fecundity	≥ 7 eggs/female over a period of 7 days; n= 30; an annual test

Description of testing methods

Quantity	Mix the contents of the package thoroughly by tumbling the product container. If the product container does not allow proper mixing use an alternative container. Weigh the contents of the package and take 4 random samples of each 3 g. Spread each sample on a white paper and count the mites by gently stirring the material (vermiculite). First, count the live predators running away; the counted mites will be killed. Next, sieve the material, rinsing it with cold water. The upper sieve opening size is 440 µm, the lower is smaller (90 µm) so that the mites will be collected in it. Count the remaining mites (only alive ones, check if they still move there legs) and add this number to the total number of mites counted earlier. Then calculate the total number of mites: (number of mites in 4 × 3 g/12) × the weight of the contents of the container. A "dry" method similar to the *Phytoseiulus persimilis* procedure can also be used; it has the advantage that one can distinguish between living and dead mites.
Sex-ratio	Identification of sex is done by mounting individuals on microscopic slides. After mounting, gently push the body contents out of the body and check the ventral side with a magnification of 100×. Adding a droplet of a lactic acid solution will facilitate the procedure. Distinguish between females and males by checking the ventral and genital shields (figure 3; source: Owen Evans G., 1953. The genus *Iphiseius* Berl. (Acarina-Laelaptidae). – *Proceedings of the Zoological Society of London* 124: 517–526).
Fecundity	See testing method for *Amblyseius cucumeris*. An ample amount of freshly hand-collected sweet pepper pollen is used as food, however. The average number of eggs per female should be ≥ 7. Do not include the eggs laid during the first day of the experiment.

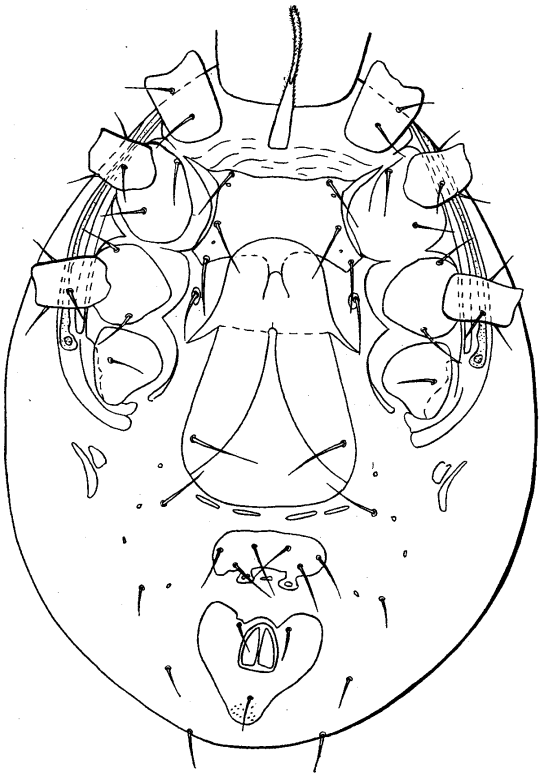


Fig. 2.—*Iphiseius degenerans* (Berl.). Venter of female.

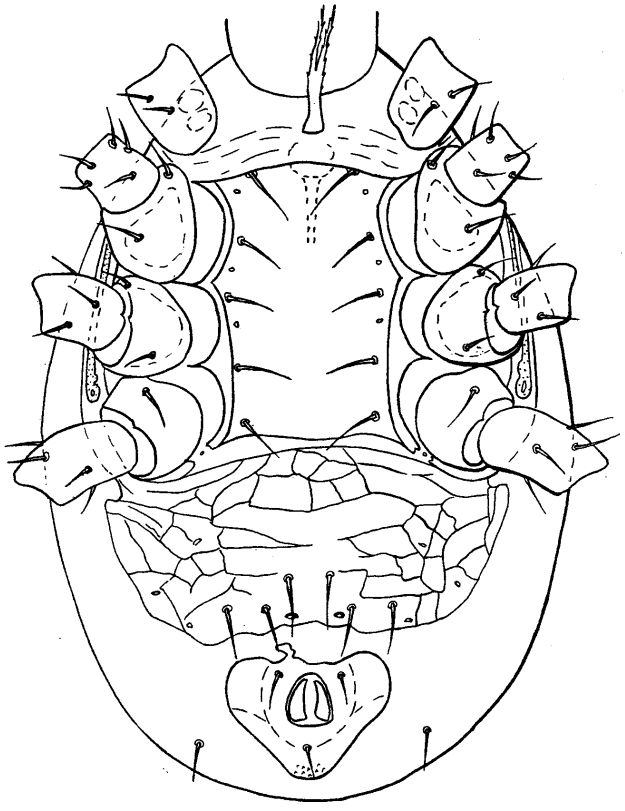


Fig. 9.—*Iphiseius degenerans* (Berl.). Venter of the male.

The female (left) and male (right) genital shields (after Evans, 1953).

Coordinator: J. Vermeulen

***Aphidius colemani* Viereck (Hymenoptera: Braconidae)**

Test conditions Temperature: 25 ± 2°C
RH: 75 ± 5%
Light regime: 16L:8D

Quality control criteria for mummies

Quantity and emergence: ≥ The number of live adults that should emerge from the package. as specified by the manufacturer. A minimum of three containers should be counted. Emergence rate ≥ 45 % (n=500). A weekly or batch-wise test.

Sex ratio: ≥ 45% females, a seasonal test, n=150

Fecundity: ≥ 60 mummies/female in the first day when tested on *Aphis gossypii*; n=30, an annual test.
≥ 35 mummies/female in the first day when tested on *Myzus persicae*; n=30, an annual test.

Description of testing methods

Quantity and emergence: Specify the number that should emerge from the mummies. Put the mummies with the carrier material in a container (height 15 cm, diameter 9 cm) with a cork in the bottom. The lid should have one or more holes with gauze for ventilation. Put some droplets of honey on the outer side of the gauze. By removing the cork, mummies and carrier material can be transferred to a new container every day. The container with emerged adults can be frozen and subsequently counted. Continue until no more wasps emerge. Run the test for a maximum of 7 days.
An alternative method for collecting the emerged adults: put the mummies with the carrier material in a ventilated container (15 cm height, 9 cm diameter) with a lid at its bottom. An inverted funnel is glued to the upper part of the container. A glass collecting tube is fitted, by mean of a cork, to the "neck" of the funnel. A standard light source, e.g. fluorescent tube, is placed c. 20 cm above the collecting apparatus. The whole system, except for the collecting tube, is covered by a dark cloth to force the emerging wasps towards the collecting tube. Change every day the tube in 7 days, count the total number of adult parasitoids caught in the tube. Add to this the number of wasps which remained at the bottom of the apparatus.
For calculating the emergence rate, count the total number of mummies in the container and figure the percent emergence according to the formula: (no. of adult wasps/ no. of mummies) x 100.

