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Rearing of parasitoid braconid wasp *Dinocampus coccinellae* in a simplified tritrophic system

Santolo FRANCATI
Dipartimento di Scienze e Tecnologie Agro-Alimentari (DISTAL), Università di Bologna, Italy

Abstract

Mass-rearing of beneficial arthropods often requires a multitrophic system for the rearing of entomophagous species. The use of artificial diets can facilitate this work by simplifying the number of elements in the system. Rearing *Dinocampus coccinellae* (Schrank) (Hymenoptera Braconidae), a parasitoid of different ladybird species, requires four elements: plants where the prey is reared, prey (i.e. aphids), hosts that feed on that prey (such as ladybirds) and the said parasitoids. This study attempted to simplify this rearing system by feeding the host, for a part of its life, with an artificial diet. A series of life history parameters was measured in 3 generations of parasitoid rearing. Results show that the host's food, parasitoid generation, or interaction between these two factors, does not affect the yield of *D. coccinellae* adults. However, the time of development became longer and the adult lifespan (of second generation) became shorter with artificial food. Overall, the data seems to suggest that it is possible to simplify a multitrophic system without great consequences on the performance of parasitoids, if the nutritional needs of the species are supported.

Key words: Mass-rearing, multitrophic system, *Harmonia axyridis*, *Ephestia kuehniella*, artificial diet.

Introduction

The mass-rearing and release of beneficial arthropods are the fundamentals of augmentative biological control (King, 1993; Elzen and King, 1999; Morales-Ramos and Rojas, 2003). In advantageous systems, the costs of mass-rearing are balanced by the economic and environmental benefits of the use of the beneficial species. Insects have similar basic nutritional requirements, regardless of the type of food consumed (Thompson and Hagen, 1999; Nation, 2002). However, rearing predatory and parasitic insects requires the additional step of rearing prey/hosts. Many of the current methods need a tritrophic system of rearing: the host plant, the natural prey (usually a herbivorous pest) and the entomophagous insect. This situation doubles the costs of rearing due the production of two species, plant and pest, in order to obtain a third one of interest (Van Driesche and Bellows, 1996). Predatory arthropods can develop and reproduce on easy-to-rear factitious prey, which has been often used in their mass-rearing (Van Driesche and Bellows, 1996). Many of these commercial natural enemies are omnivores and feed not only on different prey, but also on different plant derived food such as pollen, nectar, fruit and leaves (Dixon, 2001; Coll and Guershon, 2002; Lundgren, 2009). Examples include the insidious flower bug *Orius insidiosus* (Say) and the ladybird species *Harmonia axyridis* Pallas, *Coleomegilla maculata* (DeGeer) and *Hippodamia convergens* Guerin-Meneville (Lundgren, 2009). Parasitoids are able to complete their development in a single host, if this supplies all nutritional needs; but in many cases they are capable, by the actions of venom, of inducing changes in their host's chemistry (Morales-Ramon *et al.*, 1995; Quicke, 1997).

Developing artificial diets is a way to simplify and make more cost-effective the mass production of beneficial predators and parasitoids. However, when artificial diets are less nutritious than the natural prey or hosts (Grenier, 2009), then their quality as biological control agents decreases (Grenier and De Clercq, 2003; Riddick, 2009). In the last years, numerous studies were aimed at improving an artificial diet tailored to the development requirements of entomophagous species (Riddick and Chen 2014; De Clercq *et al.*, 2014; Dindo and Grenier, 2014), but few attempts at rearing parasitoid hymenopterans were successful (Xie *et al.*, 1989; Magro and Parra, 2004).

