Bulletin of Insectology ***66** (2): 283-290, 2013*
ISSN 1721-8861

Biochemical content in fatty acids and biological parameters of *Harmonia axyridis* reared on artificial diet

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Abstract

A flightless strain of *Harmonia axyridis* (Pallas) (Coleoptera Coccinellidae) was fed, at the larval and/or adult stage, on a liver-based artificial diet supplemented with lin-seed oil, which is rich in α-linolenic acid (C18:3n-3), an essential lipid for the fecundity of different insect species, and other unsaturated fatty acids. Biochemical analyses revealed that, compared to *Ephestia kuehniella* Zeller eggs (the control food for *H. axyridis*), the diet content of C18:3n-3 was higher ($P < 0.05$), whereas the content of linoleic acid (C18:2n-6) was not significantly different ($P > 0.05$). The composition in fatty acids of *H. axyridis* newly-emerged females, which were fed on the artificial diet during the larval stages, reflected the composition of the diet. In particular, these females showed a higher concentration of α-linolenic acid compared to control females. Moreover, no difference was found for total fatty acids between the control newly emerged females and those reared as larvae on the artificial diet. On this diet, fecund *H. axyridis* adults were obtained, but the emergence rates and a number of quality parameters (including the development times, the weights of the newly-emerged adults, the female fecundity and fertility) of the diet-fed *H. axyridis* were worse compared to the coccinellids fed on *E. kuehniella* eggs. The diet may however be utilized for adult maintenance, in order to reduce production costs, or in case of shortage of aphids or *E. kuehniella* eggs.

Key words: artificial diet, predator, fatty acid, nutrition, quality control, rearing.

Introduction

The multicolored Asian ladybeetle *Harmonia axyridis* (Pallas) (Coleoptera Coccinellidae), an active predator of aphids and scales, was introduced as a biological control agent in North America and Europe, where it has spread rapidly and is now regarded as an invasive alien species (van Lenteren *et al.*, 2008; De Clercq *et al.*, 2011). During many years, *H. axyridis* was considered as an attractive biological control agent, especially for aphid populations, because its larvae are very voracious and easy to rear (Roy *et al.*, 2006; Soares *et al.*, 2004). In most European countries, its commercialization has now been interrupted due to the concern aroused by its invasiveness and possible impact on the indigenous coccinellids and other predators (Burgio *et al.*, 2002; Santi and Maini, 2006; Kenis *et al.*, 2008; Alhmedi *et al.*, 2010). In China, *H. axyridis* is a key wild polyphagous predator, also widely released to control a lot of important agricultural, horticultural and forest pests like aphids, psyllids, and scale insects (Wang *et al.*, 2005; Qin *et al.*, 2011).

A lot of research work on the rearing technique of *H. axyridis* on alternative food, including lepidopteran eggs (Schanderl *et al.*, 1988) and artificial diets, has been carried out in the past years (Matsuka and Okada, 1975; Niijima *et al.*, 1986; Obrycki and Kring, 1998; Dong *et al.*, 2001; Specty, 2002; Sighinolfi, 2005; Sighinolfi *et al.*, 2008; Chen *et al.*, 2012). This work deserves to be continued, because, apart from any other consideration, it contributes to increase our knowledge on the entomophagous insect nutrition, especially predacious coccinellids that represent efficient biocontrol agents. Moreover some Asian countries might be interested in this rearing.

A pork liver-based artificial diet was developed to feed first through fourth instar larvae, and adults of *H. axyridis* (Sighinolfi *et al.*, 2008). This diet was tested and improved with an analytical approach and subsequent interactions, by comparing the biochemical composition of the ladybeetle larvae reared on the diet *vs.* those fed on *Ephestia kuehniella* Zeller eggs (as controls). Lower amino acid and fatty acid contents were found for the prepupae and newly-emerged females obtained from the larvae fed diet, compared to controls. The analyses of the foods showed deficiencies in the artificial diet for some amino acids, but especially for some fatty acids (C16:0, C16:1 and C18:3n-3). In the present work, a liver-based diet supplemented with lin-seed oil, rich in unsaturated fatty acids, was offered to *H. axyridis*. Biochemical analyses, concerning the body content of fatty acids, and biological parameters of the ladybeetles reared on the diet *vs.* those fed on *E. kuehniella* eggs were compared. Whole insect body analyses were performed to try to obtain information about the nutritional needs of the Asian ladybeetle as well as a necessary reference for deficiency and imbalance corrections in the food composition (Grenier and De Clercq, 2003; Cohen, 2004). The present work focused on polyunsaturated fatty acids, especially C18:3n-3, because these components were found to be essential for the fitness of a number of coleopterans, lepidopterans and orthopterans (Sivapalan and Ganapragasam, 1979; Reinecke, 1985). In particular, the coleopterans showed slow growth and decreased adult fecundity in response to deficiency in these fatty acids (Earle *et al.*, 1967; Canavoso *et al.*, 2001). In lepidopterans, abnormal pupae and adults were obtained due to deficiency of fatty acids in the food of the larvae, but these anomalies decreased when the larval food was supplemented with C18:3n-3 (Dadd, 1985). Based on these results, the purpose of the work was to check whether an improvement occurred in any biological parameter (including fecundity) of *H. axyridis* reared on a diet supplemented with a source of unsaturated fatty acids. *Ephestia* eggs were selected as a control food, because they have been commonly used in lieu of natural prey for the rearing of *H. axyridis* and other coccinellids (Specty *et al.*, 2003; Berkvens *et al.*, 2008; Michaud and Jyoti, 2008).