Sex ratio: Mix all the adult wasps from the emergence test. Take a sample of 100 adults and count the number of female wasps. Females are distinguished from males by their pointed abdomen (ovipositor). The length of the female abdomen is almost equal to wing length. The male abdomen is more rounded at the end and is always shorter than the wings. The females should amount to more than 45% of the total.

Fecundity: This test can either be done with *leaf discs on agar* or on *whole plants*.

Leaf discs on agar

Day 1: Preparing the bio-assay. The bio-assay tray consists of a round plastic petri-dish tray with a lid which can be closed tightly (∅ 77 mm; height

31mm; Bock, Art.Nr.41113). A piece of gauze is incorporated into the lid for ventilation. Pour 1 cm of water agar (1%) into the tray and cool to 30 °C. Just before it solidifies, a cucumber leaf disc (when tested on *Aphis gossypii*) or a sweet pepper leaf disc (when tested on *Myzus persicae*) is put upside down on the agar. It is very important to use a fresh leaf with maximal turgor, otherwise the life span of the leaf will be too short for the test period (11 days). It is best to pick leaves early in the morning. Put 30 adult *A. gossypii* or 30 *Myzus persicae* onto the leaf using a fine brush. Place the petri-dishes upside down, on a ventilated tray, to simulate a more natural situation for the aphids and to prevent the leaf from becoming sticky with honeydew. Remove the adult aphids after one day. By doing this, between 100 and 150 young aphids (first and second nymphal stage) per tray can be used for testing. Prepare 30 trays.
Put an ample amount of *Aphidius sp.* mummies that are close to emergence in a container. Put some droplets of honey in the container or on the gauze. Put the container in a climate room (25°C).

Day 2 — Remove the adult aphids from the dishes with a moist brush. Check the number of offspring (>100 / tray).
Place the container with emerged wasps in a cold room (8-12 °C) for 5 minutes. Tap the wasps from the container onto a smooth white surface. Place small vials over the wasps and after they have walked in, close the vial. Select 30 females by checking them under a stereoscopic microscope. Tap the vial to release individual females onto the petri-dishes in the cold room. Place the dishes upside down at 25°C for 24 hours.

Day 3 — Remove the wasps from the dishes after 24 hours.

Day 4-10 — Check the quality of the leaves. If the quality is poor remove the aphids to a new petri-dish with a fresh leaf.

Day 11 — Count the number of mummies per dish.

Comment: It is possible that *Myzus persicae* needs two days to produce enough off-spring. Adjust the scheme accordingly.

Whole plants

Day 1 — Use potted cucumber plants (30x) pruned to bear two/three leaves, or small sweet pepper plants. Place a plastic ventilated cylinder over the plant. Use a layer of vermiculite to seal the cylinder at the underside. Put 30 adult *A. gossypii* or 30 *Myzus persicae* onto each plant using a fine brush.

Day 2-12 — Follow the same protocol as described above for the system with leaf discs on agar.

Comment: It is possible that *Myzus persicae* needs two days to produce enough off-spring. Adjust the scheme accordingly.

Coordinators: J. van Schelt & S. Steinberg

Aphidius ervi **(Haliday) (Hymenoptera: Braconidae)**

Test conditions	Temperature:	22 ±2°C
	RH:	75 ± 5%
	Light regime:	16L:8D

Quality control criteria for adults

Quantity	≥ The number of live adults as specified on the container.
Adult mortality	≤ 8 % of the number of adults present in the container, based on 3 containers sampled and n = 500 or more; a weekly or batch-wise test.

Quality control criteria for mummies

Quantity and	≥ The number of live adults that should emerge from the package as specified by the manufacturer.
Emergence	Emergence rate ≥ 75 % (n=250). A weekly or batch-wise test.
Sex ratio	≥ 45% females, a seasonal test, n=150
Fecundity	≥ 35 mummies/female in 2 hrs when tested on *Macrosiphum euphorbiae*, an annual test

Description of testing methods

Quantity adults — Count the number of dead wasps. Put the containers in the freezer for a minimum of 2 hours. Count the number of wasps.

Quantity and emergence mummies

Specify the number that should emerge from the mummies. Put the mummies with the carrier material in a container (height 15 cm, diameter 9 cm) with a cork in the bottom. The lid should have one or more holes with gauze for ventilation. Put some droplets of honey on the outer side of the gauze. By removing the cork, mummies and carrier material can be transferred to a new container every day. The container with emerged adults can be frozen and subsequently counted. Continue until no more wasps emerge. Run the test for a maximum of 8 days.

An alternative method for collecting the emerged adults: put the mummies with the carrier material in a ventilated container (15 cm height, 9 cm diameter) with a lid at its bottom. An inverted funnel is glued to the upper part of the container. A glass collecting tube is fitted, by mean of a cork, to the "neck" of the funnel. A standard light source, e.g. fluorescent tube, is placed c. 20 cm above the collecting apparatus. The whole system, except for the collecting tube, is covered by a dark cloth to force the emerging wasps towards the collecting tube. Change the tube every day. Within 7 days, count the total number of adult parasitoids caught in the tube. Add to this the number of wasps which remained at the bottom of the apparatus.

For calculating the emergence rate, count the total number of mummies in the container and figure the percent emergence according to the formula: (no. of adult wasps/ no. of mummies) x 100.

Sex ratio — Mix all the adult wasps from the emergence test. Take a sample of 100 adults and count the number of female wasps. Females are distinguished from males by their pointed abdomen (ovipositor). The length of the

female abdomen is almost equal to wing length. The male abdomen is more rounded at the end and is always shorter than the wings. The females should amount to more than 45% of the total.

Fecundity test — This test is done with leaf discs on agar.