Dinocampus coccinellae (Schrank) (Hymenoptera Braconidae) (supplemental material figure S1) is a cosmopolitan solitary hymenoptera parasitoid of adult ladybirds belonging to the subfamily Coccinellinae (Ceryngier *et al.*, 2012), including *H. axyridis* both in native (Park *et al.*, 1996) and introduced areas (Firlej *et al.*, 2005; Berkvens *et al.*, 2010; Dindo *et al.*, 2016a). Females usually reproduce by thelytokous parthenogenesis (Ceryngier *et al.*, 2012) and they oviposit in adult ladybirds. However, when adult hosts are scarce, they can also parasitize larvae and pupae (Smith, 1960; Maeta, 1969; Francati, 2015). The rate of parasitism of ladybirds by *D. coccinellae* may fluctuate considerably depending on the location, season, host species and, within the same species, host age and diet (Ceryngier *et al.*, 2012; Maure *et al.*, 2016). During most of its development, the *D. coccinellae* larva does not feed directly on the host's tissues (Sluss; 1968; Dahlman *et al.*, 2003). As a consequence, most of the host's organs remain intact and the host can survive some days after the last instar larva emerges (Triltsch, 1996).

H. axyridis, originated from Asia (Kock, 2003), is an active predator of aphid and coccid pests introduced (intentionally or accidentally) in different countries (Brown *et al.*, 2011). The mass rearing of this species is relatively flexible; it can be fed on a variety of aphid species (Hodek and Evans, 2012), but also with eggs of various Lepidoptera (Specty *et al.*, 2003), pollen

(Berkvens *et al.*, 2008) and some artificial diets (Dong *et al.*, 2001; Sighinolfi *et al.*, 2008; 2013). However, different foods produce differences in the biochemical composition of the adult. Specty *et al.* (2003) found that *H. axyridis* reared on aphids *Acyrthosiphon pisum* (Harris) had lower protein content in their body than those reared on the eggs of the factitious prey *Ephestia kuehniella* Zeller, whereas Sighinolfi *et al.* (2013) found that newly emerged females reared on pork liver-based artificial diet showed a higher amount of fatty acids than those reared on *E. kuehniella* eggs.

This work is an attempt to simplify the multitrophic system required for the mass-rearing of a ladybird Hymenoptera parasitoid. It investigates the effects of rearing ladybirds on an artificial diet during their adult life, on the development and performance of the parasitoid in 3 generations. The study was performed using the braconid wasp *D. coccinellae* as a parasitoid and the *H. axyridis* as host.

Materials and methods

Rearing of *Harmonia axyridis*

A colony of *H. axyridis* was started from eggs laid by females collected in the field in Bologna (Emilia-Romagna region, Italy, 44°48'39"N 11°37'84"E) in April 2010. The colony was maintained in the laboratory of the Department of Agricultural and Food Sciences (DISTAL), University of Bologna (Italy), in a rearing chamber at 26 ± 1 °C, 65 ± 5 RH and 16:8 L:D. As described by Dindo *et al.* (2016a), all stages of a standard ladybird colony were fed *ad libitum* with *Myzus persicae* Sulzer reared on *Pisum sativum* L. (supplemental material figure S2).

Rearing of *Dinocampus coccinellae*

A colony of *D. coccinellae* was started from individuals that emerged in September 2010, from field-collected *H. axyridis*; the same species was used as host to maintain the colony. As described by Dindo *et al.* (2016a), the parasitoid adults were maintained in a Plexiglas cage (20 × 20 × 20 cm) and fed with tiny honey drops, in a rearing chamber at 26 ± 1 °C, 65 ± 5 RH and 16:8 L:D. Weekly, adults of *H. axyrydis* were exposed to *D. coccinellae* (ratio: 10 ladybirds/1 parasitoid, during 1 h), after which the hosts were removed and maintained in a plastic box until the parasitoid's cocoon detection (supplemental material figure S3). The ladybirds were fed with *E. kuehniella* eggs *ad libitum* (supplemental material figure S4), eggs were preferred to aphids because they facilitate the detection of new cocoons, having fewer places to hide.