Materials and methods

Stock culture

This work was performed using the flightless strain of *H. axyridis* selected to improve its efficacy in biological control and to restrict its capacity to find suitable places to overwinter (Ferran *et al.*, 1998; Tourniaire *et al.*, 2000). A laboratory colony of this strain, originating from INRA Antibes (France), was maintained on UV irradiated *E. kuehniella* eggs, purchased from Biotop (Valbonne, France) and stored at −20 °C until use. The stock culture and subsequent experiments were achieved in a growth chamber at 26 ± 1 °C, 60-70% RH and a 16L:8D photoperiod as described by Sighinolfi *et al.* (2008).

Artificial diet

The pork liver-based artificial diet used in the current study was composed according to the formulation described by Sighinolfi *et al.* (2008), but the Isio 4 oil (Lesieur) utilized in the previous work was replaced with a same quantity of lin-seed oil (Crudigno) which is rich in unsaturated fatty acids, in particular the C18:3n-3 (α-linolenic acid) (Choo *et al.*, 2007; Kochhar, 2011). Thus, the diet contained fresh pork liver sold as food for humans (35.8 g), lin-seed oil (2.4 g), olive oil (3.0 g), sucrose (12.0 g), glycerin (3.0 g), aqueous amino acid solution (40.0 g), yeast extract (3.5 g) and Vanderzant's vitamin mixture (Sigma®) (0.3 g). The water amino acid solution contained Tyr (0.25 mg/g), His (0.62 mg/g), Arg (0.94 mg/g) and ethanolamine (0.19 mg/g). The diet was supplied to the larvae or adults as 0.3 - 0.5 cm-diameter drops, which were dried at room temperature and stored at 4 °C until used. The ladybeetles were also provided with Eppendorf tubes (1.5 ml) filled with distilled water and plugged with cotton.

Biological parameters

Newly-hatched *H. axyridis* larvae were obtained from eggs taken from the stock colony. All eggs' parents had therefore been fed on the standard food, e.g. *E. kuehniella* eggs. The larvae were placed individually in Plexiglas cylindrical chambers (diameter 4.5 cm, height 2.5 cm) and supplied every other day with artificial diet *ad libitum* (number of larvae = 385) or *E. kuehniella* eggs as a control (number of larvae = 50). The largest number of diet-fed larvae was justified by the need to obtain enough adults for the prosecution of the experiments, considering the lower yields that are usually achieved when the larvae are fed on artificial food. Dead individuals were counted and removed daily throughout the pre-imaginal development up to adult emergence. Newly-emerged adults were counted and weighed using a Mettler microbalance (0.1 mg sensitivity). One male and one female were then paired and transferred together to Plexiglas cylindrical containers (diameter 8.0 cm, height 8.0 cm).

Paired adults obtained from artificial diet were fed *ad libitum* with *E. kuehniella* eggs (= "diet/*Ephestia*") (= 5 couples), or artificial diet (= "diet/diet") (= 15 couples). The largest number of the "diet/diet" couples was justified by the highest mortality which was expected in this treatment. Control couples (= 15) were all reared on *E. kuehniella* eggs (= "*Ephestia*/*Ephestia*").

Results were evaluated in terms of the following parameters: 1) developmental time from first instar to adult (in days); 2) number of emerged adults and % emergence (based on the original number of first instar larvae); 3) weight of the newly-emerged males and females (in mg); 4) pre-oviposition duration (in days); 5) weight daily gained by females from emergence to first oviposition (in mg/day) *i.e.* (weight at first oviposition − weight at emergence) / pre-oviposition duration; 6) number of eggs/female laid during the 10 days following the first oviposition (= E_{10}), to represent fecundity (Ferran *et al.*, 1998; Evans *et al.*, 1999); 7) number of eggs laid per mg of female weight (= E_{10} / female weight at the first oviposition); 8) % first instar larvae hatched from eggs to represent fertility; 9) adult male and female longevity from emergence (in days). For each treatment, every larva (parameters 1-3) or couple (parameters 4-9) was considered as a replicate. For parameters 4-8, the final results were evaluated taking into account the couples including females which laid at least one fertile egg.

Biochemical parameters

The fatty acid analyses were carried out on foods (*E. kuehniella* eggs and artificial diet), and on whole insect body (newly-emerged females and, for the diet/diet and the *Ephestia*/*Ephestia* ladybugs, also females at the first oviposition). Total lipids were extracted according to the method of Folch *et al.* (1957), and analyzed as stated by Specty *et al.* (2003). Briefly, after extraction with triheptadecanoin (Sigma) as internal standard, the lipids were transmethylated with 1 ml of 2% sulphuric acid in methanol for 90 minutes at 90-95 °C. Methyl esters, extracted with hexane, were analyzed using gas chromatography (Hewlett Packard 5890 Series II, Wilmington, DE) equipped with a SP2380 column (30 m, 0.32 mm, Supelco, Bellefonte, PA), a flame ionization detector and a split/splitless injector. The oven temperature was programmed from 125 to 210 °C at 1.5 °C/min. Quantification was obtained from electronic integration of the peak areas normalized to that of an external standard (methyl pentadecanoate, Sigma) added at a known amount to each assay, and finally corrected for recovery deduced from the C17:0 internal standard.