Day 1 — Preparing the bio-assay. The bio-assay tray consists of a round plastic petri-dish tray with a lid which can be closed tightly (∅ 77 mm; height 31mm; Bock, Art.Nr.41113). A piece of gauze is incorporated into the lid for ventilation. Pour 1 cm of water agar (1%) is poured into the tray and cooled to 30°C. Just before it solidifies, an aubergine leaf disc is put upside down on the agar. It is very important to use a fresh leaf with maximal turgor, otherwise the life span of the leaf will be too short for the test period (11 days). It is best to pick leaves early in the morning. Put 10 adult *Macrosiphum euphorbiae* onto the leaf using a fine brush. Place the petri-dishes upside down, on a ventilated tray, to simulate a more natural situation for the aphids and to prevent the leaf from becoming sticky with honeydew. Remove the adult aphids after one day. By doing this, between 30 and 40 young aphids (first and second nymphal stages) per tray can be used for testing. Prepare 60 trays.

Put an ample amount of *Aphidius ervi* mummies that are close to emergence in a container. Put some droplets of honey in the container or on the gauze. Put the container in a climate room (22°C).

Day 2 — Remove the adult aphids from the dishes with a moist brush. Check the number of offspring (>30 / tray).

Place the container with emerged wasps in a cold room (8-12°C) for 5 minutes. Tap the wasps from the container onto a smooth white surface. Place small vials over the wasps and after they have walked in, close the vial. Select 30 females by checking them under a stereoscopic microscope. Tap the vial to release individual females onto the petri-dishes in the cold room. Place the dishes upside down at 22°C for 30 minutes.

Bring the dishes to the cold room. Tap the wasps in a new petridish after 5 minutes. Place the dishes upside down at 22°C for 90 minutes. Remove the wasps from the dishes.

Day 3 -10 — Check the quality of the leaves. If the quality is poor remove the aphids to a new petri-dish with a fresh leaf.

Day 11 — Count the number of mummies per dish.

Coordinators: J.v. Schelt & J. Vermeulen

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

Test conditions Temperature: 22±2°C;
RH: 75±5%
Light regime: 16L:8D

Quality control criteria

Quantity	number of adult insects as specified on the label; a weekly test
Emergence rate	> 70 % emergence within 7 days; a weekly test; n=150
Sex ratio	≥ 45% females; a weekly test; n=150
Fecundity	> 40 eggs / female within 3 days; n=25; annual test.
Flight activity	Simple test, see below; annual test

Description of testing methods

Quantity

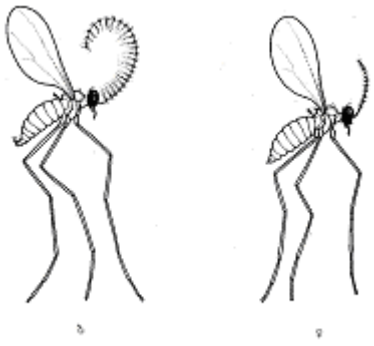
This test is made for a standard product of 1000 mummies per 0.1 litre of vermiculite (22 gram).

Estimating the number of *Aphidoletes aphidimyza* pupae per bottle: Weigh the content of the bottle. Mix carefully and take 3 samples of 1 gram from the material. Count the number of pupae per sample. Beware that pupae may be lumped together. Calculate the total number of pupae per bottle.

Number of midges and emergence rate: Put half of the original material back in the bottle. Put the bottle with open cap in a bucket. Put white paper on the bottom of the bucket. Close the bucket carefully and place it at 22°C . After 6-7 days most midges will emerge. Place the bucket in a freezer for at least 4 hours. After this period the midges can be counted. Multiply the count by 2. Dissect 150 pupae to assess percent emergence.

Sex ratio

Take at random 150 midges from the emergence test and sex them. Males have long hairy antennae, females bear short antennae without hairs. See figure.



Adult female and male of *Aphidoletes aphidimyza*

Fecundity	Estimate the number of days required till emergence of the pupae. This can be done by dissecting the pupae and checking the development of eyes, legs and wings: - no legs, no eyes 7 days - legs, white eyes 4-5 days - reticulation of the eyes clearly visible 2-3 days - wing formation: dark coloured 1 day Place an open bottle in a large cage (>40x40x40 cm). The midges can easily crawl through a layer of 12 centimetres of vermiculite. To ensure proper mating bring in some cob-webs from spiders. Cob webs can be sampled with a metal ring (diameter 20 cm). The ring should be placed horizontally in the corner at the rear end of the cage. The midges will emerge during dawn and night. Mating will take place during the first night after emergence. Put a plant with aphids in the cage (e.g. wheat with *Rhopalosiphum padi*). This will serve as a source of carbohydrates and will stimulate egg production.
Day 0	On the day you expect the emergence of the pupae, prepare 25 trays with *Aphis gossypii* (around 100) on cucumber (see testing method *Aphidius colemani*, day 1). Sweet pepper with *Myzus* sp. can also be used. Aphids may be of variable age. Midges will emerge in the large cage in the evening and night.
Day 1 (morning)	Place the cage in a cold room (5-10°C) for 10 minutes. Female midges (determine visually) can be gently tapped into the trays (25x). Place the trays upside down in the climate room. To ensure proper ventilation, place the trays on a piece of gauze.
Day 4	Count the number of eggs in the trays. Eggs are oval and orange-coloured. Inspect also the sides of the tray and the lid. Some eggs may be hatched; small larvae can hide under the aphids. Calculate the number of eggs per female. If many zero's (>5) are found and midges are still alive at the end of the test something has gone wrong in the mating and the test should be repeated.
Flight test	Put 250 pupae in a tray. Place the tray in cylinder with a diameter of approximately 25 cm. Make a small ring of grease (any kind of grease will do, as long as it doesn't melt) at 5 cm. from the bottom. Put the cylinder in a large cage or leave it opened in the climate room. When the majority of the midges have emerged count the number on the bottom of the cylinder (non-fliers) and count the number of white skins in the tray (= total emerged). Calculate the percentage of fliers.

Coordinator: J. van Schelt & B. Spencer

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

Test conditions	Temperature:	22±2°C
	RH:	60±5%
	Light regime:	16L:8D

Quality control criteria

Quantity	≥ number of live adults specified on the label; a weekly or batch-wise test.
Adult mortality	≤ 5% of the number of adults present in the container, based on 3 containers sampled and n = 500 or more; a weekly or batch-wise test.
Sex ratio	≥ 45% females ; n = 100, conducted 4 times per year.
Fecundity	≥ 45 offspring per female within 3 days ; n = 15, an annual test. Daily oviposition of single female 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 *Chromatomyia syngenesiae*).

Description of testing methods

Fecundity test: *Dacnusa sibirica* using *Chromatomya* as host.