Experimental design

For the experiment, cocoons of *D. coccinellae* from the mother colony were placed individually in a plastic cylinder (20 cm height, 9 cm diameter) and maintained in the same conditions as the colony, until the trials, 2 days after emergence. Pupae of *H. axyridis* were taken from the mother colony, placed in a plastic box and checked daily. When the ladybird adults emerged, they were divided randomly into two groups of 5 individuals. The first group was fed with a pork liver-based artificial diet, specifically developed by Sighinolfi *et al.* (2013) in order to improve the performances of a previous diet (Sighinolfi *et al.*, 2008) and to increase the yield of the rearing of this species (supplemental material figure S5). The diet was integrated with a cotton ball soaked in water. The second group was fed with frozen *E. kuehniella* eggs and maintained as control. Each group of ladybirds was reared in a plastic box (5 × 10 × 15 cm) until 2-4 days of life.

For each replicate, 10 *H. axyridis* were exposed to 1 *D. coccinellae*, in the rearing cylinder, for one hour (supplemental material figure S6). After the exposure, the ladybirds were removed, replaced in a plastic box and monitored daily for 30 days, in order to check cocoon emergence or ladybird death. In the first 20 trials (parental generation), 20 adults of parasitoid and 100 adults of ladybird, of both sex, were used. Afterwards, 10 replicates were performed for F1 generation and 10 replicates for F2 generation; in both cases 10 parasitoids and 50 ladybirds were used.

Parameters

In order to evaluate the possible effect of host food (artificial diet and *E. kuehniella* eggs) on the development of *D. coccinellae* larvae, the yields and the time of development of the parasitoid were analysed. The yields were estimated in terms of: percentage of cocoons emerged from the hosts (number of cocoons/number of ladybirds exposed); percentage of adult parasitoids (number of emerged adult parasitoids/number of cocoons); total percentages of adults (number of adult parasitoids/number of ladybirds exposed). The percentage of survival of *H. axyridis* exposed was also considered. The times (in days) were calculated: from the exposure of the hosts to the parasitoid cocoons detection; from the cocoon detection to the adult emergence; total development time (from host exposure to adult emergence); adult *D. coccinellae* lifespan.

The number of cocoons found, the adults emerged (from cocoons), the adult yields (on the original number of ladybirds exposed) and the survival of *H. axyridis* were analysed by 2 × 2 contingency tables. The development times (for all treatments) and adult lifespan were analysed by a factorial analysis of variance (Zar, 1984). The effects of food (*E. kuehniella* eggs or artificial diet) and generation (parental, F1 and F2) were tested by 2 × 3 factors analysis. All statistical tests were done with STATISTICA software for Windows (StatSoft, 2011).

Results

The yield of *D. coccinellae* adults was not statistically different among the parameters considered (figure 1). The percentages (± SE) of cocoons detected, on the total number of *H. axyridis* exposed, showed no difference in terms of the food of the hosts (F = 0.06, df = 1.73, P = 0.81), the effects of the generations of the parasitoid (F = 2.01, df = 2.73, P = 0.14) or the interaction between