Statistical analyses

Adult emergence was analyzed by a 2 × 2 contingency table. All the other biological parameters were analyzed by one-way analysis of variance or Kruskal-Wallis non-parametric procedure when heteroscedasticity occurred. For the biochemical analyses, the data were expressed as µg per mg of fresh weight or as percentages, and were analyzed by one way analysis of variance or Kruskal-Wallis test. All percentage values were transformed for the analyses using an arcsine transformation. Statistical tests were done with STATISTICA 6.0 (StatSoft, 2001).

Results

Biological parameters

Compared to those grown on *E. kuehniella* eggs, the coccinellids grown on artificial diet showed significantly longer developmental times (H = 90.7; N = 157; $P < 0.001$), lower adult emergence rates ($\chi^2 = 55.4$; $P < 0.001$) and lower weights of the newly-emerged adults (for females $F_{1,67} = 184.8$; $P < 0.001$ and for males $F_{1,79} = 218.2$; $P < 0.001$) (table 1).

Table 2 gives the results concerning the adults. The weight daily gained by females from emergence to first oviposition was significantly higher for the controls ("*Ephestia*/*Ephestia*") (H = 17.6; N = 24; $P < 0.01$) compared to the other treatments ("diet/*Ephestia*" and "diet/diet"), which did not differ significantly with each other. Conversely, the pre-oviposition duration was significantly affected by the adult food ($F_{2,21} = 23.7$; $P < 0.001$) and was significantly longer for the coccinellids fed the artificial diet rather than *E. kuehniella* eggs, regardless of the food utilized for their larval development. For the "diet/diet" females, the total number of eggs laid in 10 days (= E_{10}), the fertility and the number of eggs laid per mg of female weight were dramatically lower compared with those observed for the "diet/*Ephestia*" and "*Ephestia*/*Ephestia*" females (H = 14.9; N = 24; $P < 0.001$; H = 13.2; N = 24; $P < 0.01$ and H = 15.6; N = 24; $P < 0.001$, respectively). The number of eggs laid and the fertility of the "diet/*Ephestia*" females were higher than for the "diet/diet" females, even leading to a higher number of eggs laid per mg of female weight compared with controls, showing a good recovery for this experimental condition. Moreover the fertile eggs obtained from the diet/*Ephestia* females were even more than those obtained from the *Ephestia*/*Ephestia* females [e.g. about 142 (= 253 × 56%) *vs.* 87 (= 324 × 27%)]. The female longevity was not significantly affected by larval and

Table 1. Developmental time from egg to adult emergence, adult emergence rate and male and female adult weight of *H. axyridis* reared either on *E. kuehniella* eggs or on artificial diet.

Larval food	Developmental time (days) (mean ± SE)	Adult emergence rate (%) (mean ± SE)	Adult weight (mg) (mean ± SE)	
			Males	Females
E. kuehniella	12.5 ± 0.2a (n = 40)	86.0b (n = 50)	27.4 ± 0.6b (n = 18)	31.8 ± 0.7b (n = 22)
Artificial diet	20.5 ± 0.1b (n = 117)	31.7a (n = 385)	17.4 ± 0.3a (n = 63)	20.1 ± 0.5a (n = 47)

Number of replicates given in parentheses below the means (± SE). Values followed by the same letter in a column are not significantly different, using the Kruskal-Wallis test (developmental time), the one-way analysis of variance (male and female weight), or the 2 × 2 contingency table (adult emergence rate) (P < 0.05)

Table 2. Daily weight gain from emergence to first oviposition, pre-oviposition duration, total eggs laid in 10 days (=E_{10}), fertility (%), number of eggs laid per mg of female weight and adult longevity of *H. axyridis* reared either on *E. kuehniella* eggs ("*Ephestia*/*Ephestia*") or on artificial diet ("diet/diet"), and of *H. axyridis* reared as larvae on the artificial diet and as adults on *E. kuehniella* eggs ("diet/*Ephestia*").

Food larvae / adults	Daily weight gain (mg/day) (mean ± SE)	Pre-oviposition (days) (mean ± SE)	Total eggs laid in 10 days (=E_{10}) (mean ± SE)	Fertility (%) (mean ± SE)	Number of eggs / female weight (No/mg) (mean ± SE)	Longevity (days) (mean ± SE)	
						Males	Females
Ephestia/*Ephestia*	2.7 ± 0.2b (n = 13)	7.3 ± 0.5a (n = 13)	324 ± 30c (n = 13)	27.1b (n = 13)	6.3 ± 0.6b (n = 13)	72.3 ± 4.9a (15)	66.7 ± 4.1a (15)
Diet/*Ephestia*	1.3 ± 0.1a (n = 4)	9.8 ± 0.3a (n = 4)	253 ± 9b (n = 4)	56.0b (n = 4)	8.2 ± 0.66c (n = 4)	56.4 ± 5,9b (5)	52.6 ± 8,4a (5)
Diet/Diet	0.9 ± 0.1a (n = 7)	13.6 ± 1.0b (n = 7)	37 ± 10a (n = 7)	11.3a (n = 7)	1.1 ± 0.2a (n = 7)	93.0 ± 7.6c (15)	75.7 ± 7.5a (14)

Number of replicates given in parentheses below the means (± SE). Values followed by the same letter in a column are not significantly different using the one-way analysis of variance test (pre-oviposition) or the Kruskal-Wallis (other parameters) (P < 0.05). For all parameters except longevity the results were evaluated taking into account the couples including females which laid at least one fertile egg.

adult food (H = 2.7; N = 34; P = 0.3), whereas the "diet/diet" males lived significantly longer (H = 9.1; N = 35; $P < 0.05$) than the "*Ephestia*/*Ephestia*" and the "diet/*Ephestia*" males. The latter lived shorter than the controls (table 2).

