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## Laboratory rearing techniques for 16 beneficial arthropod species and their prey/hosts

### Labormethoden für die Anzucht von 16 Nutzarthropodenarten und ihrer Beute/Wirte

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IOBC Working Group “Pesticides and Beneficial Organisms”

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### 1 Introduction

Standardization of test methods to evaluate effects of pesticides on beneficial arthropods is one of the aims of the IOBC Working Group “Pesticides and Beneficial Organisms” (HASSAN et al. 1985).

A prerequisite for standardized, reproducible tests is to have standardized test organisms. This can only be achieved by controlled rearing of the test species under well-defined conditions. Therefore, members of the working group also develop rearing procedures in parallel to the test development.

As such procedures are often left out in publications describing the tests, this paper gives the methods for rearing of 16 beneficials. These species were reared and tested for years by several group members from eight countries (Denmark, France, FRG, Hungary, Israel, Netherlands, Switzerland und UK).

The main objectives in rearing a strain used in research should be to prevent any kind of alteration in terms of behavioural or physiological features, in our case especially sensitivity to pesticides and foraging behaviour. Besides this, the rearing must be efficient in order to enable a high daily production with a low input in labour and equipment. Several members put emphasis on this point, and several recommend regular renewal of the strains by field collection in places where no pesticide treatment takes place. For tests on resistant strains, these should be renewed with material from places where well-documented treatments have been performed.

### 2 The species reared

#### Hymenoptera

(1) *Trichogramma cacoeciae* Marchal (Chalcidoidea)

S. A. HASSAN, Biologische Bundesanstalt für Land- und Forstwirtschaft, Institut für biologische Schädlingsbekämpfung, Heinrichstraße 243, D-6100 Darmstadt (FRG).

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(2) *Encarsia formosa* Gah. (Aphelinidae)

P. A. OOMEN & G. L. WIEGERS, Plantenziektenkundige Dienst, Geertjesweg 15, NL-6706 EA Wageningen (Netherlands).

(3) *Diaeretiella rapae* McIntosh (Aphidiidae)

C. KÜHNER, Biologische Bundesanstalt für Land- und Forstwirtschaft, Institut für biologische Schädlingsbekämpfung, Heinrichstraße 243, D-6100 Darmstadt (FRG).

(4) *Aphidius matricariae* Hal. (Aphidiidae)

L. POLGAR, Research Institute of Plant Protection of the Hungarian Academy of Sciences, P. O. Box 102, H-1525 Budapest (Hungary).

(5) *Phygadeuon trichops* Thoms. (Ichneumonidae)

E. NATON, Bayerische Landesanstalt für Bodenkultur und Pflanzenbau, Abt. Pflanzenschutz, Postfach 380269, D-8000 München 38 (FRG).

(6) *Coccylomimus turionellae* L. (Ichneumonidae)

H. BOGENSCHÜTZ, Forstliche Versuchs- und Forschungsanstalt Baden-Württemberg, Abteilung Waldschutz, Wonnhaldestraße 4, D-7800 Freiburg (FRG).

**Neuroptera**(7) *Chrysoperla carnea* Steph. (Chrysopidae)

F. BIGLER, Swiss Federal Research Station for Agronomy, CH-8046 Zürich-Reckenholz (Switzerland).

**Coleoptera**(8) *Aleochara bilineata* Gyll. (Staphylinidae)

L. SAMSØE-PETERSEN, Danish Research Centre for Plant Protection, Zoology Dept., Lottenborgvej 2, DK-2800 Lyngby (Denmark).

(9) *Semiadalia undecimnotata* Schn. (Coccinellidae)

J. BRUN, Institut National de la Recherches Agronomique, Station de Lutte Biologique, Laboratoire „E. Biliotti“, Route de Biot, F-06560 Valbonne (France).

**Heteroptera**(10) *Anthocoris nemoralis* F. (Anthocoridae)

A. STÄUBLI & D. PASQUIER, Station fédérale de recherches agronomique de Changins, CH-1260 Nyon (Switzerland).

**Diptera**(11) *Metasyrphus corollae* F. (Syrphidae)

W. RIECKMANN, Pflanzenschutzamt der Landwirtschaftskammer Hannover, Wunstorfer Landstraße 9, D-3000 Hannover 91 (FRG).

(12) *Aphidoletes aphidimyza* Rond. (Cecidomyiidae)

N. HELYER, Institute of Horticultural Research, Worthing Road, Littlehampton, West Sussex, BN17 6LP (UK).

**Araneae**(13) *Chiracanthium mildei* L. Koch (Clubionidae)

F. MANSOUR, ARO, Neue yaar experimental Station, P:O: Haifa 31999 (Israel).

## Acarina

(14) *Phytoseiulus persimilis* Athias-Henriot (Phytoseiidae)

P. A. OOMEN & G. L. WIEGERS, Plantenziektenkundige Dienst, Geertjesweg 15, NL-6706 EA Wageningen (Netherlands).

(15) *Typhlodromus pyri* Scheuten and *Amblyseius potentillae* Garman (Phytoseiidae)

W. P. J. OVERMEER, Dep. of Pure and Applied Ecology, Section Population Biology, University of Amsterdam, Kruislaan 302, NL-1098 SM Amsterdam (Netherlands).

## 3 Rearing methods

### 3.1 *Trichogramma cacoeciae* Marchal (Chalcidoidea, Hymenoptera)

#### 3.1.1 General

Parasites of the genus *Trichogramma* are known world-wide as important natural enemies of pests on fruit, vegetables and field crops. The genus includes about 40 species which are exclusively egg parasites. Since this natural enemy parasitizes the eggs of important Lepidoptera pests and prevents damage caused by the larvae, it is successfully used in practical biological control. It is estimated that more than 15 million ha world-wide are annually treated with mass-produced *Trichogramma*. About 10 species are being mass-reared to control pests on corn, sugar cane, rice, cotton, soy bean, sugar beet, vegetable and pine in at least eight countries. At present, in Europe about 10 000 ha of corn are being annually treated with *Trichogramma evanescens* Westw. to control the European cornborer *Ostrinia nubilalis* Hbn. Successful attempts have been made (HASSAN 1984, 1986) to control several other pests by releasing other species in apple orchards, vineyards and field crops.

The impact of naturally occurring *T. evanescens* on cabbage pests was recently studied by ROST and HASSAN (1985).

The *Trichogramma* female deposits its egg within the egg of its host, the hatching parasite larva feeds on the contents and pupate within the host egg. The adult parasite emerges from the host egg, feeds on honeydew and pollen and searches for new hosts.

*T. cacoeciae* occurs on trees, shrubs and has a generation time of about 11 days at 25°C. This species was chosen for testing the side-effects of pesticides because of its parthenogenetic reproduction (thelytoky). The monitoring is simplified because no males occur.

*Trichogramma* has a wide range of hosts, principally Lepidoptera, that can be used to mass rear it. In this work, the rearing of a store grain insect is described.

#### 3.1.2 Rearing of *Sitotroga cerealella* Olivier

The angoumois grain moth *Sitotroga cerealella* is reared on wheat or barley. For high productivity, large grain wheat, free of pesticides should be used. In order to kill any pests present in the grain before use and to soften it, the wheat is mixed with water (100 ml water/kg wheat) and heated at 70°C for 6 h. After cooling, 6 kg of wheat is poured into a 100 × 50 × 2 cm container made from a wooden frame that is open at one end and covered at both sides with metal gauze. Infestation is accomplished by placing the container in a horizontal position and distributing 4.8 g *Sitotroga* eggs evenly over the surface. The container is then placed at about 27°C and 70 % RH (still horizontally) for about 10 days during which the eggs hatch and the larvae infest the grain. The container is then suspended in a vertical position in an adult collection unit. This unit holds 16 containers. It consists of a cage (106 × 103 × 200 cm) and a funnel. The walls of the cage are covered with nylon gauze. The cage is bottomless and is connected with the plastic funnel (106 × 103 × 110 cm) ending in a 1-litre plastic bottle. *Sitotroga* adults emerge from the grain and walk through the container wall. After spending a few hours in the cage, they collect in the plastic bottle.

A semi-automatic egg-laying unit (Fig. 1) has been developed to collect and clean the *Sitotroga* eggs. The adults are poured by hand from the plastic bottle into a cylindrical cage

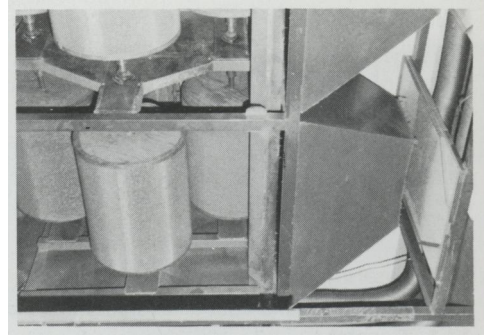


Fig. 1. A semi-automatic unit for obtaining eggs of *Sitotroga cerealella*

Abb. 1. Eine halbautomatische Apparatur zur Gewinnung von *S. cerealella*-Eiern

(with wire gauze walls, 0.8 mm mesh) that is fitted into the unit. The cylindrical cages are rotated for 10 minutes every 3 h, the outer walls being swept with built-in brushes. Air suction to remove the moth wing scales is simultaneously provided. The cleaned eggs fall through bottom openings and are collected in trays (HASSAN 1981; HASSAN et al. 1984). Using about 12 kg wheat per week in the continuous rearing of *Sitotroga*, an average of 3.5 million eggs per week are produced.

### 3.1.3. Rearing of *Trichogramma cacoeciae*

The egg parasite is reared in cylindrical glass tubes 30 cm long and 10 cm in diameter, closed at both ends by stoppers and cloth material. Every 2 or 3 days the parasites are given host eggs and honey. To facilitate handling, the *Sitotroga* host eggs are glued on strips of paper. The glue (Tragant<sup>®</sup>, E. Merck, Darmstadt, FRG), harmless to *Trichogramma*, is applied on small circular areas using a brush and a plastic frame. The eggs are then scattered on the glued areas. An egg card includes 12 circles with about 200 *Sitotroga* eggs each (Fig. 2).

Four to 5 days after parasitization, the host eggs turn black. To conduct a continuous rearing, a parasite host ratio of 1:4 should be maintained. This is done by leaving 25 % of the parasitized (black) *Sitotroga* eggs in the rearing tube.

The rearing units are kept in a climatic chamber under controlled conditions (16 h light: 50 lx, 28°C, 50–70 % RH; 8 h darkness: 18°C, 80–90 % RH) (HASSAN et al. 1984, MORRISON 1985).