Plant	*Sonchus oleraceus* grown from seed for ca. 8 weeks.
Host leafminer	*Chromatomyia syngenesiae*, a minimum of 100 2nd instar larvae per plant (ca. 8 days after eggs laid).
Apparatus	60cm perspex cube cages.
Day 0	Place a single female *Dacnusa* into a cage containing 3 plants infested with leafminer on 3 consecutive days and leave for 72 hours.
Day 3	Remove the females from the cages. Leave the plants in situ until the leafminer larvae have pupated (ca.5 days later).
Day 8+	Cut plants off, save leaf and stem tissue containing pupae and discard the rest. Store pupae in a ventilated plastic box (ca.30 x 30 x 15 cm is ideal), lined with several layers of absorbent paper, at RH of 80% and 22°C for 21 days.
Day 29	Freeze the box for 24 hours and count *Dacnusa* adults.

Fecundity test *Dacnusa sibirica* using *Lyriomyza* as host.

Plant	*Phaseolus vulgaris* grown from seed 2 weeks before starting the test.
Host leafminer	*Lyriomyza bryoniae.*
Preparations	Two weeks before starting the test sow ca. 300 *Phaseolus vulgaris* seeds in small pots with soil. Ensure that the plants can grow under optimum conditions (sufficient light and nitrogen, no Sciarid flies) as strong plants are required. After 2 weeks remove the tops of the plants leaving only the spade leaves. About 500 one day old female *Lyriomyza bryoniae* are then released on to the plants (the leafminers are provided with honey from the time they emerge from the pupae). Keep the plants in a greenhouse at about 22-25°C until the first larvae have reached 2nd instar (ca. 7 days).
Day 0	Count the approximate number of larvae on the infested plants. Place as many plants in a cage as is necessary to provide about 150 larvae per

	parasite. Do not use plants with more than 30 larvae per leaf. Collect 15 female wasps from a bottle. Put one female in each cage.
Day 3	Put / fix a sleeve or ventilated box around the leaves to collect the larvae which will pupate within about 5 days at 22°C. The RH must be 80%.
Days 5-8	Check that all the larvae have pupated. Before they hatch, collect all the pupae in a petri dish and store them at 22°C and 80% RH.
Day 20 $\pm$	Check that all the pupae have hatched and count the number of adults emerged after killing them in a freezer. Calculate the number of offspring produced per female during the three day test.

Coordinators: P. Smytheman, J. Dale & R. Greatrex

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

Test conditions	Temperature:	25±2°C
	RH:	70±10%
	Light regime:	16L:8D
	Light intensity:	< 300 lx, direct lighting to be prevented, it affects the activity of females.

Quality control criteria

Quantity	≥ number of live adults specified on the label; a weekly or batch-wise test.
Adult mortality	<= 8% of the number of adults present in the container, based on 3 containers sampled and n= 500 or more; a weekly or batch-wise test.
Sex-ratio	females >= 45% of live adults; n=100; a batch-wise test.
Fecundity	70% of females laying eggs within one week in a Petri dish test; n=30; an annual test.

Description of testing methods

Fecundity

Isolate individual females, chosen at random from the container, in Petri dishes of ca. 12 cm diameter. Use parafilm to seal the dish. For each replicate, staple a brown bean leaf (*Phaseolus vulgaris* L.) infested by ca.

10 L_2-L_3 larvae of *Liriomyza trifolii* or *L. bryoniae* to a slightly moistened filter paper and place it in a dish. Add some honey droplets to the lid of the dish. Alternatively, *Sonchus oleraceus* L. leaves infested by *Chromatomyia syngenesiae* Hardy can be used.

Replace the leaf daily for up to 7 days and check for presence of parasitoid eggs in the mines. Leaves can be stored prior to dissection for up to 24 h at 4-8°C. For each female the test is completed once the first parasitoid egg is found. At the end of 7 days, calculate the percentage of females which have laid eggs. Include the total females which died naturally. The number of females accidentally killed or lost should be omitted from the calculations, but must be recorded.

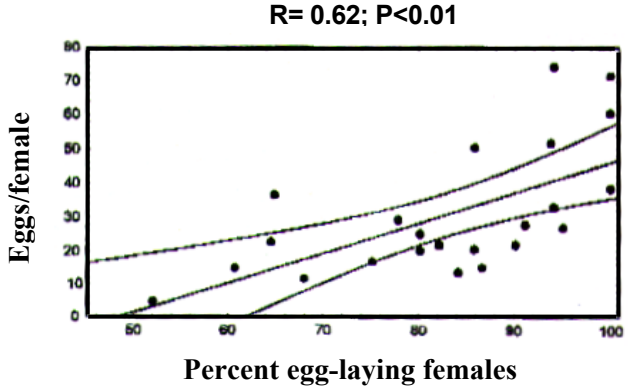


Fig. 1: Prediction of fecundity. The number of eggs laid per female in 7 days can be predicted from the relationship between this value and the percentage of egg-laying females (each replicate: n=30 females). The fecundity data were obtained using the testing method described above (G. Nicoli, unpublished data).

Coordinators: S. Steinberg, P. Smytheman & G. Burgio

***Dicyphus hesperus* Wagner (Hemiptera: Miridae)**

Test conditions	Temperature:	22°C±2°C
	RH:	75±10%
	Light regime:	16L:8D

Quality control criteria

Quantity	≥ the number of live adults and nymphs as specified on the label; weekly test
Mortality	<= 5 % of the number of live adults and nymphs as specified on the label; weekly test
Sex-ratio	≥ 45 % females; n=100; seasonal test
Fecundity	≥ 7 eggs/female/72 hours; n=30; annual test

Description of testing methods

Quantity	Place packages in the freezer for at least one hour. Count the insects.
Mortality	Count the dead insects left in the packages after live insects have been allowed to move to another container.
Sex-ratio	Take a sample of 500 insects found in the quantity test and determine sex-ratio.
Fecundity	Collect 30 females 7-10 days after their final moult from the mass rearing. Place them individually on a tobacco leaf disc (5 cm diameter) with a midrib in the middle, placed upside down on a 4mm layer of agar (1%). The container has to be ventilated with at least 2 cm between agar and the lid. Feed them *Ephestia kuehniella* eggs ad libitum. Remove the insects after 72 hours and examine leaf discs for predator eggs under a stereo microscope. Eggs will be embedded in leaf midrib and veins. The average number of eggs laid should be ≥ 7 eggs/female/72 hours.