Biochemical parameters

As regards the composition of the two foods tested, the total amount of fatty acids in the artificial diet (mean of 4 independent analyses ± SE = 133 ± 11 µg/mg) was significantly higher than that recorded for *E. kuehniella* eggs (mean of 5 independent analyses ± SE = 91.4 ± 1.2 µg/mg) (H = 6.0; N = 9; P = 0.014). With regard to the pattern of relative fatty acid content, the artificial diet showed significantly lower proportions of C16:0, C16:1 and significantly higher proportions of C18:0 and C18:3n-3. The content of linoleic acid (C18:2n-6) was not significantly different between the diet and *E. kuehniella* eggs (figure 1).

No significant difference ($F_{1,7}$ = 5.0; P = 0.06) occurred between the total amount of fatty acids in the control newly emerged females (73.4 ± 3.3 µg/mg) and females supplied with artificial diet in the larval stages (62.4 ± 3.7 µg/mg). In contrast, the patterns of fatty acids were very different between the newly-emerged females grown on the two food sources, with significantly higher proportions of C18:0 and C18:3n-3, and significantly lower proportions of C16:0, C16:1 and C18:1 for the "artificial diet" group (figure 2).

In the "diet/diet" females at the first oviposition, the total amount of fatty acids (73.0 ± 7.2 µg/mg) was higher compared with the controls (50.4 ± 4.2 µg/mg) ($F_{1,8}$ = 7.3; P < 0.05). The pattern of the relative fatty acid content of the "diet/diet" and the "*Ephestia/Ephestia*" females resembled that observed for the newly-emerged females (figure 3).

Discussion

The liver-based diet including lin-seed oil supported the development of *H. axyridis* from newly hatched larvae to adults as well as adult survival and reproduction. The biological parameters considered to evaluate fitness were however better for the ladybirds fed with *E. kuehniella* eggs. In particular, for the ladybirds fed on the artificial diet as larvae, the pre-imaginal development was longer and the percentage of emerged adults was lower as well as their weight, compared to the coccinellids reared on *E. kuehniella* eggs. Also the biological characteristics of *H. axyridis* adults were significantly affected by the food source as already shown by Specty *et al.* (2003) and Sighinolfi *et al.* (2008). The "diet/diet" females showed a longer pre-oviposition period and oviposited less eggs compared with the controls. The fertility of the eggs laid by the "diet/diet" females was clearly lower compared to the other two treatments. The lower number of eggs laid by adults fed on artificial diet has been observed by several authors for numerous predator insects (Kaliruoto, 1980; Racioppi

