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# An innovative, low-cost, small-scale rearing method for green lacewings (Neuroptera Chrysopidae)

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**ABSTRACT** In this paper we describe an innovative, low-cost, small-scale green lacewing (Neuroptera Chrysopidae) rearing method developed in our laboratories over a decade. The main simplifications of our method are represented by the replacement of a yeast-fructose liquid diet for adults with bee pollen loads and by the use of *Tenebrio molitor* Linnaeus, 1758 larvae (Coleoptera Tenebrionidae) as factitious prey for larvae. Moreover almost all the components of the rearing cages derive from common cheap materials which can be easily assembled by anybody. Our method proves to be adaptable from a small laboratory to a local farmer's insectary and its innovative aspects could be adopted in (and/or adapted to) mass rearing systems.

**KEY WORDS** bee pollen loads; mealworm beetle; factitious prey; biological control; beneficial insects.

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## INTRODUCTION

Green lacewings (Neuroptera Chrysopidae) have long been recognized as effective biological control agents of a wide variety of arthropod pests, but their use has been directed almost exclusively toward the augmentative method for years (Canard et al., 1984; McEwan et al., 2001). So a method of mass-rearing was developed and a few species of the genus *Chrysoperla* Steinmann, 1964 became commercially available during the 1990s in the USA, Europe and China (Wang & Nordlund, 1994; Hunter, 1997). However, despite many promising laboratory tests and several instances of successful field releases, failures often occurred probably due to the lack of integration between research and commercial development (Tauber et al., 2000).

In the last decade, the amount of research on lacewing mass-rearing techniques has been stationary or even in decline and this is linked to the trend of augmentative methods in biological control (Warner & Getz, 2008; van Lenteren, 2012). So reading the recent review of Pappas et al. (2011) on the role of green lacewings in biological control, we did not find any true innovations compared to similar papers written more than 10 years ago (Tauber et al., 2000).

Thirty five years ago, one of us (RAP) began to rear green lacewings on a very small-scale for taxonomic purposes (e. g. in order to obtain all the stages of a given species or the adults from field collected larvae). Working on the staff of Maria Matilde Principi at the Alma Mater Studiorum, University of Bologna, Italy, his starting point was the method described in Pasqualini (1975). This method was subsequently modified in small steps in order to make it less laborious, less expensive and to save time. Recently, in the laboratory of the ISE-CNR Sassari, Italy, other changes have been made so that it can be used on a laboratory-scale for experimental purposes.

In this paper we want to describe our practices in lacewing rearing because:

i) the well-known large-scale technology is often not suitable when producing lacewings for laboratory purposes and very few papers describe small-scale rearing methods (McEwen et al., 1999);

ii) our method proves to be easily adaptable from a small laboratory to a local farmer's insectary being a simple, low-cost technique;

iii) its innovative aspects could be adopted in (and/or adapted to) mass rearing systems.

## ADULT REARING

Over the years we have reared many Euro-Mediterranean species with glyciphagous adults, scilicet pollen-and-nectar-feeders (Pantaleoni, 2014), excluding the genus *Chrysopa* Leach in Brewster, 1815 which is predaceous. We have never had problems obtaining a new generation from gravid wild females, very few species had difficulty in mating and laying eggs. All of the *Chrysoperla* and almost all of the *Pseudomallada* Tsukaguchi, 1995 species that we have dealt with have been reared continuously for several generations, sometimes for years.

For laboratory purposes, specimens were maintained in a climatic chamber with 20 ± 2°C Temperature, 65 ± 5% Relative Humidity, and a 16:8 h Light:Dark Photoperiod. Among the rearing conditions the key factor was the photoperiod, at Mediterranean latitude almost all species enter in diapause under short days.

The adult rearing unit is a cylinder, 100 mm in height and 80 mm in diameter open at both ends, obtained from a plastic water bottle. The inner part of the cylinder was lined with type "Bristol" yellow cardboard, by rolling up a 100 per 300mm rectangle fixed by two clips. Both bases were closed with square tulle mesh (1.4 mm openings), about 150 mm inside, secured with rubber bands. The cages were kept vertically resting on a base. A water dispenser, containing cotton wool with a plastic cover (half petri dish), was placed on the tulle netting at the top of the container. The unit was put on a tray covered with paper towels to absorb excrement.

Food consisted of honeybee pollen loads. About fifty pollen loads, weighing more or less 300 mg, were put on the bottom of the cage. Water was supplied by dampening the cotton in the dispenser, making sure that the water did not drip down. Every unit hosted no more than four lacewing pairs. The females laid eggs both on the paper and on the tulle netting.

The maintenance of the adult rearing unit took place twice a week. Eggs were isolated as necessary in order to obtain larvae both for experimental purposes and to renovate the laboratory colony. The egg surplus had to be destroyed in order to prevent hatching and subsequent attacks of larvae against adults. With the same interval of time, twice a week, the pollen was renewed and the cotton refilled with water. The cotton, tulle netting and paper towels were substituted at regular but longer intervals.

An experienced technician can easily manipulate the adults by transferring them into a glass tube during rearing unit maintenance, otherwise, with caution, it is possible to anaesthetise them (Loru et al., 2010).