Fig. 2. *Trichogramma* rearing, glass cylinder, host eggs (*Sitotroga cerealella*), stripes of paper, glue brush and plastic frame

Abb. 2. Anzucht von *Trichogramma*; Glaszylinder; Wirtseier (*S. cerealella*); Papierstreifen; Leimpinsel und Kunststoffrahmen

To control the quality of the rearing, monitoring of parasitism and adult emergence should be done at intervals. If the parasites are to be used for biological control, *Trichogramma* should be reared on the eggs of the target pest at intervals of about 6 months.

### 3.2 *Encarsia formosa* Gahan (Aphelinidae, Hymenoptera)

#### 3.2.1 General

*E. formosa* is an endoparasite of the greenhouse whitefly (*Trialeurodes vaporariorum* Westw.). It is used in most European countries for biological control of whiteflies in glasshouses and can replace pesticides during the entire growing season. Males occur rarely and they develop as hyperparasites of female larvae. Males are recognized by their abdomen which is brown and somewhat larger than the yellow female abdomen. The predominance of females is a suitable characteristic for a test species. *E. formosa* searches for hosts randomly on the leaves and is able to discriminate between unparasitized and parasitized hosts and to avoid oviposition in the latter. The 3rd and 4th larval instars and the prepupae are preferred for oviposition. However, parasitized scales of these instars may be rejected at first encounter. Adult parasites feed both on honeydew and hosts. Host feeding accounts for a considerable part of the larval mortality of the whitefly. The wasps can eat hosts of all stages, but 2nd instars and pupae are preferred for host feeding. Thus, *E. formosa* is both a parasite and a predator. It was observed that *E. formosa* used 7 % of the hosts encountered for feeding and 35 % for oviposition. In tests on parasitization, a certain surplus of scales has to be offered to compensate for host feeding and host rejection.

The first eggs can be laid without previous feeding.

#### 3.2.2 Rearing of host plants

Bean seeds of *Phaseolus vulgaris* of a large-leaf cultivar (e. g., 'Blokker' or 'Kogelboon') are sown in growing soil in 6-cm pots. The pots are placed in a separate glasshouse compartment (approx. 4 m<sup>2</sup>) at 20–30°C, 50–80 % RH and 12–16 h natural light. After the primary leaves have unfolded, the plants are ready for host rearing. Plants are topped regularly during the complete rearing period so that only the two primary leaves remain.

#### 3.2.3 Rearing of *Trialeurodes vaporariorum*

Three times per week, a batch of 30 plants is placed in a cage (0.15–0.25 cm<sup>3</sup>) with whiteflies, to be infested with whitefly eggs. The cage is situated in a glasshouse compartment at 20–25°C, 50–80 % RH and 12–16 h light. After 2 days (at the weekend 3 days), each batch of plants is taken out of the cage and the whitefly adults are blown back by mouth into the cage onto new plants. The infested plants are then transferred to the larval development compartment, where temperature is about 22°C, RH 50–60 % and day length is 12–16 h, natural light.

About half the number of plants are kept intact; these are intended for mass-rearing of whiteflies and parasites. The other half have the leaves cut off, these leaves are put in trays of tap water for rooting and are intended for testing. This procedure gives a steady supply of infested plants. After 17 days, most scales have reached the 4th instar (3rd or 4th instar scales are preferred for oviposition by *E. formosa*). The plants are then ready to be used for rearing *E. formosa* or the leaves can be used in parasitization tests.

Before use, a check should be made on the density of scales. The number on the central part of the leaf (exposed to the parasite in the cage test) should be 250–350 (about 12 per cm<sup>2</sup>). More scales cause premature leaf wilting, while fewer could result in poor parasitization or even hyper-parasitization. To ensure a sufficient number of vigorous leaves carrying the right number of scales for the tests, allowance should be made for 50 % more plants. Part of the whitefly-infested plants are used to maintain the population of adults in the whitefly cage; the other part is used for rearing the parasite.

### 3.2.4 Rearing of *Encarsia formosa*

The breeding stock of *E. formosa* is maintained in a cage (approx. 0.25 m<sup>3</sup>) in a separate parasite rearing compartment in the glasshouse (22°C, 60–70 % RH and approx. 16 h, natural light). Two tomato plants in 20-cm pots, infested with whitefly, serve as a source of honeydew and small larvae to keep the *E. formosa* adults in the cage in a good condition.

Bean plants with whitefly scales are placed in the parasite rearing cage to be parasitized. By regular introduction of new bean plants with scales (four plants three times a week) a steady supply of parasitized scales for testing can be assured. After 2 days, the plants with parasitized scales are taken out, and the parasite adults are blown back into the cage. The plants are then kept in the same room. In about 9 days, the parasitized scales will turn black and 9 days later the new adults emerge. Leaves with pupae at the stage 2 or 3 days before emergence are used for testing. The surplus of parasite-infested plants are used to maintain the population of adult parasites in the cage.

## 3.3 *Diaeretiella rapae* McIntosh (Aphidiidae, Hymenoptera)

### 3.3.1 General

*Diaeretiella rapae*, an internal parasite, is one of the important antagonists of the green peach aphid, *Myzus persicae* Sulz. and the cabbage aphid, *Brevicoryne brassicae* L. and is found world-wide.

*M. persicae* hibernates in the egg stage on peach trees. In spring, fundatrix larvae emerge and reproduce by parthenogenesis. The winged forms leave the winter host to fly to their different summer hosts. On these plants (many cultivated, for example potato, cabbage, lettuce, turnip), several generations of aphids develop by parthenogenesis. At the end of September, gynoparae and males develop and fly to the winter host. The offspring of the gynoparae are females that mate and lay eggs for hibernation.

*M. persicae* causes serious damage as a transmitter of many phytopathogenic viruses (more than 100 different species). Therefore, it may be considered one of the most important agricultural pests in the Federal Republic of Germany (HOFFMANN and SCHMUTTERER 1983).

*D. rapae* attacks all instars of *M. persicae* (including the winged forms), but prefers the younger ones (2nd and 3rd instar larvae). The whole development of the parasite takes place inside the aphid and includes four larval and one pupal stage. The adult parasite emerges through a hole in the abdomen of the aphid mummy, and a few hours later, the females start laying eggs. Fertilized eggs develop into females and unfertilized into males. There is often a surplus of females, and the sex ratio of 1:1 is seldom reached. Superparasitism occurs, but only one parasite larva develops into an adult. Longevity under field conditions is 2–3 weeks. Hibernation takes place in the fourth larval or pupal stage (HAFEZ 1961).

### 3.3.2 Rearing of host plants

Cabbage plants *Brassica oleracea* L. (var. *medullosa* Thell or var. *gemmifera* DC) are used as hosts for *M. persicae*. The plants are cultivated in plastic flower pots (Ø: 10 cm, H: 8 cm) in a humus/sand mixture (3:1).

Plant rearing is carried out in a room with controlled environment, a temperature of 20–22°C, a relative humidity of 50–60 % and 16 h light. The light intensity should not be lower than 2500 lx.

2–3 g of the cabbage seed is sown in the humus/sand mixture. Ten days after germination, young plants of 6–8 cm are isolated in the above mentioned pots, and kept for the next 6–8 weeks in the plant breeding room. Watering is carried out every 3rd day. When the plants have reached the five leaf stage (a size about 15 cm), they can be used for aphid rearing.

### 3.3.3 Rearing of *Myzus persicae*

Rearing of aphids takes place in cages (a wooden frame with sides of gauze, 1.5 × 1.3 × 1.2 m)

in a greenhouse, where the average temperature is about 20–22°C, the relative humidity is 60–80 % and the day length 16 h.

The 6- to 8-week-old cabbages from the plant rearing room are placed in the cages in the greenhouse (60 plants/cage) and are infested with aphids. Ten to 14 days later, when the aphids are distributed over the plants, and a density of 100–120 aphids per leaf is reached, the plants are used for the parasite rearing.

### 3.3.4 Rearing of *Diaeretiella rapae*

*D. rapae* is reared in a room with controlled environment at the most favourable conditions for *D. rapae*: 22–23°C, 60–70 % RH, and 16 h light, 2500–3000 lx.

The parasites are offered *M. persicae* on cabbage plants, standing on a shelf. The shelf is closed on the sides with curtains made of gauze.

Development from egg to adult takes about 10–16 days under these conditions. To guarantee a continuous supply of adult parasites, unparasitized aphids are offered for oviposition in a one-week rhythm.

Plants with parasitized aphids are cut, and part of the mummies can be stored at a temperature of 4°C. The emerged parasites of the remaining mummies are released again to the rearing stock to obtain a continuous production. If it is necessary to rear mummies of uniform age, the parasite are allowed to parasitize aphids for only 24 h. Then the aphids are isolated in a parasite-free room until mummification.

The relation between the number of parasites and hosts is very important for the rearing. If the density of *D. rapae* is too high, the behaviour of *M. persicae* is affected. Many aphids leave the plants, and the rate of mummification decreases. An optimal parasite:host ratio for *D. rapae* is about 1:150.

## 3.4 *Aphidius matricariae* Hal. (Aphidiidae, Hymenoptera)

### 3.4.1 General

This aphid parasite has a world-wide distribution and is known to parasitize almost 40 aphid species. Its hosts belong mainly to Aphidinae. Adult females are about 1.5–2.5 mm long. The size of the mummy depends on the host and varies between 1.5 and 2.5 mm. The colour is yellowbrown, but the diapausing mummy is dark brown. The sex ratio is about 1:2 (male:female). Development from egg to adult takes 13–14 days at 20°C with *Myzus persicae* as host. Females live 5–10 days at temperatures between 15 and 25°C, males live longer (GIRI 1982, SHALABY and RABASSE 1979).

*A. matricariae* is a solitary aphid endoparasite. Superparasitism is rare because the wasp is able to distinguish parasitized from unparasitized hosts (HART et al. 1976). Females prefer 3rd instar hosts, but accept all larvae and adults. Adults feed on honeydew. The optimal conditions for development are 21°C, 70 % RH and 16 h light.

### 3.4.2 Rearing of host plants

Tobacco plants for *M. persicae* and pepper plants for the parasites are sown in pots and kept separately in a roughly controlled cabinet at 18–25°C and 16 h light. At the fourth to six-leaf stage, both plant species can be used for the rearing.

### 3.4.3 Rearing of *Myzus persicae*

The *M. persicae* rearing takes place in a controlled environment cabinet on tobacco seedlings under the following conditions: 20–25°C, 70–80 % RH and 16 h light, > 2000 lx.