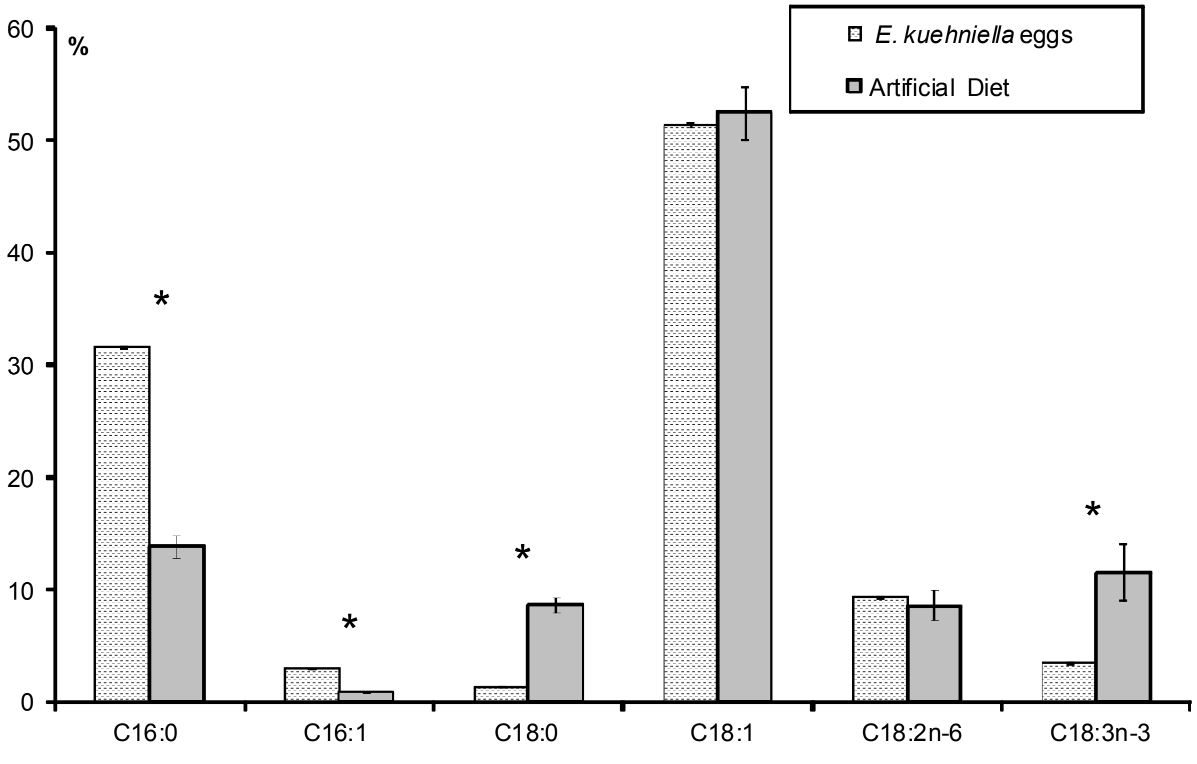

Figure 1. Relative fatty acid contents of *E. kuehniella* eggs and artificial diet. The mean value of 5 (*E. kuehniella*) or 4 (artificial diet) independent analyses are given together with their confidence intervals. * Significant difference ($P < 0.05$). Abbreviations for fatty acids: C16:0 = palmitic acid; C16:1 = palmitoleic acid; C18:0 = stearic acid; C18:1 = oleic acid; C18:2n-6 = linoleic acid; C18:3n-3 = α-linolenic acid.

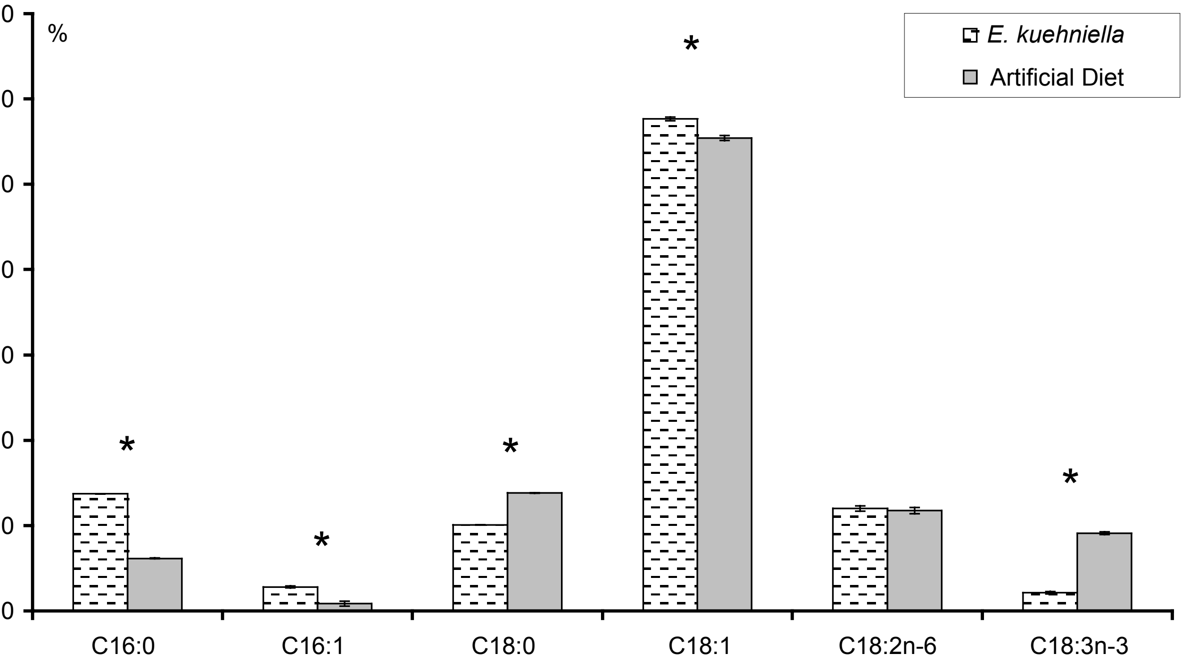

Figure 2. Relative fatty acid contents of *Harmonia axyridis* newly-emerged females. Larvae were reared on control (*Ephestia kuehniella*) or on artificial diets (Artificial Diet). The mean value of 6 (*E. kuehniella*) or 4 (Artificial Diet) independent analyses are given together with their confidence intervals. * Significant difference (P < 0.05). See figure 1 for abbreviations of fatty acids.