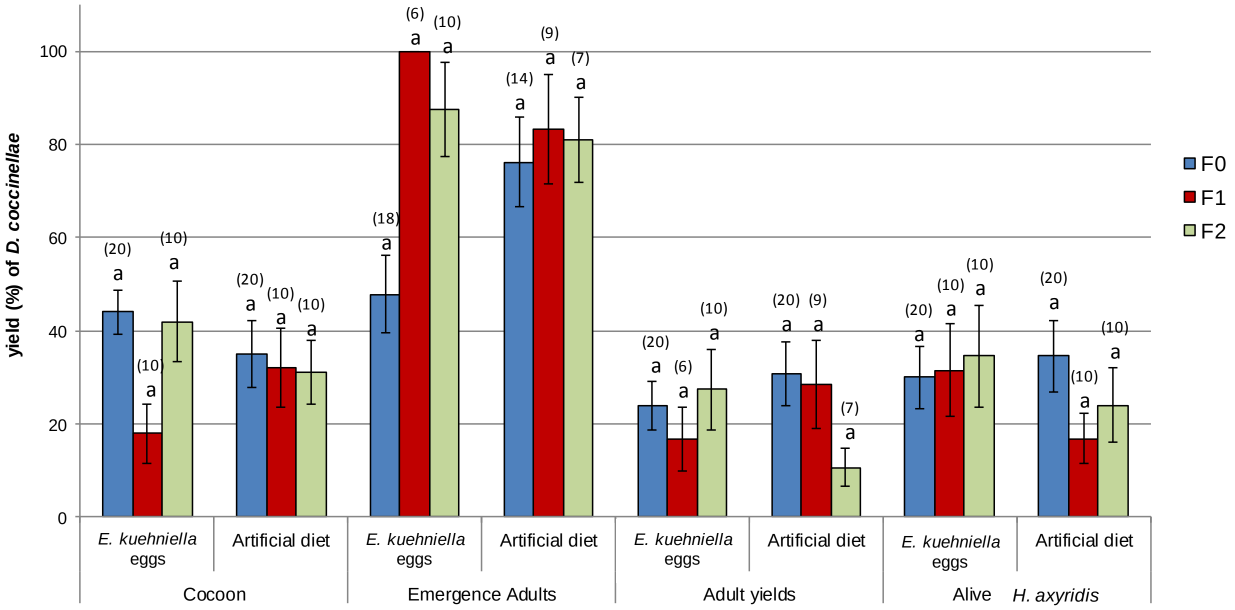

Figure 1. Percentage yield (± SE) of *D. coccinellae* in *H. axyridis* reared on two different types of food (frozen *E. kuehniella* eggs or artificial diet). F0 = parental generation; F1 = first generation; F2 = second generation. Cocoon = cocoons found on total number of ladybirds exposed; Emergence adults = parasitoid adult emergence from the cocoons obtained; Adult yields = adult yields on total number of ladybirds exposed; Alive *H. axyridis* = percentage of ladybirds alive at the end of experimentation. The number of replicates is given in parenthesis, means in a column followed by the same letters are not significantly different.

these two factors (F = 1.83, df = 2.73, P = 0.16). Similarly, the yield of adults from these cocoons was high (figure 1) and was not influenced by the food of the hosts (F = 0.34, df = 1.58, P = 0.56), the generations of the parasitoid (F = 0.35, df = 2.58, P = 0.71) or the interaction between these two factors (F = 0.07, df = 2.58, P = 0.93). The percentages of parasitoid adults obtained (± SE), on the total number of *H. axyridis* exposed, was also unaffected by the food type of the hosts (F = 0.90, df = 1.66, P = 0.35), the generations of the parasitoid (F = 0.60, df = 2.66, P = 0.55) or the interaction between these two factors (F = 0.16, df = 2.66, P = 0.85). Finally, the survival percentages of *H. axyridis* was low (figure 1) but was not influenced by the food type (F = 0.19, df = 1.73, P = 0.66). Moreover, no significant differences on the efficacy of the parasitoids, on the number of exposed hosts, were found between the generations (F = 0.65, df = 2.73, P = 0.53) or interaction between the factors (F = 0.43, df = 2.73, P = 0.64).

Results show that the time of development and adult lifespan were, to some extent, affected by the food type, the generations or their interaction (figure 2). The data concerning the mean time (± SE) from host exposure to *D. coccinellae* cocoon detection did not show any statistical difference between the two groups if we consider the food of the hosts (F = 0.25, df = 1.59, P = 0.62), the effects during the generations of the parasitoid (F = 0.57, df = 2.59, P = 0.95) or the interaction between the two factors (F = 0.75, df = 2.59, P = 0.48). However, the mean time (± SE) from cocoon detection to parasitoid adult emergence was statistically different between generations (F = 4.86, df = 2.52, P = 0.01), but not due to the host's food type (F = 0.26, df = 1.52, P = 0.61) or the interaction between the two factors (F = 1.69, df = 2.52, P = 0.19). The mean time from cocoon to adult was longer in F1 and shorter in F2 (F = 4.73, df = 2.55, P = 0.012) (figure 3). The total development time was influenced both by the food effect (F = 4.27, df = 1.52, P = 0.04) and by the generation effect (F = 6.36, df = 2.52, P = 0.003). Development time was longer for the parasitoids reared on diet-fed *H. axyridis* (F = 5.49, df = 1.56, P = 0.02) and shorter for the F2 parasitoids (F = 6.69, df = 2.55, P = 0.0025) (figure 3). Lastly, the parasitoid lifespan was significantly affected by the generation (it was shorter in F2 parasitoids, F = 15.07, df = 2.51, P = 0.00001) and less by the interaction between food and generation (F = 3.33, df = 2.51, P = 0.04); no effect was found for the host's food (F = 0.72, df = 1.51, P = 0.40).