Coordinator: J. Klapwijk and K. Jans

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

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

Quality control criteria

Emergence rate	≥ the number of adults specified on the label which will emerge over a two-week period; n=1000; a weekly or batch-wise test
Sex-ratio	≥ 98% females; n=500; an annual test
Fecundity	≥ 7 eggs/female/day for days 2, 3 and 4 after emergence of the adult; n=30 females; an annual test
Flight test	a design of a flight test for *E. formosa* is given at the end of this report

Description of testing methods

Emergence	Specify the number of adults that should emerge before conducting the test. Take 3 sub samples which make up 1,000 or more full black pupae in total. Put the samples in a closed container for two weeks and then determine the number of emerged adults. This can be done by counting the number of emerged adult parasites or by comparing the number of empty pupae at the start and at the end of the test. A combination of both counting methods will give the most reliable results. The quantity of emerged adults should achieve the number specified on the label.
Sex-ratio	Take a sample of 500 of the adults from the emergence test and count the number of male wasps. These are completely black and easily distinguished from the females which have a yellow abdomen. The number of females should be ≥ 98%.
Fecundity	
Day 1	Put an ample amount of black pupae which are close to emergence in a container. Remove all adult parasites at the night before the day on which the test animals will be collected from the container.
Day 2	Collect 30 freshly emerged females at about 10 o'clock; put each into a small container with a droplet of honey until the following day. This is to feed them and to get them through the pre-oviposition period.
Day 3	The test is conducted on individual females in small round plastic "petri dish type" trays (min. ϕ 35 mm; height 15 mm) which can be closed very tightly. A nylon mesh is incorporated into the lid to facilitate air exchange. Trays are filled with agar solution (1%) to a depth of 10 mm. Just before the agar solidifies a tobacco leaf disc is placed with its upper surface in contact with the agar. The leaf disc should contain at least 25 whitefly larvae (*Trialeurodes vaporariorum*) in the 3rd and 4th instar. To insure an optimum quality of the leaf disc pick leaves, prepare trays early in the morning and use leaves with a density of max. 3 larvae/ cm² . Prepare 30 trays in total and release 1 female per tray.
Day 4	Provide the female with a new supply of whitefly larvae by placing her in a new tray. Do this around 10 0'clock in the morning, again.
Day 5	Repeat day 4.

Day 6	Remove the parasites from the whitefly larvae. Keep all whitefly that were exposed to *E. formosa* in closed containers to prevent unwanted parasitism after the test. Count all black pupae after 14 days. The average number of black pupae per female per day should be ≥7. This test should be performed in the period August to October.

Coördinators: J.Klapwijk, P.Smytheman and A.Luczynski

***Eretmocerus eremicus (Rose)* (Hymenoptera: Aphelinidae)**

Test conditions	Temperature:	22°C±2°C
	RH:	75 +/- 10 %
	Light regime:	16L:8D

Quality control criteria

Emergence	≥ the number of adults as specified on the label which will emerge over a two-week period; n=1000; a weekly or batch-wise test
Sex-ratio	≥ 45% females; n=500; an annual test
Fecundity	≥ 45 eggs/female/72 hours for day 2 - 4 after emergence of the adult; n=30 females; an annual test

Description of testing methods

Emergence

Take at least 3 sub samples which make up 1,000 or more yellow pupae in total. Put the samples in a closed, ventilated container for two weeks and then determine the number of emerged adults. This can be done by comparing the number of empty pupae at the start and at the end of the test. The quantity of emerged adults should achieve the number specified on the label.

Sex-ratio

Take a sample of 500 of the adults from the emergence test and count the number of female wasps. These can be distinguished from the males by the shape of the antennae (see fig.) and the brighter yellow color. The number of females should be ≥ 45%.

Antennae of *Eretmocerus eremicus* male (left) and female (right)

Fecundity

Day 1

Put an ample amount of yellow pupae which are close to emergence in a container. Remove all adult parasites at the night before the day on which the test animals will be collected from the container.

Day 2

Collect 30 freshly emerged females and males in the morning; put them together ina container with a droplet of honey until the following day. This is to allow them to mate, to feed them and to get them through the pre-oviposition period.

Day 3

The test is conducted on individual females in small round plastic "petri dish type" trays (min. ϕ 50 mm; height 15 mm) which can be closed very tightly. A nylon mesh is incorporated into the lid to facilitate air exchange. Trays are filled with agar solution (1%) to a depth of 10 mm. Just before the agar solidifies a tobacco leaf disc is placed with its upper surface in contact with the agar.

The leaf disc should contain at least 60 whitefly larvae (*Trialeurodes vaporariorum*) in the 2nd and 3rd instar. To insure an optimum quality of the leaf disc pick leaves early in the morning and prepare trays

	immediately. Use leaves with a density of max. 3 larvae/cm². Prepare 30 trays in total and release 1 *Eretmocerus eremicus* female per tray.
Day 4	Provide the female with a new supply of whitefly larvae by placing her in a new tray. Do this around the same time in the morning.
Day 5	Repeat day 4.
Day 6	Remove the parasites from the whitefly larvae. Keep all whitefly that were exposed to *E. eremicus* in closed containers to prevent unwanted parasitism after the test. Remove adult whitefly emerging from unparasitised pupae to keep the leaf disc in optimum condition.
Day 20	Count all yellow pupae.
	The average number of yellow pupae per female per day should be ≥15

Coördinators: J.N. Klapwijk and Pete Smytheman

***Leptomastix dactylopii* Howard (Hymenoptera: Encyrtidae)**

Test conditions	Temperature:	25°C±2°C
	RH:	70±5%
	Light regime:	16L:8D

Quality control criteria

Quantity	≥ the number of live adults as specified on the label; a weekly or batch-wise test
Adult mortality	≤ 10%, based on 3 containers sampled and n=500 or more; a weekly or batch-wise test
Sex-ratio	≥ 45% of the number specified on the label should be females; the sex ratio does not necessarily have to be 45% as long as there are enough females in the container; a four-weekly test
Fecundity	≥ 40 offspring/female/14 days; n=30 females; an annual test

Description of testing methods

Fecundity	Place a single potato tuber with short sprouts and infested by an ample amount of L3 females of citrus mealybug, *Planococcus citri*, in a ventilated container. Introduce a single pair of *Leptomastix dactylopii* into the container. Leave the system as it is for 14 days. By the end of the 14 days, take out the pair of wasps from each container. Collect emerging adults of *L. dactylopii* from the 21st day up to the 31st day from the beginning of the experiment. Calculate the cumulative number of adults emerging during this period.