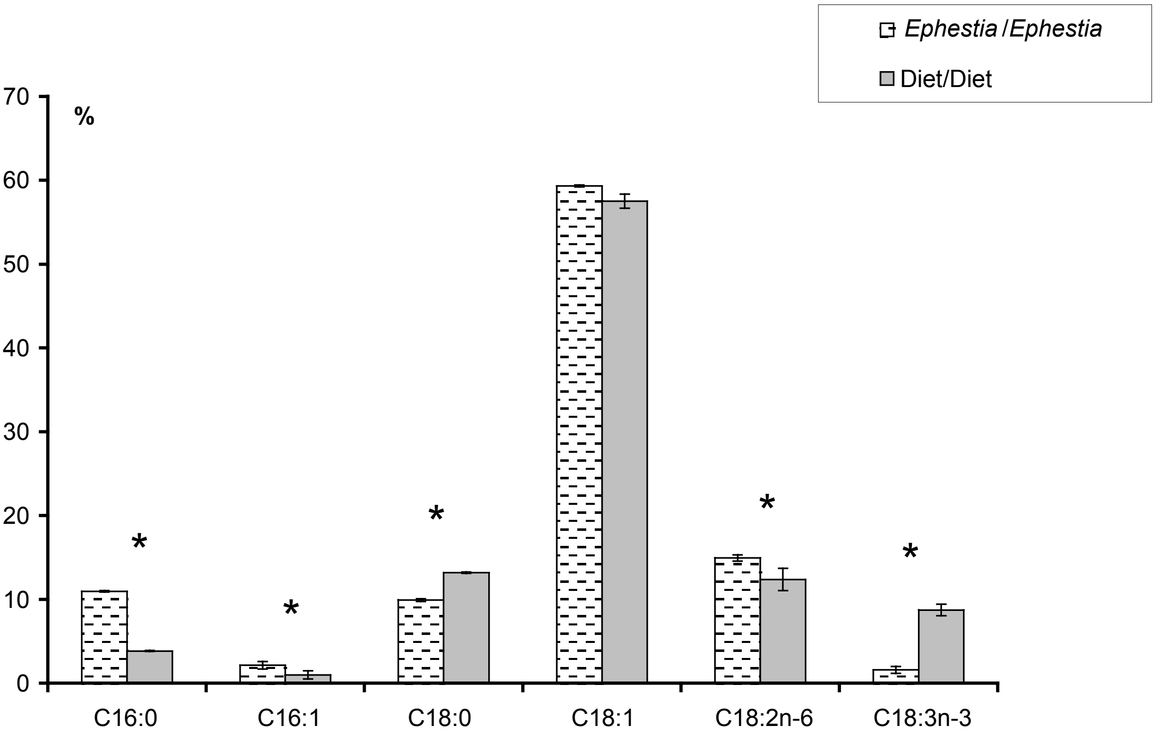

Figure 3. Relative fatty acid contents of *Harmonia axyridis* females at the first oviposition. *H. axyridis* was reared either on *E. kuehniella* eggs ("*Ephestia/Ephestia*") or on artificial diet ("diet/diet").The mean values of 5 independent value analyses are given together with their confidence intervals. * Significant difference (P < 0.05). See figure 1 for abbreviations of fatty acids.

et al., 1981; Jalali *et al.*, 2009). However, in the case of the predatory mirid bug *Dicyphus tamaninii* Wagner, the fecundity of females reared on meat-based artificial diets during the nymphal stages has been found to be comparable to that of conventionally reared insects (Zapata *et al.*, 2005).

The female longevity was not significantly affected by larval and adult food, contrary to some heteropteran predators grown on artificial diet (De Clercq *et al.*, 1998; Adams, 2000). The "diet/diet" males even lived significantly longer than the "*Ephestia*/*Ephestia*" and the "diet/*Ephestia*" males.

Similarly to the study conducted by Sighinolfi *et al.* (2008), females fed artificial diet as larvae showed an important fitness recovery when supplied with *E. kuehniella* eggs during their adult life. This was observed for the pre-oviposition period and the number of eggs per mg of female weight, the latter being significantly higher compared to the controls. Both in the present study and in that of Sighinolfi *et al.* (2008), the recovery was however incomplete and differences remained for absolute daily weight gains and the total number of eggs laid in ten days. Otherwise, in the present study the fertile eggs were more for the diet/*Ephestia* females than for the control females. The fertility observed for the control females was however considerably lower in this study compared to the previous one for unknown reasons.

Different studies showed that a correlation exists between weight and individual fitness for insect predators (reared on natural or factitious food), with particular regard to the duration of the pre-oviposition period and the number of eggs laid (Grenier and De Clercq, 2003; Omkar and Strivastava, 2003). Therefore, the incomplete recovery observed in the "diet/*Ephestia*" females may have been linked to the low weight at emergence of these females.

The biochemical analyses of the artificial diet revealed that the content of α-linolenic acid (C18:3n-3) was higher compared to *E. kuehniella* eggs, whereas the diet developed by Sighinolfi *et al.* (2008), which did not include lin-seed oil, almost completely lacked this compound. Moreover, the content of linoleic acid (C18:2n-6) was clearly lower in the present diet, with a value similar to that of *E. kuehniella* eggs. Nevertheless, the diet contained a significantly higher total amount of fatty acids (= 133 µg/mg) than *E. kuehniella* eggs (= 91.4 µg/mg), but there was no difference for total fatty acids between the control newly emerged females and those reared as larvae on the artificial food.