Discussion and conclusions

Mass-rearing is a complex process that involves a multidisciplinary approach and requires a considerable economic investment in technology, facilities and qualified personnel. Developing an artificial rearing system for parasitoids involves more than supplying a high quality artificial diet to the immature stages or to their hosts; it also requires understanding the nutritional needs imposed by host-parasitoid interactions (Consoli and Parra 1999; Dindo *et al.*, 2016b).

This experiment was aimed at improving and simplifying the parasitoid's rearing, building on previous successful studies where *E. kuehniella* eggs were used as food for the hosts instead of aphids (Dindo *et al.*, 2016a).

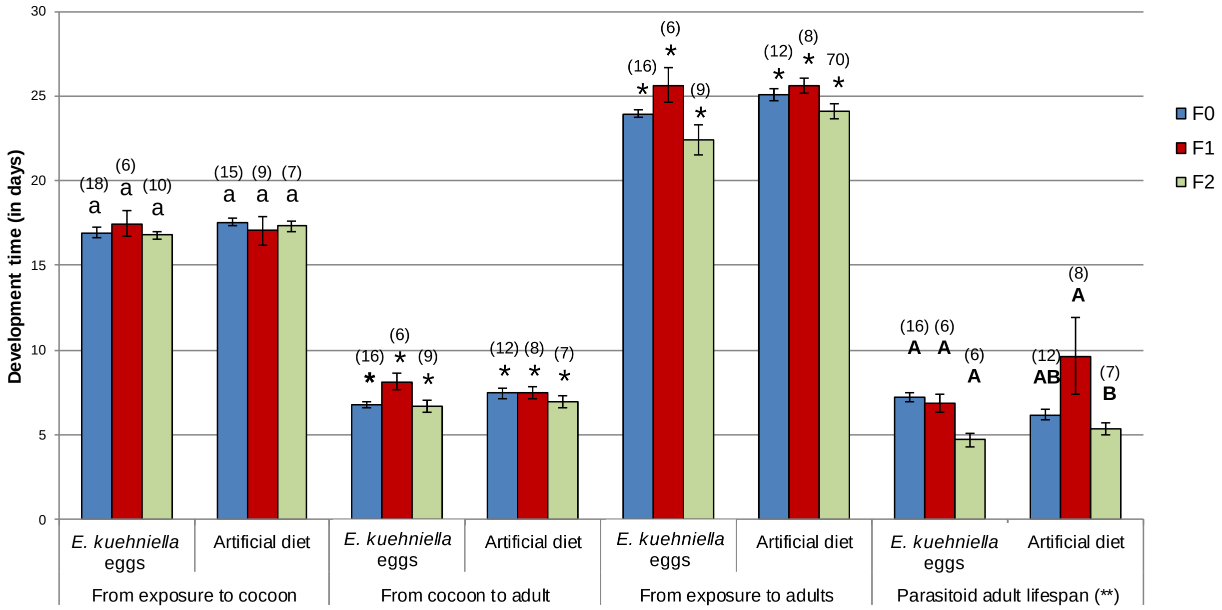

Figure 2. Mean times in days (± SE) of development of *D. coccinellae* in *H. axyridis* reared on two different types of food (frozen *E. kuehniella* eggs or artificial diet). F0 = parental generation; F1 = first generation; F2 = second generation. From exposure to cocoon = time from ladybird exposure to parasitoid cocoon detection; From cocoon to adult = time from parasitoid cocoon detection to adult emergence; From exposure to adults = time from ladybird exposure to parasitoid adult emergence; Parasitoid adult lifespan = time of life of adult parasitoids from emergence to death. The number of replicates is given in parenthesis, means in a column followed by the same letters are not significantly different. (*) The grand means for the generation effect and for the food and generation effect are shown in figure 3. (**) Adult parasitoid lifespan as related to the combination of the factors "food" and "generation", the means followed by same capital letters were not significantly different.

These *E. kuehniella* eggs were chosen because contain more amino acids and lipids compared to the aphid *A. pisum* used to rear *H. axyridis* (Specty *et al.*, 2003), although this aphid contains a greater percentage of glycogen. In addition, the eggs of this Lepidoptera have a positive effect on the growth and reproduction of this ladybird (Specty *et al.*, 2003). However, if fed over many generations on *Ephestia* eggs, it could show a reduction in the capacity of larvae to search and kill its natural prey (Ettifouri and Ferran, 1993; Ferran *et al.*, 1997).