The aphids disperse themselves from old to young plants, so that the rearing is continuous. For parasitization, aphids are transferred to pepper plants in the four to six-leafstage. Tobacco leaves, heavily infested with aphids, are picked off and put on the pepper plants where they remain for 24 h.



#### 3.4.4 Rearing of *Aphidius matricariae*

*A. matricariae* are reared in a glasshouse, where the environment is roughly controlled at 18–23°C, 60–80 % RH and 16 h light. For easier population density control and to avoid attacks of hyperparasites, it is preferable to rear the parasites inside rearing cages. The cage contains two trays with pepper plants infested with aphids. One of them is for continuous rearing and the other for stage rearing. If aphid mummies with known age are needed, the trays with infested pepper plants have to be placed in the cage for 24 h only. After this, the parasitized aphids are kept separately in a parasite-free cage until mummification. Because of the deposition of honeydew and in order to prevent hyperparasites, the cage must be changed monthly and a new parasite stock be established. The parasite density in the cages must be carefully controlled, it should neither be too low nor too high. A high parasite density can result in elimination of the aphids, because they are continuously disturbed by ovipositing and searching parasite adults. The optimal aphid:parasite ratio for a continuous rearing is 100–150:1 (POLGAR 1985).

### 3.5 *Phygadeuon trichops* Thoms. (Ichneumonidae, Hymenoptera)

#### 3.5.1 General

*Phygadeuon trichops* is a parasite of different *Delia*-species (Diptera, Anthomyiidae). Among these are some important species for agriculture, which can cause considerable damage (the cabbage root fly (*Delia brassicae* Bché.), the onion fly (*D. antiqua* Mg.), the wheat bulb fly (*D. coarctata* Fall.), the bean seed fly (*D. florilega* Zett. a. o.). The larvae mine in their host plants, but pupate in the soil. The ichneumonids search for the host pupae in the soil, lay their eggs in the pupae, and, as a consequence, the development of the pest is stopped. Sometimes several eggs are laid in one pupa – especially under the rearing conditions in the laboratory, however, only one wasp develops. Because *D. antiqua* is most easy to rear, the culture of the parasite is based on this host.

#### 3.5.2 Rearing of host plants

The onions used are bought. It is important that these onions are free of residues of pesticides and that they have a suitably firm consistence. Experience has shown that onions from Hungary are the best. However, it is recommended, to perform a short test of suitability by rearing some larvae in an incubator (24–27°C, 10 days) before the use of a new lot of onions.

#### 3.5.3 Rearing of *Delia antiqua* (See also 3.8.2) Flies

The climatic conditions for the rearing are: 20–22°C; illumination by fluorescent tubes (Osram L 36 W/25; 16 h/day; two tubes/cage; about 30 cm above the cage); no humidity control is necessary. The imagines and the larvae are reared in different rooms for hygiene reasons. The flies live in large cages (1.20×0.85×0.55 m), made of a plastic frame with a fly screen (plastic, approx. 6 meshes/cm<sup>2</sup>). A foil (about 15 cm broad and 0,2 mm thick) is hung under the ceiling like a hammock and food is distributed over the surface. This food consists of soybean flour and dried yeast (5:1 v/v) mixed with honey to make a paste that can be smeared on the foil. At the bottom of each cage, there are two large Petri dishes (12–15 cm diameter) with dry food: sugar, milk powder, soybean flour and dried yeast (10:10:1:1). Equally large Petri dishes, but a little higher, are filled with wet cotton wool that serves as water supply. Whereas the food is sufficient for the life span of one generation of flies in the cage (4 weeks), the watering dishes must be renewed weekly. Two sand-filled dishes (18 × 12 × 2.5 cm) each with two onion halves, cut surface upwards, are provided as ovoposition sites. The dishes are exchanged twice a week, and the sand in them is always kept moist to prevent the eggs from drying out.

## Larvae

The only climatic condition controlled is the temperature (20–22°C), supplementary illumination is not necessary. As mentioned above, onions are used for the rearing of the larvae. For the eggs from the two oviposition dishes, a large pan (32 × 32 × 14 cm) is needed, which is covered with a thin layer of moist sand (about 2 cm). On this sand, halved onions are placed, close together in an upright position. The eggs and the sand from the two oviposition dishes are dispersed uniformly over these. Onions and eggs are then covered with a further layer of moist sand. It is necessary to check four to five times a week whether the rearing dishes are too moist or too dry or whether fresh halved onions should be added (as soon as the onions first given are much gnawed or rotten). The optimum moisture content is similar to that of good garden soil. Accordingly, dry sand or water must be added. The development takes about 3 weeks. After this, the pupae are washed out from the sand by tap water (20°C) and are spread on filter paper for drying.

### 3.5.4 Rearing of *Phygadeuon trichops*

The climatic conditions are the same as for the flies, but with a moisture content of the air above 50 % RH. At lower humidities, mortality of the parasites increases. The parasites are reared in a room, in which pesticides are not allowed. The imagines are reared in glass cages (50 × 35 × 30 cm) with aluminium frames. One of the front plates has a round opening (15 cm diameter), closed by a cloth sleeve. One third of the long walls is screened with a plastic mesh (0.65 mm). The lid can be removed; it is edged with foam rubber to prevent escaping of insects and has, in one corner, a small opening (about 3.5 cm), closed by a cork stopper. To this stopper, a filter paper is attached (7 × 15 cm) smeared with the food mixture as for flies. For additional feeding, a little plastic dish with dry food mixture, also similar to that given to the flies, is offered. Water is supplied by a beaker standing upside down in a Petri dish filled with cotton wool. The food, paper and water are renewed weekly. Three times per week, young, pale pupae of *D. antiqua* are offered for parasitization. About 15 cm<sup>3</sup> of pupae are placed in Petri dishes (9 cm diameter) on moist sand. After 2 or 3 days of exposure, the pupae are stored in the now closed Petri dishes, at the same climatic conditions. They are examined daily and about 1 week later, the flies emerge from the nonparasitized pupae. These must be removed. About 2 weeks later, the parasite start emerging.

## 3.6 *Coccygomimus turionellae* L. (Ichneumonidae, Hymenoptera)

### 3.6.1 General

*Coccygomimus turionellae* is a polyphagous parasite of lepidopteran pupae. About 100 host species belonging to 23 families are known. Among them are a number of agricultural and forest pests. *C. turionellae* is neither bound to one definite biotope nor to a certain season. The number of generations per year depends on the hosts present. In the laboratory, up to 12 generations may be reared in 1 year without any interruption. The size of the imagines depends on the size of the hosts in which they develop. The females usually measure between 8 and 18 mm from their head to the end of their ovipositor. The total number of eggs per female depends on the number of pupae at disposal.

Both longevity and number of eggs laid during their lifetime differ from female to female (BOGENSCHÜTZ 1971). The highest frequency of egg-laying is between the 15th and 30th day of their life, possibly also later. Only one *C. turionellae* larva can develop in each host pupa. The females can distinguish already parasitized hosts from unparasitized ones, so that superparasitism is avoided. However, this is only the case if more than 24 h have passed between the first and the second attempt of parasitizing (BOGENSCHÜTZ 1975). In nature, this period is enough. With the close contact between the parasite and the host in the laboratory, superparasitism occurs regularly. The development takes about 3 weeks. A suitable host for laboratory breeding of *C. turionellae* is the greater wax moth, *Galleria mellonella*.

The method for examining the effects of pesticides on *C. turionellae* was described by BOGENSCHÜTZ (1984) and BOGENSCHÜTZ et al. (1986).

### 3.6.2 Rearing of *Galleria mellonella*

The wax moth larvae feed on old honeycombs and are kept in moulded glass boxes (24 cm × 18 cm × 22 cm) at 30°C and constant darkness. The lid consists of a mesh mounted on a wooden frame. It is also possible to use tight plastic containers ventilated through holes covered with wire mesh.

In order to get eggs, fairly old pupae, which have already got a dark pigmentation, are placed in 2-litre bottles with wide necks, where the emergence, mating and oviposition of the moths take place. A folded piece of flimsy is used for the oviposition. The paper is folded several times and stapled at one end to give it a concertina-like shape. After 1 or 2 days, it contains enough eggs to start breeding in a glass container with a honeycomb. During the feeding period, the larvae are fed as required.

For parasitization, *G. mellonella* pupae are cut out of the cocoon. Pale pupae whose eye pigmentation can just be discerned through the pupal case are best suited for breeding and experiments.

This method of getting host pupae is very laborious. In the literature, easier ways of doing this are suggested, but none have given satisfactory results in practice. This was the case both with artificial food (MARSTON and CAMPBELL 1973), dissolution of the cocoon in a buffered sodium hypochlorite solution (DUTKY et al. 1962), and the production of pupae by use of metal sheets (RYAN 1968).

### 3.6.3 Rearing of *Coccygomimus turionellae*

Normal cages for rearing insects can be used for *C. turionellae* (size for max. 40 females: 30 cm × 30 cm × 60 cm) at 16 h light, 2000–4000 lx, 55–70 % RH, temperature: 23°C during the day and 15°C at night. Water and food must be available all the time. A bird's drinking trough with a cotton wad pushed into the opening is used for water provision. The food for the parasite is prepared as follows: Soak 1 g agar-agar in 100 ml distilled water. Add 50 g sugar, 20 g honey and 1 g yeast extract, heat while stirring continuously. Use a pipette for dripping on a cardboard box and leave to cool. To induce oviposition and cover the hemolymph requirement, a couple of host pupae are always present in the rearing cages.

Forty host pupae are placed for a period of 5 to 15 min with about 40 females ready for oviposition. Up to 40 % of the hosts remain unparasitized after this, but if the pupae are exposed for a longer period the rate of superparasitism will increase (HUANG 1976). In order to increase parasitism, it is advisable to expose pupae once more for oviposition if they do not show any signs of successful parasitization after 48 h; paralysis of the abdominal musculature and melanized spots. The parasitized pupae are placed in cages where hatching starts after about 21 days. The emerged males and females are transferred daily to new breeding cages. In this way, a sex ratio of 1:1 is maintained. However, mating does not have any influence on the oviposition (BOGENSCHÜTZ 1975).

## 3.7 *Chrysoperla carnea* Steph. (Chrysopidae, Neuroptera)

### 3.7.1 General

*Chrysoperla carnea* – the common green lacewing – is considered to be one of the general entomophagous predators, it is present in most agricultural crops and forests around the world. The predacious larvae attack a wide range of phytophagous insects, such as aphids, young larvae of lepidopterans and other softbodied arthropods. The adults of *C. carnea* are not predacious. Their food sources consist mainly of honeydew, flower nectar and other sweet secretions of plants as well as pollen (PRINCIPI and CANARD 1984).