## LARVAE REARING

Larvae were reared individually in order to avoid cannibalism. We used transparent, plastic, cylindrical containers both 25mm in height and in diameter with a plastic lid. The eggs were isolated by cutting the cardboard or tulle on which they had been laid and were put singularly into the containers using tweezers. As food we used mealworm beetle larvae (the Coleoptera Tenebrionidae *Tenebrio molitor* Linnaeus, 1758) previously killed with ethyl acetate. Also a drop of fructose solution was provided to the newly-hatched larvae (Pantaleoni, 2014). Different lacewing larvae instars were fed on mealworms of the appropriate size, in particular 4–5 mm long and 0.5–1 mg in weight vs 1st instar, 6–8 mm long and 2–4 mg in weight vs 2nd instar, 9–12 mm long and 6–12 mg vs 3rd instar. Nevertheless an expert technician is perfectly able to sort them by eye. Three mealworms were given to each lacewing larva twice a week and every time residues of the previous meal were eliminated. Initially a little piece of paper towel was put into the container in order to absorb exudates from dead mealworms and offer a suitable support during cocooning. When the cocoon was about one week old, the residues of its last meal were eliminated. At the same time a 30mm high piece of "Bristol" cardboard was put into the container in order to provide support for pharate adults and facilitate adult emergence. Adults which had just emerged were sexed and put into the container described above.

## DISCUSSION

Our adult rearing unit (Fig. 1) can be easily assembled by anybody. All the components derive from common cheap materials and a few simple tools such as scissors, a paper cutter, a ruler, a set square and a pencil are needed. Only the cap of the water dispenser is a specialized laboratory item (half of a petri dish), but this is also easily replaceable. Neither individual containers nor possible communal cages for larvae have been developed although there are many opportunities for improvement.

The replacement of a liquid meridic diet for adults with a solid oligidic one, such as pollen loads, is the main simplification of our method. According to our experience the management of a liquid yeast-fructose diet is laborious. This diet requires frequent cleaning or changing of the dispenser. It is both rapidly perishable and filthy, insects often get themselves dirty becoming incapable of flying or even moving. On the contrary pollen loads are clean, easy to give and to remove and more durable. In nature adult lacewings are essentially pollen-feeders and pollen, as stated by Nordlund et al. (2001), has good nutritional qualities. Moreover adult lacewings harbour mutualistic yeasts (*Turolopsis* sp.) that synthesize essential amino acids which are missing from their diet (Hagen et al., 1970). The pollen loads contain these yeasts, collected in the environment by bees (Gilliam, 1979). The slight disadvantage of using pollen is the dramatic difference in quality of the various kinds of pollen derived from different plants (Nordlund et al., 2001).

Just as the pollen-feeder habit of adults drove us towards our choice of pollen loads, the occasional scavenger habits of lacewing larvae (repeatedly

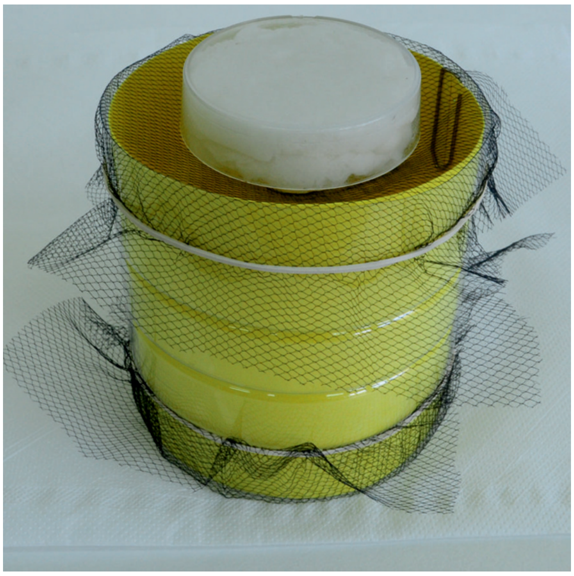

Figure 1. Adult rearing unit.

observed in nature by one of us (RAP), made us choose the killed mealworms. The use of *T. molitor* as factitious prey for lacewings was reported for the first time at the IX International Symposium on Neuropterology held in Ferrara, Italy, in 2005, but it remained unpublished. Since then, only Pappas et al. (2007) tested mealworms as a prey for lacewings, but using "second instar mechanically injured" instead of killed larvae of a larger size. Although the aim of the paper was completely different, and the use of *T. molitor* as factitious prey was not even cited, Loru et al. (2010) published the same kind of data. Pappas et al. (2007), rearing a species of *Pseudomallada* (then *Dichochrysa* Yang, 1991), found a short lifespan and low female fertility in specimens fed with mealworms. Loru et al. (2010), rearing a species of *Chrysoperla*, found a much longer lifespan (max 120 days vs max 45 days) and a higher fecundity (recalculated in order to compare the two papers) (about 650 eggs/female vs 192).

The great advantages in the use of mealworms as factitious prey are the minimal physical space required for their rearing, their high conversion efficiency, their potential for massive production and the chance to use organic waste materials as a food source (Ramos-Elorduy et al., 2002).

The future of biological control in agriculture will be played in two different arenas: the enhancement of "big" technology (biotechnology, industry, worldwide market) to apply to large scale productions, and the increase of “sustainable” technology to apply to local development policies. In both cases, the lacewings will be able to give a little help as beneficial through their mass production or their conservation by means of habitat management, but in any case we should be able to rear them.

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