The artificial diet tested (Sighinolfi *et al.*, 2013) is easy to prepare and cheap compared to *E. kuehniella* eggs. Results showed that it is possible to rear *D. coccinellae* on *H. axyridis* adults fed with an artificial diet and this may allow to speed up and facilitate parasitoid rearing. The yield and the survival of the parasitoid were not influenced by the host's food, and *D. coccinellae* could develop up to 2 generations without major impact on the adult yields. Notwithstanding, the total development time (from host exposure to adult emergence) and the adult parasitoid lifespan were influenced by the host's food. The total development time was longer in the group fed with artificial diet and the adult parasitoid lifespan was longer for parasitoids obtained from *H. axyridis* fed on an artificial diet, in F1 and F2 generations.

A possible explanation for these differences could relate to a different chemical composition of the hosts, although this hypothesis would require further study. In this study, the *H. axyridis* were 2-4 days old and fed on an artificial diet or *E. kuehniella* eggs, in which time the host's gonads matured and the body fats stored lipids (Kock, 2003). The diet used to feed *H. axyridis* adults in this research was an attempt to improve a previous diet (Sighinolfi *et al.*, 2008) and it has a different composition in fatty acids compared to the eggs of *E. kuehniella* (Sighinolfi *et al.*, 2013). Lipids are mainly considered as a source of metabolic energy, but they are also necessary for development and reproduction, and are essential structural components of the cell membrane (House, 1961; Agosin, 1978; Downer, 1978; Chapman, 1998). As reported by different authors, the fatty acid composition of adult insects reflects the composition of their diet (Cohen, 1990; Iriarte and Castane, 2001; Specty *et al.*, 2003; Zapata *et al.*, 2005; Sighinolfi *et al.*, 2008). Moreover, some species of parasitoid show a similarity of lipid composition with their host and this suggests that host lipids may be absorbed with few modifications (Delobel and Pageaux, 1981; Grenier, 2012). During their development, *D. coccinellae* larvae feed on the gonads and fat bodies of their host (Triltsch, 1996) and it is possible that the different quality of fatty acids present in the two groups affected over time the development of the larvae and their adult lifespan.

This experiment was not aimed at making a comparison with aphid-fed adults of *H. axyridis* or at rearing ladybirds for their whole development on an artificial diet or *E. kuehniella* eggs, although this would be an interesting approach for future tests. However, it does