*C. carnea* has attracted much interest with respect to mass-rearing and inundative or inoculative releases in both greenhouses and field crops. Techniques for mass-rearing larvae

and adults have been developed and reported by FINNEY (1948, 1950), HAGEN and TASSAN (1970), RIDGWAY et al. (1970), MORRISON et al. (1975), HASSAN (1975), TULISALO (1978) and MORRISON (1985 a). Nowadays, mass-rearing is generally accomplished by feeding the adults on artificial diet and by rearing the larvae on eggs and larvae of various lepidopterans, e. g., *Sitotroga cerealella* (see 3.1.2), *Ephestia kuehniella* Zell. and *Phthorimaea operculella* Zell. Many attempts have been made to develop artificial diets for larvae (VANDERZANT 1973; PONOMAREVA and BEGLIAROV 1973; BIGLER et al. 1976), but so far, none has led to practical application. The rearing method reported here has not been developed for commercial mass-rearing purposes, but for a continuous maintenance of a *C. carnea* strain which is used for research purposes, especially for measuring the effects of pesticides on this predator.

### 3.7.2 Rearing of *Ephestia kuehniella*

Rearing as described in section 3.10.3 will be suitable.

### 3.7.3 Rearing of *Acyrtosiphon pisum* Harr.

The pea aphid, *Acyrtosiphon pisum*, is used as the main larval food. They are reared on broad beans, *Vicia faba* L. Broad bean seeds are soaked in water for 24 h, for pregermination. Afterwards, 150 seeds are sown in trays (30 × 25 × 6 cm) with sterilized soil and left at 23–25°C for 7–10 days. After that, the plants have developed four to six leaves (5 to 10 cm high) and are ready for aphid rearing.

According to the needs, a certain number of trays are infested with pea aphids. Approximately 0.5 g of aphids, collected from the previous rearing, are evenly distributed over the plants of one tray. They are left at 22 ± 1°C, 50–70 % RH and 16 h light, 4000 lx, for about 7 days before harvesting the aphids for larval food. It is important to discard the harvested trays immediately in order to prevent problems with aphid parasites and diseases.

### 3.7.4 Rearing of *Chrysoperla carnea*

#### Larvae

Larvae are reared under fluctuating temperatures (16 h at 25°C, 8 h at 18°C), RH at 70 ± 10 % and natural daylight from April to September. This is supplemented by white fluorescent light (2000 lx) to 16 h day length from October to March. Under these conditions, the preimaginal life cycle of *C. carnea* requires about 30 days (egg: 5–6 days, larva: 14–16 days, pupa: 8–10 days).

The rearing cage consists of two parts: the bottom is a container (65 × 45 × 12 cm) which is partly filled with Vermiculit®, approx. 3 mm diameter. This material offers the larvae hiding places, and most of the larvae pupate within the Vermiculit. In that way, cannibalism is drastically reduced. The upper part of the container is a transparent frame of acrylic glass (60 × 40 × 40 cm) with an opening on one side (diameter 17 cm) for handling from outside. The lid consists of a 20-mesh cotton gauze fixed by an acrylic frame which is tightly fastened with clips.

Approximately 500 eggs of *C. carnea* (24–48 h old), attached to the cotton gauze, are placed in each cage. As soon as the first larvae hatch, eggs of *E. kuehniella* (see 3.10.3) are added.

First instar larvae are fed with UV-irradiated, killed eggs of *Ephestia kuehniella*. These eggs are either fresh or stored at 2°C, 80 % RH for at most 2 months. Approximately 4 ml (ca. 100 000) eggs are loosely distributed over the cotton gauze towels where *C. carnea* had previously oviposited. Part of the eggs can be glued with gum Arabic to paper strips in order to increase the inner surface of the cage and consequently reduce cannibalism. Pea aphids, *Acyrtosiphon pisum*, are provided on potted broad bean plants, *Vicia faba*, as soon as the more cannibalistic 2nd instar larvae appear. From that time on, an amount of fresh aphids are added daily according to the density of the larval population. In order to minimize cannibalism, it is important to maintain high aphid populations and dense plant foliage. If available, other aphids, e. g., cereal aphids, green peach aphids etc., can be fed.

The success of the rearing depends very much on the amount of aphids available during the 2nd and 3rd larval stages. After approx. 14 days, pupation starts. It occurs almost exclusively within the Vermiculit layer. The first adults emerge approx. 1 week later.

### Adults

At this moment, adult food is offered on a paper strip and water on a cotton pad. Adult lacewings are collected daily or at least twice a week with an aspirator. One rearing unit produces 120–150 adults, that is 25 %–30 % of the eggs introduced in the cage.

The adults are maintained under constant conditions of  $22\pm 1^\circ\text{C}$ ,  $60\pm 10\%$  RH and 16 h white fluorescent (2000 lx) light. The temperature can easily be raised to  $25\text{--}26^\circ\text{C}$  if high numbers of eggs are needed within a short period.

The adult oviposition and feeding container consists of a 1.5-litre, transparent plastic container. A hole (diameter 1 cm) is cut in the centre of the bottom, and a cotton plug for water supply is inserted. The soft plastic lid is cut so that a 1 cm edge is left. A piece (diameter ca. 15 cm) of a 20-mesh cotton gauze is placed over the container top and held in place by the modified lid. Three strips (ca.  $5 \times 1$  cm) of a one-side adhesive paper or plastic are glued to the inside of the container and a thin layer of adult food is smeared on them. The container is then placed in a shallow, water-filled cup from which fresh water is permanently supplied.

The adult diet consists of brewer's yeast flakes, honey and water (4:7:5 w/w). This paste can be stored in a closed glass jar at  $4^\circ\text{C}$  for approx. 4 weeks.

About 50 adults (sex ratio 1:1) are caged in one 1.5-litre container. Higher densities negatively affect longevity and fecundity. The pre-oviposition period lasts approx. 6–7 days at  $22\pm 1^\circ\text{C}$ . The eggs are laid on the cotton gauze on the top of the container. The cotton towels are changed at least twice a week or daily according to the utilization of the eggs and the prevailing temperature. The adults are transferred to a previously prepared new container in order to prevent hatching of scattered eggs laid on the side wall or at the bottom. Such larvae would destroy newly laid eggs or even kill adults. For transferring the adults, the modified lid with the cotton gauze is opened, the new container is placed upside down over the open top and then turned  $180^\circ$ . The adults are then moved down into the new container by gently tapping the sides of the old container. In case the adults are too active and tend to escape, they can be cooled at  $12^\circ\text{C}$  for 20–30 min. According to the utilization of the eggs, they can be stored at  $12^\circ\text{C}$ ,  $80\pm 5\%$  RH and 16 h light for approximately 2 weeks without loss of viability.

The normal egg production at  $22^\circ\text{C}$  is reached 10–14 days after adult emergence. It stays at a constant level for approx. 5 weeks and drops slowly afterwards. At the age of approx. 10 weeks, the oviposition becomes insignificant and the adults, although still alive, can be discarded. An average daily egg production of about 10 eggs/female can be obtained during the 5 weeks peak. The average total egg production per female ranges from 300 to 400 eggs.

## 3.8 *Aleochara bilineata* Gyll. (Staphylinidae, Coleoptera)

### 3.8.1 General

*A. bilineata* adults are polyphagous predators living in and on the soil near cabbage, onion, beet, bean and lupin plants. The larvae are predatory against the pupae of flies found living on these plants. They are especially important as natural enemies of cabbage root flies (*Delia brassicae* Bchè.). Their life span may be as long as 3 months.

Eggs are deposited in the soil near fly-infested plants, and the newly hatched larvae (carrying resources for about 3 days of activity) move around until they encounter a host pupa. The pupa is entered and development and pupation takes place inside the host puparium from which the adult emerges about 6 weeks later.

### 3.8.2 Rearing of *Delia antiqua* Meig. (See also 3.5.3)

This rearing is based on the description by TICHELER (1971). The cages with adult onion flies are kept in a room with controlled environment:  $22 \pm 2^\circ\text{C}$ , RH = 65–85 % and strong light (approx. 2000 lx.) for 18 h a day. The rest of the rearing takes place in controlled environment cabinets at  $22 \pm 0.7^\circ\text{C}$ ,  $90 \pm 10$  % RH and 16 h light (50–75 lx.).

#### Adults

The adult onion flies are kept in plastic cages ( $32 \times 22 \times 16$  (h) cm) with a sleeve inserted in one wall. The watering device is a small glass (such as a 50 ml beaker) filled with water covered with soaked kitchen paper and placed upside down in a Petri dish. The flies must have access to onions in order to lay eggs. This is achieved in the following way: In the bottom of a Petri dish (9 cm diameter) a piece of onion is placed and covered by a small earthenware flowerpot (approx. 7 cm diameter) turned upside down and with a rubber stopper in the hole. Thus, the smell of onion reaches the flies which deposit their eggs in the Petri dish close to (or under the edge of) the flowerpot.

Adult onion flies are fed on a powdery diet which consists of yeast extract (Difco yeast extract technical), soy flour, sugar and milk powder (1:1:10:10). The milk powder is mixed from baby milk powder (NAN no. 1) and skim-milk powder, to make a fat content of 10 %.

250 onion fly pupae in an open plastic box with moist Vermiculit (10 % v/v) are placed in a cage with 11 g food and water. Ten days later the flies start to emerge. A further eight days later, onion is added to the cage, and the egg-laying starts about 10 days after adult emergence. Eggs are collected and the old onion replaced by a fresh piece twice a week. Food and water is renewed weekly. The flies lay eggs for 4–5 weeks after which the cage is emptied and washed. As a new cage is started each week, there are always five to six cages with onion flies.

#### Eggs and larvae

Onion fly larvae and pupae are kept in plastic boxes ( $18 \times 13 \times 4$  (h) cm) with closely fitting lids solid or open. The open lids have a fine nylon gauze inserted in a hole covering most of the area.

The larvae are reared on an artificial medium made of (for 2000 eggs): 160 g carrot powder, 57.6 g yeast extract, 2.0 ml 30 % formaldehyde solution, 73.6 ml of 1 mol/l hydrochloric acid, 22.4 ml benzoate-sorbate solution and approx. 800 ml of demineralised water. The benzoate-sorbate solution is made from: 10 g methyl-4-hydroxybenzoate in 90 ml 96 % ethanol and 10 g potassium sorbate in 90 ml demineralised water.