The similarity of lipid composition between parasitoid and host in some species suggests that host lipids may be absorbed with few modifications, especially neutral lipids (Delobel and Pageaux, 1981). Specty *et al.* (2003) established that the fatty acid composition of *H. axyridis* larvae copies the pattern of their prey (either *E. kuehniella* eggs or pea aphids). In the same way, as observed with the herbivore heteropteran *Lygus hesperus* (Knight) (Cohen, 1990), the composition in fatty acids of *H. axyridis* newly-emerged females reflected the composition of the diet. In fact, these females showed an even higher concentration of α-linolenic acid (C18:3n-3) compared to control females. On the contrary, the females obtained from larvae fed on the diet developed by Sighinolfi *et al.* (2008) completely lacked this fatty acid. In *D. tamaninii* reared on a meat-based artificial diet (Iriarte and Castane, 2001), Zapata *et al.* (2005) used a reformulated diet with new sources of proteins and lipids, leading to improvement of the biochemical content (including total fatty acids and fatty acid profile), as well as of some biological parameters of the mirid bugs produced. Conversely, in our study, the biological parameters of *H. axyridis* reared on the diet were worse than those of the coccinellids fed on *E. kuehniella* eggs. In particular, despite the presence of C18:3n-3 (in the diet and in the newly-emerged females), the number of eggs laid by the diet-reared *H. axyridis* females was considerably lower compared to controls. Contrary to our expectations, therefore, the correction of the diet imbalance concerning C18:3n-3, found in the trials of Sighinolfi *et al.* (2008), did not improve either oviposition or other quality parameters of the diet-fed coccinellids. A possible explanation is that the fatty acid content only reflects passive fatty acid storage in the artificial diet-reared coccinellids. A difference in biochemical conversion during the pupal stage between the coccinellids fed the two foods may be hypothesized. It may be interesting to perform analyses of fatty acids in neutral lipids and phospholipids, besides total ones, in order to deep our knowledge on this aspect. According to Grenier and De Clercq (2003) the composition of key components in living organisms might be a good criterion for estimating the quality of artificially reared predators and parasitoids. The biochemical analyses of insects grown on deficient diet may suggest improvements by modification of some components, possibly leading to better results with the new diet. Nevertheless, it is necessary to be careful, and verify that biochemical but also biological parameters would be improved. Future research will be aimed at finding correlations between biochemical and biological parameters, so as to facilitate both the improvement of the artificial diet for *H. axyridis* and the assessment of its overall quality, because it is maybe faster to evaluate biochemical than biological characteristics. This approach could be also applied for other coccinellids or other insect predators to be used as biocontrol agents, which could be conveniently maintained on an artificial diet at least for part of their life cycle, to reduce production costs or in case of shortage of preys or alternative foods, such as *E. kuehniella* eggs.

The maintenance of *H. axyridis* on artificial diets may also facilitate the management of colonies of parasitoids of this ladybug. The study of the biology of these beneficial insects [including *Dinocampus coccinellae* (Schrank)] may be of interest in the view of investigating their potential as control agents of *H. axyridis*, which is sometimes considered as an invasive insect in the countries of introduction (Berkvens *et al.*, 2010; Francati, 2013).

Acknowledgements

This research was partially funded by the Italian Ministry of Education and Research (PRIN Project 2010-2011: "Insects and globalization: sustainable control of exotic species in agro-forestry ecosystems" GEISCA). The *Alma Mater Studiorum* Università di Bologna supported Luca Sighinolfi's stage at INSA-Lyon, Université de Lyon, INRA via the funds of the Ph.D program and the "Marco Polo" project.

References

Adams T. S., 2000.- Effect of diet and mating status on ovarian development in a predaceous stink bug *Perillus bioculatus* (Hemiptera: Pentatomidae).- *Annals of the Entomological Society of America*, 93: 1288-1293.

Alhmedi A., Haubruge E., Francis F., 2010.- Intraguild interactions implicating invasive species: *Harmonia axyridis* as a model species.- *Biotechnology, Agronomy, Society and Environment*, 14: 187-201.

Berkvens H., Bonte J., Berkvens D., Deforce K., Tirry L., De Clercq P., 2008.- Pollen as an alternative food for *Harmonia axyridis*.- *BioControl*, 5: 201-210.

Berkvens N., Moens J., Berkvens D., Samih M. A., Tirry L., De Clercq P., 2010.- *Dinocampus coccinellae* as a parasitoid of the invasive ladybird *Harmonia axyridis* in Europe.- *Biological control*, 53: 92-99.

Burgio G., Santi F., Maini S., 2002.- On intra-guild predation and cannibalism in *Harmonia axyridis* (Pallas) and *Adalia bipunctata* L. (Coleoptera:Coccinellidae).- *Biological Control*, 24: 110-116.

Canavoso L. E., Jouni Z. E., Karnas K. J., Pennington J. E., Wells M. A., 2001.- Fat metabolism in insects.- *Annual Review of Nutrition*, 21: 23-46.

Chen J., Qin Q. J., Liu S., Ye Y. Z., 2012.- Effect of six diets on development and fecundity of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae).- *African Entomology*, 20: 85-90.

Choo W. S., Birch J., Dufour J. P., 2007.- Physicochemical and quality characteristics of cold-pressed flaxseed oils.- *Journal of Food Composition and Analysis*, 20: 202-211.

Cohen A. C., 1990.- Fatty acid distribution as related to adult age, sex and diet in the phytophagous heteropteran, *Lygus hesperus*.- *Journal of Entomological Science*, 25: 75-84.

Cohen A.C., 2004.- *Insect diets, science and technology*.- CRC Press, New York, USA.

Dadd R. H., 1985.- Nutrition: organisms, pp. 313-390. In: *Comprehensive insect physiology biochemistry and pharmacology. Vol. 4. Regulation: digestion, nutrition, excretion* (Kerkut G. A., Gilbert G. I., Eds).- Pergamon Press, Oxford, UK.

De Clercq P., Merlevede F., Tirry L., 1998.- Unnatural prey and artificial diets for rearing *Podisus maculiventris* (Heteroptera: Pentatomidae).- *Biological Control*, 12: 137-142.

De Clercq P., Mason P., Babendreier D., 2011.- Benefits and risks of exotic biological control agents.- *BioControl*, 48: 39-45.

Delobel B., Pageaux J. F., 1981.- Influence de l'alimentation sur la composition en acides gras totaux des Diptères Tachinaires.- *Entomologia Experimentalis et Applicata*, 29: 281-288.

Dong H., Ellington J. J., Remmenga M. D., 2001.- An artificial diet for the lady beetle *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae).- *Southwestern Entomologist*, 26: 205-213.