Coordinators: M. Mosti and M. Kole

Macrolophus caliginosus **Wagner (Hemiptera: Miridae)**

Test conditions	Temperature:	22°C±2°C
	RH:	75±10%
	Light regime:	16L:8D

Quality control criteria

Quantity	≥ the number of live adults and nymphs as specified on the label; weekly test
Mortality	<= 5 % of the number of live adults and nymphs as specified on the label; weekly test
Sex-ratio	≥ 45 % females; n=100; seasonal test
Fecundity	≥ 7 eggs/female/72 hours; n=30; annual test

Description of testing methods

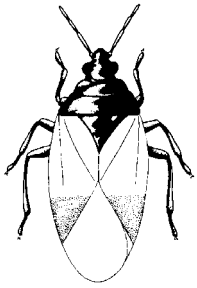
Quantity	Place packages in the freezer for at least one hour. Count the insects.
Mortality	Count the dead insects left in the packages after live insects have been allowed to move to another container.
Sex-ratio	Take a sample of 500 insects found in the quantity test and determine sex-ratio.
Fecundity	Collect 30 females 7-10 days after their final moult from the mass rearing. Place them individually on a tobacco leaf disc (5 cm diameter) with a midrib in the middle, placed upside down on a 4mm layer of agar (1%). The container has to be ventilated with at least 2 cm between agar and the lid. Feed them *Ephestia kuehniella* eggs ad libitum. Remove the insects after 72 hours and examine leafdiscs for predator eggs under a stereo microscope. Eggs will be embedded in leaf midrib and veins. The average number of eggs laid should be ≥ 7 eggs/female/72 hours

Coordinator: J. Klapwijk and K. Jans

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

Test conditions

Temperature:	22-25°C
RH:	70±5%
Light regime:	16L:8D



Quality control criteria

Quantity	The number of live adults/nymphs as specified on the container. Species name(s) to be indicated on the label. A weekly test.
Sex-ratio	≥ 45% females; n=100 (picked at random; to distinguish between male and female, see figure); a seasonal test
Fecundity	≥ 30 eggs/female/14 days; n=30 pairs; an annual test

Description of testing methods

Counting method

After a short period (ca. 20 min.) at 8°C, the material in the container is sieved to separate vermiculite, buckwheat husk and insects; thereafter all the individuals can be counted using an aspirator.

Fecundity

Take about 100 *Orius* adults from a bottle ready for shipment or from a rearing cage with adults which emerged less than 24 hours ago. Maintain these individuals for 2-3 days in a cage feeding them with *Ephestia kuehniella* eggs and supplying a French bean pod. Then determine their sex under stereomicroscope (see figure). Subsequently, put a pair of predators in a ventilated transparent container of ca. 75 ml (3-4 cm of diameter), which contains a piece of French bean pod. The bean pod has to be cut between two seeds, to prevent egg-laying on the inside of the bean pod. Provide new *E. kuehniella* eggs *ad libitum*.

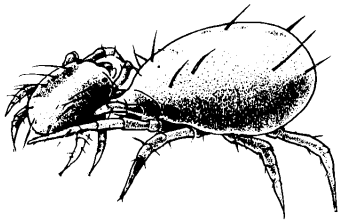
Every 2-3 days the bean pod is substituted with a fresh one; eggs are counted and new prey is added.

The total number of eggs is calculated for a 14 days oviposition period. Exclude data from females which have died accidentally or got lost during the test period.

Figure: Abdomen of *Orius*. Males have a curled, swollen abdomen (A), females have an elongated ovipositor (B).

Coordinators: J. Vermeulen & M. Mosti

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



Test conditions

Temperature: 22 - 25°C
RH: 70±5%
Light regime: 16L:8D.

Quality control criteria

Quantity	Average number of live predators as specified on the container; a weekly or batch-wise test; n= 3 containers.
Sex-ratio	≥ 70% females; once a year; n= 100; for identification of sex, mount the individuals on microscopic slides.
Longevity	Minimum 5 days, reached by at least 80% of the females examined in the fecundity test; n= 30; a seasonal test.
Fecundity	≥ 10 eggs/female/5 days; n= 30; a seasonal test.

Description of testing methods

Quantity

Mix the contents of the package thoroughly by tumbling the product container. If the product container does not allow proper mixing, use an alternative container. Take a minimum of 5 samples per container, each sample consisting of 2% of carrier weight or volume for 200-500 ml containers or minimum 5% of carrier weight or volume for <200 ml containers. Make sure that the material is remixed immediately before taking each sample.

Analyze the samples one by one. Spread the sample on a white sheet of paper under a warm bulb. Count the live predators running out of the material. Add to it the count of live predators remaining in the material. From these, estimate the total number of *P. persimilis* per container by dividing x – the mean number of adults per sample by f – the fraction of a total contents of the vermiculite carrier in one sample (x/f).

Fecundity

Prepare leaf discs of 2.5-3.5 cm diameter of brown beans (*Phaseolus vulgaris*) infested with an ample amount of the two-spotted spider mite (*Tetranychus urticae*, all developmental stages).

Place the discs on Agar, their infested side facing upward, in small plastic containers of the same diameter and of 2 cm height. The container lid should fit tightly to prevent escape of mites and should allow proper ventilation.

Take females of *P. persimilis* at random from the rearing unit. Put a single adult female on each leaf disc (= one replicate) by allowing mites to walk onto a fine brush. Place the containers with leaf discs upside down, i.e. infested side facing downward, so as to simulate the true orientation of the predatory mites in the field. After 48 h. transfer the female to a freshly prepared container. Count the eggs and/or larvae present in the old container. Leave the female predator in the new container for another 72 h. Then count the number of eggs and/or larvae. Add up the two counts to get the total fecundity for 5 days. Calculate the mean and standard error for the 30 replicates. Exclude individuals that do not lay eggs throughout the test, but indicate their number.

Preparation of agar substrate for fecundity tests, quantities for 6 containers:

- Boil 100 cc of plain tap water
- Add 1 gr of Bacto Agar when water temperature is 65°C

- Boil solution again while stirring
- Cool the Agar solution to 40-45°C (keep on stirring while cooling)
- Pour Agar solution in containers.
- Put the leaf discs on the Agar when it is still liquid. Make sure the margins of the leaf discs are slightly dipped in the Agar. This will prevent the disc from dehydrating prematurely.

Coordinators: S. Steinberg, J. Dale & A. Luczynski

***Neoseiulus californicus* McGregor (Acarina: Phytoseiidae)**

Test conditions	Temperature:	25 ± 1 °C
	RH:	75 ± 5 %
	Light regime:	16L:8D

Quality control criteria:

Quantity: Average number of living predators as specified on the container. Every week or batch-wise check.