When the eggs are collected, they are weighed, the number calculated (14 eggs/mg) and the medium for the larvae is prepared. It is stirred for about 10 min with a handmixer. The water content of the medium is very important for the development of the larvae, but it is not possible to give a precise amount: Water should be added until the medium is wet, but firm enough not to flow out when the bowl is shaken.

The medium is put into a plastic box with a solid bottom, the eggs are spread out on the surface and a tight lid is put on. 2–4 days later (when the larvae start crawling around in the box), the lid is exchanged for one with gauze. In this way, the air is saturated with water during the hatching of eggs and about 70 % RH for the rest of the larval development. Nineteen days after the start, the larvae are ready to pupate and assemble in the corners of the box.

#### Pupae

The medium is washed out through a  $1550 \mu\text{m}$  sieve that retains the larvae. A gentle shower is used to avoid injury to the larvae. These are placed in moist (10 % v/v) Vermiculit (0–1.5 mm) in a box with a gauze lid. 5 and 9 days later, the pupae are collected, whereafter the Vermiculit is discarded. The pupae are dried and can be stored at least 1 month in the refrigerator. Pupae to be used for parasitization – whether for rearing or for experiments – should be 3–4 days old:

At this age, pupation has been completed, and the remaining pupal development time is long enough to allow a rove beetle larva to establish before the metamorphosis of the fly.

### 3.8.3 Rearing of *Aleochara bilineata*

The rearing takes place under the same conditions as those used for onion fly larvae. The procedure is based on the description by HERTVELD et al. (1984).

#### Adults

The adult rove beetles are kept in circular acrylic cages (22 cm diameter, 9 cm height). The bottom of the cage is a fine nylon gauze (630  $\mu\text{m}$  mesh aperture) glued to the walls on the slant 1–5 cm above the lower edge of the ring. On the lower part of this gauze, 15–20  $\text{cm}^3$  of expanded clay granules (“Leca”, grain size 1.6–3.0 mm) are placed. The lid of the cage is an acrylic ring (22 cm by 1 cm) to which a very fine nylon gauze (200  $\mu\text{m}$ ) is glued (Fig. 3). Each cage contains up to 80 rove beetles.

The adults are fed with minced beef. The meat is bought fresh, packed in little pieces of tin foil (about 1 g in each) and frozen.

The Leca is changed once a month. Fresh minced beef is provided every day. Water is supplied as the eggs are washed out (see below). The adult beetles live for 2–3 months, and egg production starts when they are 4–5 days old. Both egg production and lifespan depend on the food offered: If the beetles eat fly eggs or larvae, the daily egg production is high (5–6 eggs/female/day), and the lifespan 1–2 months. If they eat minced meat, the egg production is only 3–4 eggs/female/day, but lifespan is 4 months.

Once every month, a new cage with adults is started and this is kept for about 4 months (until the egg production is too low). In this way, there are always at least four cages with up to 80 rove beetles in each.

#### Eggs, parasitization

To wash out eggs, the following is needed: A plastic funnel (inner diameter >22 cm), a 10 cm piece of plastic tube with a diameter larger than the tip of the funnel to which a piece of 200  $\mu\text{m}$  gauze is glued or melted. An acrylic cylinder (e. g., 20 cm high with a diameter of 15 cm) is very useful for holding the funnel.

Twice every week, the cages are washed out with water (approx. 20°C) from a shower. The eggs end in the fine gauze (200  $\mu\text{m}$ ) form which they are collected and counted (Fig. 4).

For parasitization, the eggs are placed in small plastic boxes (7 × 11 × 5 (h) cm) with a tight lid, Vermiculit (0–1.5 mm, with 10 % v/v water) and 3–4 days old pupae of the onion fly. Four fly pupae per five rove beetle eggs are used. The plastic box is tightly closed, as

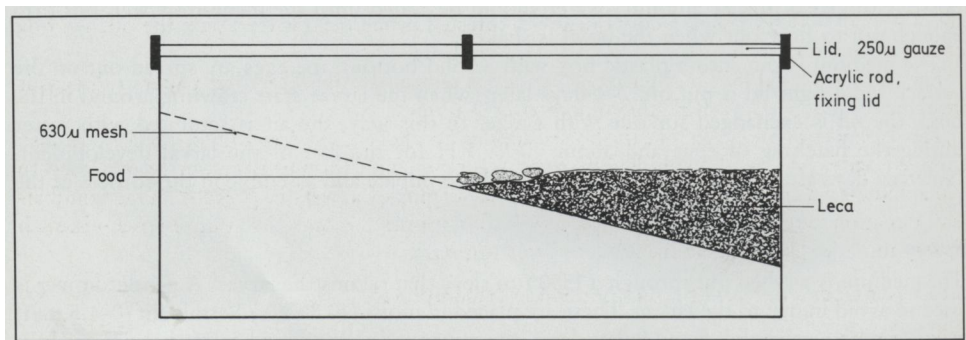


Fig. 3. Cage for 80–100 adult rove beetles (*Aleochara bilineata*)  
Abb. 3. Käfig für 80–100 adulte Raubkäfer (*A. bilineata*)

the hatching and parasitization are promoted by high humidity. After 10 days, the lid is exchanged for a gauze lid, resulting in 70 % RH for the rest of the period.

### Emergence

The emerging rove beetles are collected in “emergence boxes”: Two plastic boxes placed on top of each other, of which the upper has a bottom made of 1550  $\mu\text{m}$  mesh gauze and the lower is closed. The parasitized pupae are placed in the upper box, and when the rove beetles emerge (5–6 weeks after parasitization), they fall through the holes into the lower box, from which they are easily collected daily. Before collection, the box is shaken vigorously to make the rove beetles leave the empty puparia in the upper half. Onion flies emerging during this period stay in the upper box, and may be collected.

## 3.9 *Semiadalia undecimnotata* Schn. (Coccinellidae, Coleoptera)

### 3.9.1 General

The tribe Coccinellini includes many species, mainly feeding on aphids. *S. undecimnotata* above all is the aphidophagous species. It preys mainly on aphid populations developing on low vegetation. Eggs are deposited on the underside of leaves, near aphid colonies, throughout the spring and summer. It has one generation per year. Adults overwinter on hilltops near crop zones, in crowds under stones or at the foot of vegetation.

### 3.9.2 Rearing of *Vicia faba*

Germination and growth of broad bean take place in a controlled-environment chamber at 20°C and 12 h light, 2000 lx. The plants are grown in 4-litre plastic boxes on moist pinewood chips. The seeds pregerminate in water for 24 h, before they are sown in the plastic boxes. After 8 days, the plants are 5 cm high and suitable for aphid infestation.

### 3.9.3 Rearing of aphids

Three species of aphids are used: *Aphis fabae* Scop., *Myzus persicae* and *Acyrtosiphon pisum*. They are reared in a growth chamber at 20°C, 12 h light, 2000 lx, and 70 % RH. Plants and aphids are placed in cubic cages with a side of 40 cm covered with muslin. Infestation is performed by infested cuttings of broad bean put on the healthy plants. The aphid populations are suitable for the ladybird rearing after 1 week.

### 3.9.4 Rearing of *Semiadalia undecimnotata*

The ladybirds are reared in growth chambers at 20°C, 16 h light, 500 lx, and 70 % RH.

Two-litre plastic boxes (26 × 13 × 7 cm) with two circular pieces of cotton mesh inserted in the lid and a filter paper sheet covering the bottom are used for both adults and larvae.

Eighteen females and seven males are placed in each box, and about 200 aphids per ladybird are provided daily. For that, a container with infested broad bean is held over the opened box of ladybirds, and aphids are quickly removed by brushing.

Eggs are deposited on the filter paper, which is removed daily. The filter paper with eggs is incubated at 20°C, 70 % RH; they hatch after 5–6 days.

The larvae are reared in plastic boxes similar to those used for adults, aphids are also supplied daily. At first, 20 aphids/larva/day is enough. The amount is gradually increased to 100 aphids/larva/day. It is necessary to supply a surplus of aphids to prevent larval cannibalism. Pupation takes place in the rearing boxes after approx. 15 days. The pupae remain in such boxes, and adults emerge about 25 days after egg hatch.

## 3.10 *Anthocoris nemoralis* F. (Anthocoridae, Heteroptera)

### 3.10.1 General

*A. nemoralis* is one of the principal natural enemies of the pear psylla (*Psylla pyri* L., *P. pyricola* Först, *P. pyrisuga* Först). It is also a predator of aphids and phytophagous mites.



The bug hibernates as an adult in different shelters such as dead leaves or bark crevices. Activity begins towards mid-March, when the temperature exceeds 10°C, and egg-laying starts on the first leaves or sometimes on the buds. The eggs are inserted under the epidermis of the leaves leaving a small whitish operculum. The first larval generation appears in mid-May. There are five larval stages, and three generations per year – sometimes even four. *A. nemoralis* consumes a large number of pear psylla eggs as well as larvae which it pierces with a powerful stylet and empties. In laboratory tests, the eggs of the pear psylla are replaced by eggs of the flour moth (*Ephestia kuehniella*) as prey.

### 3.10.2 Rearing of *Pelargonium hederacifolium* Salisb.

For egg laying, leaves of geranium (*Pelargonium hederacifolium*) are used. The cuttings are placed in containers filled with “Perlite” in a humidity controlled room (17–25°C, 75–85 % RH) and 1 month later, when the roots have started to grow, they are transferred to pots with soil.

The pots are left for another week in the humidity controlled room before being placed either in a greenhouse (winter) or outside in wire cages (spring, summer and autumn). For the egg-laying of *A. nemoralis*, the leaves are picked fairly young after having had time to toughen (third to fifth leaf from the apex).

### 3.10.3 Rearing of *Ephestia kuehniella*

One rearing method for a large production of *E. kuehniella* has been published by DAUMAL et al. (1975). Recently, a plan for reduced and automatic rearing has been developed and described by DAUMAL et al. (1985). The system is inspired by the behaviour of *E. kuehniella*: The chrysalis is placed in a very restricted space connected to a more spacious cage. The emerging adults go towards the cage at once to spread their wings and let them dry. In the cage, mating and egg-laying takes place. A slope allows the eggs to be collected in a small channel after passing a fine wire grill.