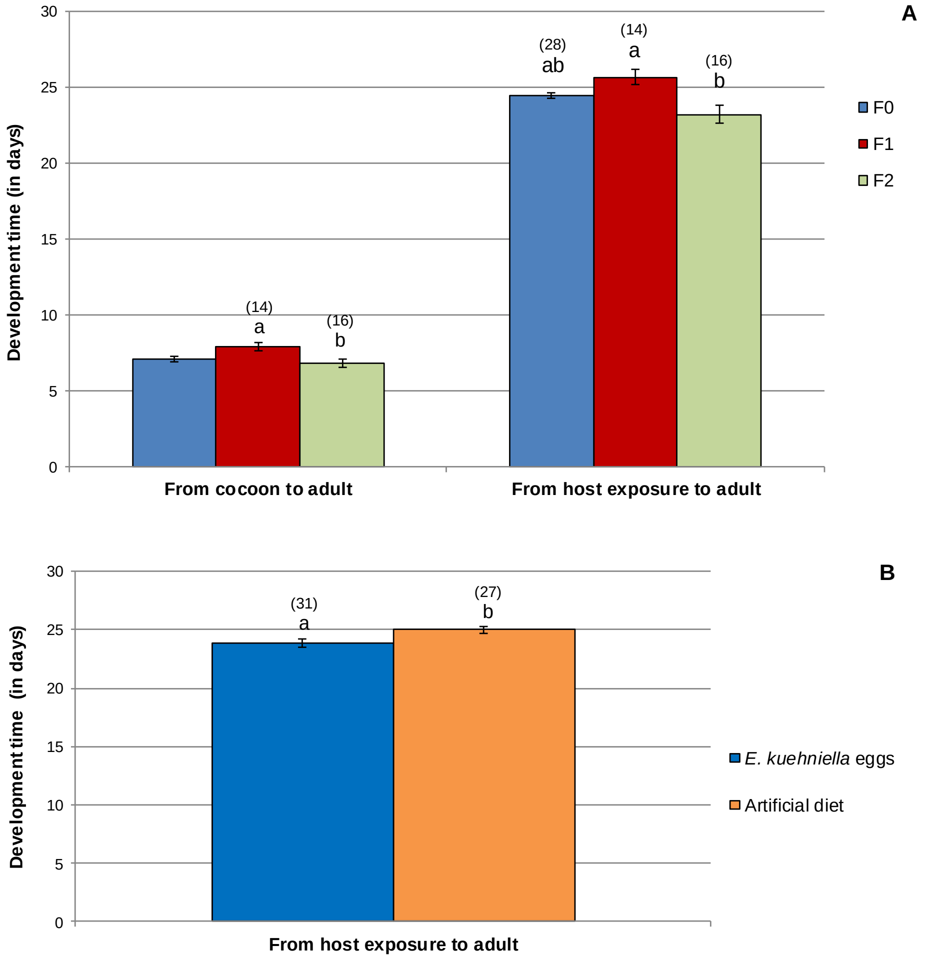

Figure 3. Grand means, on the number of replicates, (**A**) for the generation effect on *D. coccinellae* development time (1) from cocoon detection to adult emergence and (2) from host exposure to adult (total development) and (**B**) for the food effect on *D. coccinellae* development time from host exposure to adult (total development). Means in a column followed by the same letters are not significantly different.

lay the basis for such studies since the results show that it is possible to rear a parasitoid in a simplified multitrophic system, without significant quality reductions in the "fitness/yield" of parasitoids, at least in a first generation that might be relevant for biological control programs. Parasitoids reared on artificial media for a long time often show a reduction in quality (Grenier and De Clercq, 2003; Marchetti *et al.*, 2008; Riddick, 2009). To reduce or eliminate this risk, it would also be interesting to analyse in future studies if, as some research (Dheilly *et al.*, 2018) suggests, the *Dinocampus coccinellae* paralysis virus (DcPV), injected by this parasitoid inside the host, is really able to manipulate the physiology of the host.

Artificial diets for entomophagous arthropods have seldom been used in commercial mass production, but rather to facilitate biological research (Cohen, 2001). Nevertheless, a good knowledge of the biological and physiological necessity of these species is the key to subsequently improve their mass production.

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Author's address: Santolo Francati, Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* Università di Bologna, viale G. Fanin 42, 40127 Bologna, Italy (santolo.francati2@unibo.it).

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