Sex ratio: ≥60% females; once a year; n=100; for identification of sex, mount the individuals on microscopic slides.

Fecundity: ≥7 eggs per female, during a period of 5 days (n=30). A seasonal test.

Longevity: ≥5 days reached by 80% of the females examined in the fecundity test. n=30. A seasonal test.

Description of testing methods:

Quantity: Empty the contents of a bottle into a container, weigh the contents. Mix thoroughly with a spoon. Take three samples of 2 grams (for instance in a small cup). Empty the small cup in the upper sieve of a set of two sieves (upper one 315 μm, lower one 90 μm). Wash out the cup through the sieves. Run cold water through the sieves for a few minutes. All stages of the mite pass through the upper sieve and remain in the lower sieve. Trickle hot water on the sieve so that the mites are dead but not completely destroyed. Submerge the lower part in a shallow dish with a bit of water and detergent, and move the sieve gently. The mites in the sieve are evenly distributed now. Remove the sieve, and weep the underside of the sieve dry, with a piece of tissue paper. Place the sieve on top of a graph-paper circle covered by plastic. Count the predatory mites in the 2-gram sample. Take the weighted mean of the 3 samples to calculate the amount of predatory mites in the bottle.

Fecundity: Prepare leaf discs of 2.7 cm diameter of brown beans (*Phaseolus vulgaris*) or sweet pepper (*Capsicum annum*) infested with 10-15 mobile stages of the two-spotted spider mite (*Tetranychus urticae).* An ample amount of spider mite eggs should also be present. Place the discs on Agar, their infested side facing upward, in small plastic containers (diameter 32mm and 15mm height). The container lid should fit tightly to prevent escape of mites and should incorporate proper ventilation. Take females of *Neoseiulus californicus* at random from the bottle. Put a single adult female on each leaf disc (=one replicate) by allowing mites to walk onto a fine brush. Place the containers with leaf discs upside down, i.e. infested side facing downward, so as to simulate the true orientation of the predatory mites in the field. After 48 h. transfer the females to freshly prepared containers. Count the eggs and/or larvae present in the old container. Leave the female predator in the new container for another 72 h. Then count the number of eggs and/or larvae. Add up the two counts to get the total fecundity for 5

days. Calculate the mean and standard error for the 30 replicates. Exclude individuals that do not lay eggs throughout the test, but indicate their number.

Coordinator: S. Mulder/E. van Baal

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

Test conditions

Temperature:	22 ± 1 °C
RH:	70 ± 5%
Light regime:	16L:8D

Quality control criteria

Quantity	The number of live predators as specified on the label, excluding eggs, at the time of delivery (both for containers and controlled release systems)
Sex ratio	≥ 50 % females; n=100, an annual test
Fecundity	≥ 7 eggs/ female over a period of 7 days. Count from the second day of testing; n=30 females; an annual test

Description of testing methods

Quantity

Neoseiulus cucumeris is normally sold as a mix of bran, bran mites (as a food source) and the predatory mites themselves. Both the ratio of the two mite species and the concentration can vary considerably, depending on the product and the producer.

Mites can be washed out of the material with (hot) water, though counting is not easy because of reflection and difficult identification of the mite species. A more accurate method is to use a "Berlese technique" as described below. Mites are driven out of the material with the heat of a lamp. The advantage is that the mite species are clearly visible and dead mites will remain behind in the sieve. Make sure to allow for a "warming up time". This gives the small bran mites the chance to walk downwards before getting burned. Full heat is needed to drive the predatory mites out of the sieve.

Use material from 1 container or 4 sachets, depending on the product. Empty the contents into a bucket and weigh the content. Mix thoroughly with a spoon to get a homogenous mixture. According to the density take the following samples:

Density of *N. cucumeris*	sample size
1000 / 5 gr.	0.5 gr.
500 / 5 gr.	0.5 gr.
250 / 5 gr.	1.0 gr.
100 / 5 gr.	1.0 gr.

Put the material directly in a sieve of 6 cm. diameter, 2.5 cm height, mesh width 333 µm, 42% open. Spread the material as evenly as possible. Place the sieve at a distance of 4 cm. under a lamp of 150 Watt. The warming up time should take 5 minutes. Full power for an extra 10

minutes. (see figs. 1 and 2). Put a piece of black sticky tape under the sieve to trap the falling mites.
The number of mites can be counted directly with a grid, or if the mites still can walk over the glue, kill them in the freezer (20 minutes). Use cold light to prevent melting of the glue. The stickiness of the glue is very important. When the tape is not sticky many mites will walk off the tape and onto the holders. An alternative for the black sticky tape is to use a black plate with a ring of pure detergent as a barrier. Mites must be killed in the freezer immediately after extracting them. The humidity of the material is also very important. Within the range of 16,5-19% there does not seem to be a difference in the counting. At a higher humidity of the material there may be a different total heating time because mites stay longer in the material.

Fig. 1 Set-up of the mite extractor

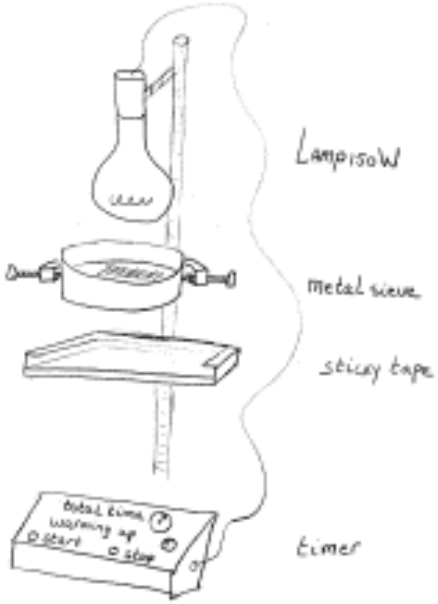
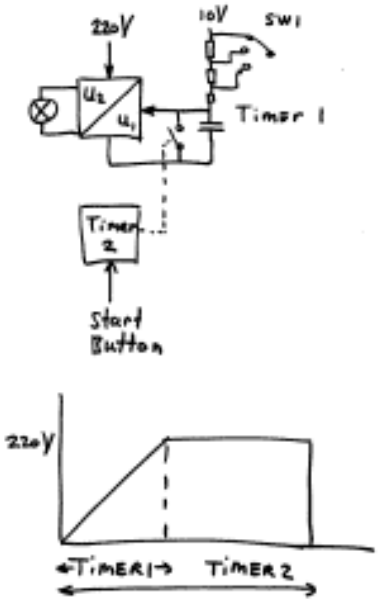