The larvae are fed on a diet of wheat semolina (130 g per unit of production) which may be enriched by 1–2 % of brewers yeast. The exploitation of one production unit during 30 days with 480 moths allows a production of 20 000 to 30 000 eggs at 25°C (16 h light) based on 600 eggs at the beginning with a yield of 80 %. Temperatures outside the range of 15–26°C must be avoided. The units of production can be increased readily depending on the number of eggs required. It is possible to delay the use of produced eggs by storage at 4°C, 80 % RH. Eggs of *E. kuehniella* that are used as food for *A. nemoralis* are sterilized in a ventilated cabinet with an ultraviolet lamp (length of exposure 45 min).

### 3.10.4 Rearing of *Anthocoris nemoralis*

#### Adults

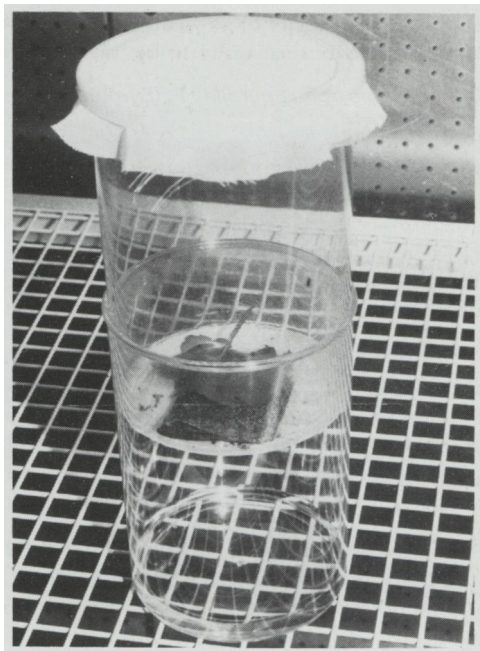
For egg-laying, the adults are placed in a cage made of two round slightly conical polystyrene boxes (upper diameter 11 cm, lower diameter 10.5 cm, height 15.5 cm) that are inserted one into the other (total height 27.5 cm). The lower box contains approx. 200 ml of a saturated solution of NH<sub>4</sub>Cl. The upper box, with a fine nylon gauze (330 μm) replacing the base, contains the adults, food and geranium leaves. It is closed by a round piece of nylon gauze (330 μm) held by a plastic lid cut away in the centre (Fig. 4).

Egg-laying takes place in a controlled environment cabinet at 22±1°C, 80±5 % RH, 17 h light, 2500–3000 lx. It is imperative that the bugs can hide from the light (under a geranium leaf or in the cardboard for food described below).

Strips of black cardboard (0.3 mm thick, 10 cm wide) serve to support the sterilized eggs off *E. kuehniella*. They are folded lengthwise, the top part (5 cm wide) is moistened with demineralized water, then sprinkled with eggs using a salt shaker. After drying, the eggs stay stuck to the strips of cardboard which are then cut into 5-cm pieces (giving a surface of approx. 25 cm<sup>2</sup>) covered with eggs. Fifty adults (sex ratio approx. 1:1) are put in an egg-laying

Fig. 4. Egg-laying cage for 25 couples of *Anthocoris nemoralis*

Abb. 4. Käfig für die Eiablage für 25 Paare von *A. nemoralis*



cage with two geranium leaves placed on the bottom of the upper box, the stem pointing upwards. Two strips with food are placed on either side of the leaf (after 2 weeks of egg-laying, one is sufficient).

The eggs of *A. nemoralis* are usually laid on the hidden side of the geranium leaves, i. e., the side turned against the wire. They are inserted beneath the epidermis of the leaf. Egg-laying starts when the female is 5–6 days old and continues for about 2 months. In ideal conditions (only five couples per cage), the average daily production may be as high as 6.6 eggs per female. The total number of eggs laid by a female during 58 days being 229 with an average rate of hatching of 80 %.

Twice a week, the leaves carrying the eggs are transferred to the rearing boxes and replaced by fresh leaves. The strips are also changed at this time.

From one cage with 25 couples, it is possible to obtain, in 10 weeks, 1500–2000 new adults. Adults can be stored at 6°C for 4 weeks without food supply.

#### Larvae

For the hatching of eggs and rearing of larvae, a round plastic box (10 cm diameter, 5.5 cm high) is used. This is closed with a round piece of nylon gauze held by a lid pierced with holes. Another box, identical to this but having the inside wall coated with Fluon (polytetrafluoroethylene), allows the adults to be counted and selected before being transferred into other cages without risk of escaping.

In these boxes, the first larva does not appear until the 5th day after the eggs have been laid. The larvae are fed until they are fully developed (five larval stages) in the same way as the adults (one cardboard with *E. kuehniella* eggs twice a week). The geranium leaf is removed after 1 week when the food is changed. The cardboards and the leaf are shaken to make the larvae fall to the bottom of the box. Larvae can be stored at 10°C for 3 weeks with food

supply. The majority of new adults appear between the 16th and 19th day after hatching of the larvae. They are then ready to be transferred to new cages for egg-laying.

### 3.11 *Metasyrphus corollae* F. (Syrphidae, Diptera)

#### 3.11.1 General

In Central Europe, zoophagous syrphids are among the most important species for regulation of aphid populations. They are very specialized organisms; larvae which feed on aphids do not accept other food (i. e., eggs of Lepidoptera, Coleoptera, etc.). Up to 850 aphids are eaten by a larva from first instar until pupation. Adult syrphids generally feed on nectar and pollen. Females deposit their eggs among or near aphids. The total number of eggs is very variable and depends on food uptake.

#### 3.11.2 Rearing of *Vicia faba*

Broad bean is used for rearing aphids (*Acyrtosiphon pisum* and *Aphis fabae*). Cultivars which develop big flat beans are more suitable than those giving smaller roundish seed. Beans are grown in pots or transplant dishes standing in a greenhouse. The soil is fertilized with 3 g of compound fertilizer (12N-12P-17K-2Mg) per kg of soil. When the seed has germinated, temperature should not rise above 22°C in order to prevent etiolation of the plants.

#### 3.11.3 Rearing of *Acyrtosiphon pisum* and *Aphis fabae*

The pea aphid (*Acyrtosiphon pisum*) is a very suitable prey for syrphid larvae. However, this species is very active and it may be difficult for first instar larvae to prey on them. Black bean aphids (*Aphis fabae*) are more sedentary and should be preferred for first instar larvae. Both aphid species can be reared under the same conditions.

Plants are infested with aphids when the second leaf has unfolded and shoots begin to elongate. It is advisable to take care that the beans do not grow up too fast: Aphids grow and reproduce better if host plant growth is inhibited. Therefore, the temperature should not rise above 22–24°C, and light intensity should exceed 2000 lx (bulb: SON-T 400). It is recommended to illuminate plants for 14 to 16 h daily. 10–14 days after infestation, the aphid populations are used for the predator rearing.

#### 3.11.4 Rearing of *Metasyrphus corollae*

Rearing of all stages takes place at 22–24°C. For adults, humidity must be controlled to stay between 65–70 % RH and 14–16 h light (500–4500 lx) is necessary.

#### Adults

Adult hover-flies are reared in wooden frame cages (70 × 50 × 50 (h) cm) with gauze sides (0.2 mm) and glass ceiling. In the front door, a sleeve is fitted to prevent escape of the syrphids. Glass jars with a 3 cm bottom of moist gypsum and covered with gauze can be used as smaller rearing units.

Syrphid pupae are placed in plastic Petri dishes which have a bottom of moistened gypsum or filter paper to achieve a high humidity. If humidity is lower than 65 % RH, the adults have problems with hatching. When flies are to be reared in jars, one of the wings is cut off immediately after emergence while they are still sluggish. This is necessary to prevent escape, and the operation does not affect mating. If syrphids are reared in larger cages, it is not necessary to cut their wings. Six to eight flies are kept in each jar and a cage contains up to 100 adults.

Before release of the flies, each cage or jar is supplied with the following:

- (1) Pure honey, offered on a beaker cover. Gauze is pressed on to the honey to prevent the syrphid wings from sticking together.
- (2) 10 % honey-water solution offered on a cotton pad.
- (3) Pollen (hazel, willow, sun flower). Flowers of Apiaceae (Umbelliferae) are sometimes offered for pollen removal.

(4) Pure water, offered on a cotton pad.

The pad with the honey-water solution should be changed once or twice a week, and it is advisable to change the pure honey every fortnight. Pollen must be added daily in small portions, in order to enable syrphids to eat nearly all of it within 24 h.

From 4–5 days after emergence, the females are able to start oviposition. About 7 days after emergence, broad beans infested with black bean aphids should be placed in the cage or jar for egg deposition. Two days later, these plants are exchanged for new ones and kept in another cage for 3–4 days until larval hatch.

No cage should be stocked permanently. After the lapse of one generation, a careful cleaning is recommended (use customary disinfectant solutions) followed by 2–3 weeks emptiness.

Adults survive 3–5 weeks if reared in the laboratory. Life span depends upon food quality and suitable environmental conditions. 400–600 eggs may be laid by each female during this period, but extreme quantities of about 1500 eggs have also been observed. Larvae hatch from about 75 % of these eggs.

### Larvae

To protect the larvae from pathogens, they are reared in small containers. Crystallization dishes 18–20 diameter and 5 cm high are used. The bottom of the dishes is covered with 1 cm moistened gypsum. It is advisable to lay a plastic sheet between dish and gypsum to make cleaning easier. To cover the rearing container, a perforated thin sheet, fixed with an elastic band, can be used. Plants with eggs and aphids are cut and transferred to the crystallization dishes (about 500 eggs per dish). First instar larvae are fed with black bean aphids on broad beans. Aphid-infested plants are cut and placed in the rearing cages daily. After moulting into the second instar, the larvae are fed with pea aphids. For good nourishment, aphids must be offered ad libitum (late second instar larvae eat up to 200 pea aphids each day). Dishes must be cleaned every day. It is recommended to change the gypsum bottom once during the larval development. Gypsum and sheet are only used once. The dish must be sterilized carefully between generations. A small paint brush can be used to remove the first instar larvae from plants or gypsum. For late second instar or third instar, a spring steel tweezer is suitable.

Syrphid larvae need 7–10 days from hatch until pupation. About 1.5 days before pupation, defecation starts. It is recommended to change the dish (gypsum bottom is no longer necessary) in order to prevent the larvae from sticking in their feces or becoming contaminated with those of others. Three days after pupation, pupae are removed from the dishes and placed in the emergence dishes as already described.

## 3.12 *Aphidoletes aphidimyza* Rond. (Cecidomyiidae, Diptera)

### 3.12.1 General

The aphid midge *Aphidoletes aphidimyza* has been used commercially on vegetables in Finland since 1978 (MARKKULA and TIITTANEN 1982) and is currently being evaluated in the UK. As part of this programme, a laboratory rearing method to produce various stages of the midge was developed.

Aphids, along with spider mites and whitefly, are the most serious pests of protected crops. Pesticide resistance is becoming more of a problem; recently, *Aphis gossypii* on cucumber was found to be tolerant to pirimicarb in the UK (ANONYMOUS 1987). It is becoming increasingly more important to find aphid control measures which will integrate with other biological control agents. *A. aphidimyza* is a non-specific aphid predator, its prey includes all the common aphid species found on glasshouse crops, e. g., *A. gossypii*, *Myzus persicae*, *Macrosiphum rosae*, *M. euphorbiae* and *Macrosiphoniella sanborni*. The midge will survive on low aphid populations, requiring only five to eight aphids (UYGUN 1971) to complete its life cycle but, due to a voracious nature, will kill much greater numbers (ROBERTI 1946).

### 3.12.2 Rearing of plants

Chinese cabbage (*Brassica pekinensis* cv. 'Tip-Top') are used to rear *M. persicae* and cucumber (*Cucumis sativus* cv. 'Perfection') for *A. gossypii*. The Chinese cabbages are sown weekly in a peat and grit compost and are kept at 20°C throughout the germination period. After germination, 36 seedlings are transplanted to 12.5-cm plastic pots filled similarly. These plants are then kept at a minimum of 15°C for 4 weeks before being used for aphid rearing. Cucumbers are germinated at 22°C in a peat and grit compost after which eight are transplanted to 12.5-cm pots and kept at 18–20°C for 3–4 weeks before use. Both cabbages and cucumbers are grown with a minimum of 16 h light, supplemented when required by either 400 W mercury vapour or 65/85 W. fluorescent lamps (180 lx). The plants have a basic liquid NPK feed which changes for winter and summer. Winter feed is, in mg/l: 150 N + 15 P + 150 K + 20 Mg. Summer feed is: 200 N + 30 P + 150 K + 20 Mg + 1 Fe. Both feeding programmes are started 2 weeks after transplanting to 12.5-cm pots.

### 3.12.3 Rearing of host aphids

Both species of aphids are kept in a constant temperature room (22°C) with 16 h light, 300 lx and no humidity control. The aphids are reared on plants kept in perspex cages (90 × 40 × 50 cm) (SCOPEs et al. 1974). Each cage is ventilated by ducted air entering from the rear and escaping through a nylon-mesh front. Between cultures, the cages are washed with hot soapy water and then sprayed internally with alcohol. This kills any pathogens which may contaminate future insects.

*M. persicae* are cultured weekly by placing one adult per leaf on nine plants in each of four cages. *A. gossypii* are cultured similarly, but the aphids are placed on the underside of each leaf on all eight plants in a single cage. After 2.5–3 weeks, the plants have sufficient aphids for use.

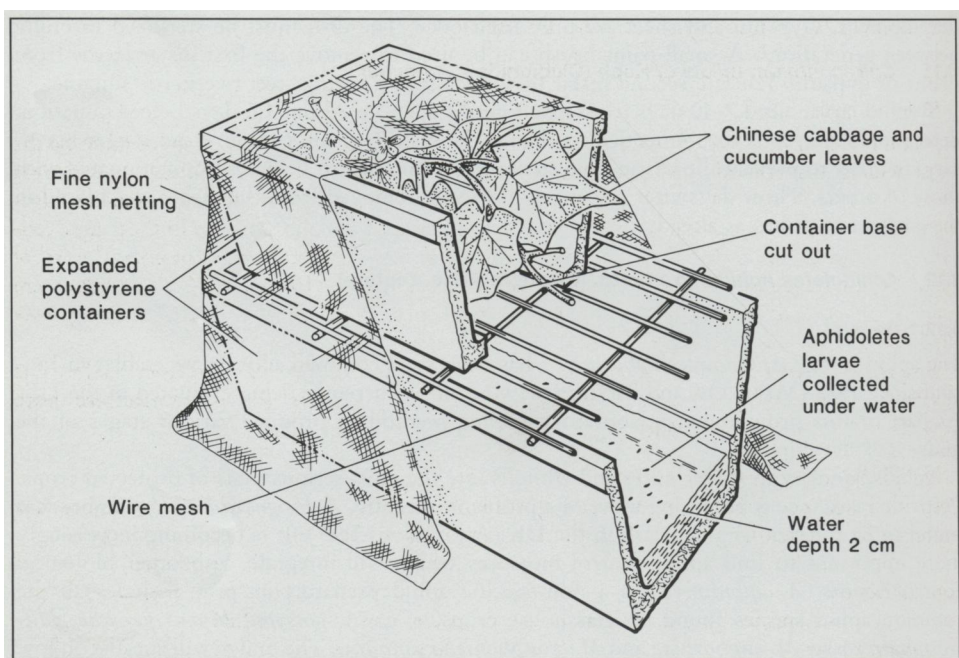


Fig. 5. Cut-away diagram of polystyrene collecting troughs for *Aphidoletes aphidimyza*  
 Abb. 5. Schnittbild durch Sammeltrough aus Polystyren für *A. aphidimyza*

### 3.12.4 Rearing of *A. aphidimyza*

The predatory midge is kept in a separate rearing chamber with constant temperature (22°C), rearing and harvesting processes are carried out on a Monday, Wednesday and Friday. The larvae are light-sensitive and are kept at a photoperiod greater than 15.5 h (300 lx) throughout the rearing period to prevent diapause (HAVELKA 1980). Three Chinese cabbages and one cucumber plant, all infested with aphids, are transferred to each of two perspex cages where they are introduced to adult *A. aphidimyza*. After 2–3 days with the adults, the plants are moved to clean cages for larval development. Extra Chinese cabbages, infested with *M. persicae*, are added 3–4 days later to supplement the food source.

After a further 7 days, larvae are harvested by cutting the leaves off the plants and placing them on a wire grid over a trough of water (Fig. 5). Extra aphids may be added if necessary to allow late developing larvae to mature. The collecting troughs are loosely covered with a fine nylon-mesh to prevent aphids and larvae from escaping. The larvae drop into the water where they can survive for several days (VAN LIEBURG and RAMAKERS 1984) before being transferred to capillary matting for pupation. The clean, grey/white capillary matting (Fibertex superflor SF 250) is cut into 3 × 5 cm strips, laid side-by-side to facilitate handling and placed in a shallow polystyrene tray. Excess water is decanted off and the larvae are washed onto the matting where they form cocoons in which they pupate. Matting and tray is then placed in a dated polythene bag which is kept under 16 h fluorescent light (300 lx) for 7–9 days. The advantage of using a light-coloured capillary matting as a pupation medium compared with peat or sand (MARKKULA et al. 1979) is that the cocoons can be easily observed. Thus, emergence can be quickly assessed and the matting cut to provide known numbers for trials work.

Cocoons bearing pupae can be cold stored at 2–5°C for up to 10 days before use. To collect freshly emerged adults, the capillary matting is placed over wet tissue paper in a plastic pot with a gauze lid. Alternatively, the moist matting can be placed in an inflated polythene bag. Since the bag is sealed after inflation, the matting should not be too wet or condensed water may accumulate in the bag and drown the adults.

## 3.13 *Chiracanthium mildei* L. Koch (Clubionidae, Araneae)

### 3.13.1 General

*C. mildei* is a hunting spider (not web building) active at night. This spider was found to be the most widely distributed species in apple orchards (MANSOUR et al. 1980), in citrus groves (MANSOUR and WHITCOMB 1986) and cotton fields (MANSOUR 1987) in northern Israel. Spiders were found to play an important role in the suppression of injurious insects in these agroecosystems. It was shown that they, and especially *C. mildei*, were important biological control agents of the larvae of *Spodoptera littoralis* Bois. In collections from mid-spring, adults were much more common than immature stages, which probably indicates that *C. mildei* passes the winter mainly as adults or in the penultimate stage.

Empirical observations indicate that *C. mildei* is an indiscriminate and voracious feeder. The spider rarely rejects prey except a few days before every molting. Gravid females are more voracious than at any other stage.

### 3.13.2 Rearing of *Spodoptera littoralis*

Prey is reared on artificial media. For the Egyptian cotton leaf worm *S. littoralis*, such a medium is described by NAVON (1985). The medium is made from:

	Amounts	% (estimated)
(a) Nutrients		
Soybeans (autoclaved with water)	900 g	12.86
Alfalfa meal	170 g	2.43
Yeast powder	300 g	4.28
L-ascorbic acid CP	26 g	0.37

	Amounts	% (estimated)
(b) Antimicrobials		
Methyl-p-hydroxybenzoate	7.5 g	0.11
Propyl-p-hydroxybenzoate	2.5 g	0.03
Chloramphenicol	3.0 g	0.04
Formaldehyde 37 %	25.0 ml	0.36
(c) Alginate-gel mixture		
Sodium-alginate "Protanal SF"	130 g	1.86
Calcium carbonate (precipitated)	6 g	0.09
Citric acid CP	70 g	1.0
Distilled water	<u>5360 ml</u>	<u>76.57</u>

Apart from that, different insects which can be collected by sweep net in any habitat could serve as food for the spiders.

### 3.13.3 Rearing of *Chiracanthium mildei*

To avoid cannibalism, the spiders are kept singly in 30 cm<sup>3</sup> translucent plastic containers, with a perforated top covered with cloth for ventilation. Every second day, they are fed small, 1 to 6-day-old larvae of the Egyptian cotton leaf worm *S. littoralis* and adults of the fruit fly *Drosophila melanogaster*. Adults, larvae and eggs of spider mites *Tetranychus* sp. could also be used. The rearing takes place in a room with controlled environment: 24±1°C, 50–60 % RH and a 16 h light, approx. 2000 lx.

Under standard laboratory conditions, *C. mildei* shows 87 % survival from egg to fertility. Males require a mean of 182 (137–207) days after hatching to reach maturity, become adults following seven to eight molts, and live for an average of 73 days as adults. Females require a mean of 231 (191–286) days after hatching to mature, reach adulthood after nine to ten molts, and live for an average of 240 days as adults. Females mate only once and oviposit from one to five times (average 1.8) at 30-day intervals. They produce a mean of 35 eggs in the first batch and 31 eggs in the second.

### Eggs and eclosion

The female spins a thin and strong egg sac, into which she encloses herself and lays the eggs. The female stays in the sac until the eggs have hatched, and the spiderlings have started growing.