Fig. 2 Electric scheme of the mite extractor



Fecundity

Day 0

The test is conducted on individual female mites in small round plastic petri-dish trays (32 mm diameter; 15 mm height), which can be closed tightly. A nylon mesh is incorporated into the lid for ventilation. Trays are filled with agar solution (1%) to a depth of 5 mm. Just before the agar solidifies a sweet pepper leaf disc is placed with its upper surface in contact with the agar. Care should be taken to ensure that a leaf disc has a vein and some hairs for egg deposition. Good contact between the leaf disc and the agar solution is also necessary to prevent predatory mites from hiding. Thirty "big" mated females of *N. cucumeris* are taken from the commercial product. An ample amount of killed fresh *Ephestia kuehniella* eggs is added as food every day. Place the trays upside down in the climate room to simulate the natural leaf position.

Day 1

Remove the eggs laid on the first day. Do not include them in the total number of eggs laid.

Day 2	Count the egg laying of the predatory mites and provide the females with new trays as described on day 0.
Day 3	Count the eggs while removing them.
Day 4	Repeat day 2
Day 5	Repeat day 3
Day 6	Repeat day 2
Day 7	Repeat day 3
Day 8	Repeat day 3. The average number of eggs per female should be ≥ 7. Do not include eggs that are laid on the first day of the test.

Coordinators: S. Mulder & A. Hale

***Trichogramma brassicae* Bezd. (=*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 is specified on the label and veryfied by the producer[1]

Quality control criteria

Sex-ratio	≥ 50% females; 100 adults assessed on 10 release units each or 5 x 100 adults of bulk material; at least weekly or batch-wise test if batches were exposed to special treatments (e.g. storage)
Number of females[2]	As indicated on label; determined as for sex-ratio
Fecundity and longevity	≥ 40 offspring / 7 days / female; 80% of females should live at least 7 days; monthly or batch-wise test; n=30.
Natural host parasitism	≥ 10 parasitized hosts / 4 hours / female

Description of testing methods

Fecundity and longevity	30 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 day 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 day 7 is 40 offspring / female; mortality after day 7 is < 20%; at least monthly test or batch-wise if batches were exposed to special treatments (e.g. storage procedures, long-range shipments).
Natural host parasitism	30 females (age 24 hrs) are confined individually in tubes; two fresh egg-masses of at least 20 eggs/egg-mass of *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 the females they are incubated for 3 days; the number of black eggs is counted; the mean number of black eggs is ≥ 10 per female. The host cluster acceptance rate (= females parasitizing at least one host egg) should be ≥ 80%. This measure is important because parasitism drops drastically if a high proportion of females does not accept their hosts. This is especially true at low host densities and when hosts occur in batches. Often, parasitoids find only one egg mass during their lifetime and a high percentage acceptance is therefore crucial. This test is an indirect measure of the acceptance and suitability of the natural host egg. The test should be performed 2 to 4 times/year depending on the rearing system (number of generations reared on the factitious hosts).

Comments

[1] Molecular techniques are available at INRA Antibes France. Test necessary once a year, sample size min. 30 individuals

[2] The emergence period and pattern depend on the mixture of developmental stages released together and must be specified on the label.

Coordinator: S. Hassan

Trichogramma cacoeciae **Marchal (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 is specified on the label and veryfied by the producer[1]

Quality control criteria

Sex-ratio	100% females; 100 adults assessed on 10 release units each or 5 x 100 adults of bulk material; at least weekly or batch-wise test if batches were exposed to special treatments (e.g. storage)
Number of females[2]	As indicated on label, determined as for sex-ratio
Fecundity and longevity	≥ 30 offspring / 7days / female; 80% of females should live at least 7 days; monthly or batch-wise test; n=30.
Natural host parasitism	≥ 5 parasitized hosts / 4 hours / female

Description of testing methods

Fecundity and longevity

30 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 day 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 day 7 is 30 eggs/female; mortality after day 7 is < 20%; at least monthly test or batch-wise if batches were exposed to special treatments (e.g. storage procedures, long-range shipments).

Natural host parasitism

30 females (age 24 hrs) are confined individually in tubes; approx. 40 fresh eggs of natural host (*Cydia pomonella* or *Adoxophyes orana* as available / < 24hrs old) are added for 4 hrs; honey and water are provided as described above; after the separation of the eggs from the females, they are incubated for 3 days; the number of black eggs is counted; the mean number of black eggs ≥ 5 per female. This test is an indirect measure of the acceptance and suitability of the natural host egg. The test should be performed 2 to 4 times/year depending on the rearing system (number of generations reared on the factitious hosts).

Comments

See *T. brassicae*

Coordinator: S.A. Hassan

Trichogramma dendrolimi Matsumura (Hymenoptera: Trichogrammatidae)

Test conditions

Temperature: 23±2°C
RH: 75±10%
Light regime: 16L:8D
Rearing host: *Ephestia kuehniella*
Sitotroga cerealella

Species identification

The species is specified on the label and verified by the producer[1]

Quality control criteria

Sex-ratio	≥ 50% females; 100 adults assessed on 10 release units each or 5x100 adults of bulk material; at least weekly or batch-wise test if batches were exposed to special treatments (e.g. storage)
Number of females[2]	As indicated on label; determined as for sex-ratio
Fecundity and longevity	≥ 75 offspring / 7days / female; 50% of females should live at least 7 days; monthly or batch-wise test; n=30.
Natural host parasitism	≥ 10 parasitized hosts / 4 hours / female

Description of testing methods

Fecundity and longevity

30 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 day 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 day 7 is 75 eggs/female; mortality after day 7 is < 50%; at least monthly test or batch-wise if batches were exposed to special treatments (e.g. storage procedures, long-range shipments).

Natural host parasitism

30 females (age 24 hrs) are confined individually in tubes; approx. 40 fresh eggs of natural host (*Cydia pomonella* or *Adoxophyes orana* as available / < 24hrs old) are added for 4 hrs; honey and water are provided as described above; after the separation of the eggs from the females, they are incubated for 3 days; the number of black eggs is counted; the mean number of black eggs ≥ 10 per female. This test is an indirect measure of the acceptance and suitability of the natural host egg. The test should be performed 2 to 4 times/year depending on the rearing system (number of generations reared on the factitious hosts).

Comments

See *T. brassicae*

Coordinator: S.A. Hassan