About 10 days after oviposition, the eggs hatch. The first postembryonal period lasts an average of 36 h and the second about 7 days. The spiderlings reach the first true molt 16 days after oviposition. The duration of all subsequent instars vary widely – even between individuals of the same brood reared under standardized conditions.

It has been thought that first instar spiderlings do not feed but are sustained by the yolk that remains in their body. *Chiracanthium* spiderlings appear to feed on the infertile eggs in their egg mass. After the first stage, they are able to feed on larvae of *Tetranychus cinnabarinus* Boisd. and on 1-day-old larvae of *S. littoralis*. After their first feedings, the spiderlings are strong enough to overcome larger prey with no difficulty.

## 3.14 *Phytoseiulus persimilis* Athias-Henriot (Phytoseiidae, Acarina)

### 3.14.1 General

*Phytoseiulus persimilis* is a predatory mite which feeds on all stages of the spider mite (*Tetranychus urticae* Koch). Sex determination is parahaploid (all eggs are fertilized, but males become haploid while females remain diploid) and mating is necessary for oviposition.

At 25°C, the oviposition rate is three to four eggs per female per day. About 20 % of the offspring are males. At this temperature, mature females eat 40–45 spider mite eggs or 10–15 mature spider mites per day. Males and younger stages eat only about a quarter of this. When food shortage occurs, egg-laying decreases proportionally with food intake. Cannibalism arises when there is a total lack of prey. The development from egg to the egg-laying female takes 5–6 days and passes through a larval, protonymphal and a deutonymphal stage.

#### 3.14.2 Rearing of *Phaseolus vulgaris*

The bean plants are grown in a small separate glasshouse compartment (approx. 4 m<sup>2</sup>) at 20–30°C, 50–80 % RH and 12–16 h light (>1000 lx). Bean seeds (most cultivars are suitable) are sown in 6-cm pots in growing-soil in the glasshouse. After the primary leaves have unfolded, the plants are ready for host rearing. The plants are topped regularly during the complete rearing procedures so that only the two primary leaves plus one or two secondary leaves remain.

#### 3.14.3 Rearing of *Tetranychus urticae*

The spider mites are reared in a glasshouse compartment like the one used for beans, but with temperature controlled at 20–25°C. Twice a week, potted bean plants are transferred to this compartment. Batches of 6–20 potted plants, placed in a water-surrounded tray, are infested with spider mites by placing some well-infested leaves from the previous lot on top of the plants. After several days, the plants are well infested and may be used for the mass-rearing of *P. persimilis* or for collecting spider mites as prey.

#### 3.14.4 Rearing of *Phytoseiulus persimilis*

The predatory mites originate from the same source as used by growers. They are reared in a third small glasshouse compartment, where temperature is kept constant at 22°C, RH is 60–70 % and the light is 16 h, 25 lx. Trays (30 × 45 × 8 cm), surrounded by a water barrier, are used to isolate the mites. In the centre of each tray, four bricks (20 × 10 × 5 cm) are placed and the tray is filled with water. Twelve potted bean plants infested with spider mites and predators are placed on the bricks. Twice a week, half of all bean plants are replaced by new plants well infested with spider mites. Leaves of the oldest plants are detached and pegged on a central pin on the brick to retain predators and eggs within the rearing stock.

### 3.15 *Typhlodromus pyri* Scheuten and *Amblyseius potentillae* Garman (Phytoseiidae, Acarina)

#### 3.15.1 General

*Typhlodromus pyri* and *Amblyseius potentillae* are species of predatory mites which are capable of controlling spider mites in orchards and vineyards. *T. pyri* is the more successful species in northwestern Europe, whereas *A. potentillae* seems more effective in southern regions of the continent. Both species feed on spider mites, in particular on *Panonychus ulmi* Koch, but *Tetranychus urticae* is also accepted as food. However, both predators dislike the webbing of *T. urticae*. Rust mites are also fed upon, e. g., *T. pyri* feeds to a large extent on the apple rust mite, *Aculus schlechtendali* Nalepa, thus keeping this pest species under an economic threshold. Both species of predatory mite also feed on pollen grains of a variety of plants. Both species occur on various kinds of trees and *A. potentillae* is also encountered on herbs.

Diapause is induced in autumn. Adult females overwinter; mating takes place in fall. Diapause is terminated in the field in early spring, and in this period both species feed on rust mites and on pollen. By the end of May, *P. ulmi* will become available and then both predators feed on this species of mite as well.

*T. pyri* is not so capable of suppressing sudden outbreaks of spider mites as *A. potentillae*. On the other hand, *T. pyri* will stay in the orchard when prey densities are low, whereas under



such a condition *A. potentillae* may migrate to other areas. Resistance to some carbamates and O-P compounds is commonly found in *T. pyri* in orchards (OVERMEER and VAN ZON 1983) and vineyards. O-P resistance in *A. potentillae* is known from Italy (IVANCICH-GAMBARO 1975).

A wealth of information on the biology of phytoseiid mites was recently brought together by HOY (1982) and HELLE and SABELIS (1986).

### 3.15.2 Rearing of *Phaseolus vulgaris* and *Vicia faba*

*Phaseolus vulgaris* is used for spider mites and *Vicia faba* for pollen production. The bean plants are grown in the greenhouse at 20–25°C and with additional light in winter to 14 h light, > 1000 lx.

Seeds of *P. vulgaris* are placed in soil in pots or plastic trays, or laid on top of the soil and then covered with wet Perlite (2 cm deep). The young plants will appear after 1 week, and after about 10 days, the plants can be used for infestation with spider mites.

Seeds of *V. faba* are placed 2 cm deep in soil in pots. At 20°C, the plants start flowering after 6 weeks. Flowering plants should be kept for 3 weeks at the most to keep the risk of infestation with thrips (*Thrips tabaci* Lind.) as low as possible. Thrips will feed on the pollen in the flowers. Good results for *T. pyri* and *A. potentillae* have been obtained with pollen of broad bean, *Vicia faba*, pollen of the ice plant, *Dorotheanthus bellidiformis* Burman (*Mesembryanthemum criniflorum* L.), and with pollen of the Peruvian lily, *Alstroemeria* sp. The two latter species are best bought from growers. Ice plants can be grown further in the greenhouse starting from young plants. With sufficient sunshine (or additional light) and a temperature of 25–30°C, flowers will soon appear. The plants will last for more than 6 weeks. Daily picking of flowers is necessary; once seed is formed, the plants will cease to flower. *Alstroemeria* is bought as a cut flower and placed in vases.

Collecting pollen of *V. faba* is a time-consuming work. Young flowers are collected and by bending the petals of the flower backwards small clumps of pollen will drop out. Pollen of ice plant and *Alstroemeria* is collected by removing the anthers with forceps.

After collection, the pollen is placed in an oven at 35–40°C for 24 to 48 h. The collected material is then purified by sieving it through a fine gauze (50 meshes/cm) with the aid of a firm artist's brush. The pollen can be stored in small vials in a refrigerator at 4°C for more than 1 year.

### 3.15.3 Rearing of *Tetranychus urticae*

Spider mites are easily mass-reared on young potted bean plants (*P. vulgaris*) in a rearing cabinet or rearing chamber at about 25°C, a RH of 50–80 % in 14 h light, 1000 lx. The population will increase very rapidly in size under these conditions. Old plants should be replaced by fresh ones at least every 14 days. The fresh plants are infested by placing some leaves with mites from the old plants onto the fresh plants. The number of mites in the colony will double roughly every 3 days. See also section 3.14.3.

### 3.15.4 Rearing of *Typhlodromus pyri* and *Amblyseius potentillae*

The phytoseiid mites are reared on arenas modified (by OVERMEER et al. 1982) from the one described by MCMURTRY and SCRIVEN (1965). The arena consists of a 8 × 15 cm tile of black plastic, 5 mm thick, laid on a thick piece of foam plastic (3 cm) saturated with water. The foam is placed in a small plastic tray (14 × 20 × 3 cm) that contains water. Strips of wet tissue paper are stretched round the periphery of the tile and folded over the edges. In this way, about 1.5 cm of the outer margin of the tile is covered and the rest of the strips hang down in the water. The tissue paper barrier thus remains wet and also serves as a drinking water supply. On the tissue paper, near the edges of the tile, an extra barrier is added consisting of a rectangle of sticky material (e. g., Tanglefoot®). This is placed there with the aid of a syringe. The tray must be filled with water regularly.

Predator cultures on arenas are placed in a climatic cabinet or a rearing chamber at 20–25°C. The relative humidity must be above 70 % to ensure egg hatching. However, if pollen is used as food for the predators, the RH should not surpass 90 % because otherwise the pollen will attract mould. A photoperiodic regime of 14 h light is necessary to prevent diapause 25 lx is used.

It is, in general, advisable to renew colonies on an arena frequently and to avoid overlapping generations on the same arena. This makes rearing much more efficient. By transferring eggs to fresh arenas with the aid of an artist's brush, all specimens that develop on the arena are approximately of the same age. This will prevent cannibalism. When adults emerge and start laying eggs, it is best to stop collecting eggs till the 2nd or 3rd day of oviposition, since the first egg laid by a female develops into a male and hence a lot of male progeny is to be expected in the "egg wave" of the first day of oviposition. Eggs of the 2nd or 3rd days of oviposition can be transferred to fresh arenas. The same can be done with eggs produced the next day (or eggs produced over the next 48 h if that is more convenient) and so on, depending on how many arenas one wants to keep. Old colonies can be destroyed. In this way, most of the material in culture consists of young stages and these do not consume as much food as adult females. Moreover, the mass-rearing is at the same time a stage-rearing. Food is added to the arenas, preferably three times a week. When predators are fed on pollen, a small amount is taken from the vials stored in the refrigerator and placed on the rearing arena with the aid of an artist's brush. If the predator is fed on *T. urticae*, either infested bean leaves are laid on the arena, or prey is brushed off the leaves with a stiff artist's brush. The use of a funnel, above which the mites are brushed off the leaves, makes placing of the food easier.

Except for cases where detached leaves are added to the arena, it is necessary to provide the predatory mites with some kind of shelter. Small, 2 cm<sup>2</sup> pieces of thin, transparent plastic cut from overhead transparencies folded to roof-shaped shelters are very suitable. They are also preferred by the mites as oviposition site.

Females of *T. pyri* will produce about 0.5–1 egg per day during the oviposition period which is about 3 weeks. *A. potentillae* produces approximately 2–3 eggs per day for some 2–3 weeks. The development from egg to egg-laying female takes 12–14 days for *T. pyri* and 7–9 days in the case of *A. potentillae*.

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