Earle N. W., Slatten B., Burks M. L. J., 1967.- Essential fatty acids in the diet of the boll weevil, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae).- *Journal of Insect Physiology*, 13: 187-200.

Evans E. W., Stevenson A. T., Richards D. R., 1999.- Essential versus alternative foods of insect predators: benefits of a mixed diet.- *Oecologia*, 121: 107-112.

Ferran A., Giuge L., Tournaire R., Gambier J., Fournier D., 1998.- An artificial non-flying mutation to improve the efficiency of the ladybird *Harmonia axyridis* in biological control of aphids.- *BioControl*, 43: 53-64.

Folch J., Lees M., Sloane-Stanley G. H., 1957.- A simple method for the isolation and purification of total lipids from animal tissues.- *Journal of Biological Chemistry*, 226: 497-509.

Francati S., 2013.- New associations between native parasitoids and exotic insects introduced in Italy and in Europe. 92 pp., *PhD Thesis in Agricultural Entomology*, *Alma Mater Studiorum* University of Bologna, Italy.

Grenier S., De Clercq P., 2003.- Comparison of artificially vs. naturally reared natural enemies and their potential for use in biological control, pp. 115-132. In: *Quality control and production of biological control agents: Theory and testing procedures* (van Lenteren J. C., Ed.).- CABI Publishing, Cambridge, UK.

Iriarte J., Castane C., 2001.- Artificial rearing of *Dicyphus tamaninii* Wagner (Heteroptera Miridae) on a meat-based diet.- *Biological Control*, 22: 98-102.

Jalali M. A., Tirry L., De Clercq P., 2009.- Food consumption and immature growth of *Adalia bipunctata* (Coleoptera: Coccinellidae) on a natural prey and a factitious food.- *European Journal of Entomology*, 106: 193-198.

Kaliruoto K. T., 1980.- Survival and fecundity of *Adalia bipunctata* (Coleoptera: Coccinellidae) and some other predatory insect species on an artificial diet and a natural prey.- *Annales Entomologici Fennici*, 46: 101-104.

Kenis M, Roy H. E., Zindel R., Majerus M. E. N., 2008.- Current and potential management strategies against *Harmonia axyridis*.- *BioControl*, 53: 235-252.

Kochhar S. P., 2011.- Minor and speciality oils, pp. 291-342. In: *Vegetable oils in food technology: composition, properties and uses*, 2nd edition (Gunstone F., Ed.).- Wiley-Blackwell, Chichester, UK.

Matsuka M., Okada I., 1975.- Nutritional studies of an aphidophagous coccinellid, *Harmonia axyridis*. 1. Examination of artificial diets for the larval growth with special reference to drone honeybee powder.- *Bulletin of the Faculty of Agriculture, Tamagawa University*, 15: 1-9.

Michaud J. P., Jyoti J. L., 2008.- Dietary complementation across life stages in the polyphagous lady beetle *Coleomegilla maculata*.- *Entomologia Experimentalis et Applicata*, 126: 40-45.

Niijima K., Matsuka M., Okada I., 1986.- Artificial diets for an aphidophagous coccinellid, *Harmonia axyridis*, and its nutrition, pp. 37-50. In: *Ecology of aphidophaga* (Hodek I., Ed.).- Academia, Prague & Dr Junk W, Dordrecht.

Obrycki J. J., Kring T. J., 1998.- Predaceous Coccinellidae in biological control.- *Annual Review of Entomology*, 43: 295-321.

Omkar, Strivastava S., 2003.- Influence of six aphid prey species on development and reproduction of a ladybird beetle, *Coccinella septempunctata*.- *BioControl*, 48: 379-393.

Qin Q. L., Wang H. T., Chen X. Z., Zheng J. F., Miao L., Zhang H., Li X., Zhang J. H., 2011.- A pilot-scale plant being established to produce the multicolored Asian beetle based on the larvae on beet armyworm as a diet, p. 63. In: *Proceedings of international symposium on mass production and commercialization of arthropod biological control agents (ISMC)*, Beijing, China.

RACIOPPI J. V., BURTON R. L., EIKENBARY R., 1981.- The effects of various oligidic diets on the growth of *Hippodamia convergens*.- *Entomologia Experimentalis et Applicata*, 30: 68-72.

REINECKE J. P., 1985.- Nutrition: artificial diets, pp. 391-420. In: *Comprehensive insect physiology biochemistry and pharmacology. Vol. 4. Regulation: digestion, nutrition, excretion* (KERKUT G. A., GILBERT G. I., Eds).- Pergamon Press, Oxford, UK.

ROY H., BROWN P., MAJERUS M., 2006.- *Harmonia axyridis*: a successful biocontrol agent or an invasive threat?, pp. 295-309. In: *An ecological and societal approach to biological control* (HOKKANEN J., EILENBERG H. M. T., Eds).- Springer, Dordrecht, Netherlands.

SANTI F., MAINI S., 2006.- Predation upon *Adalia bipunctata* and *Harmonia axyridis* eggs by *Chrysoperla carnea* larvae and *Orius laevigatus* adults.- *Bulletin of Insectology*, 59: 53-58.

SCHANDERL H., FERRAN A., GARCIA V., 1988.- L'élevage de deux coccinelles *Harmonia axyridis* et *Semiadalia undecimnotata* à l'aide d'oeufs d'*Anagasta kuehniella* tués aux rayons ultraviolets.- *Entomologia Experimentalis et Applicata*, 49: 235-244.

SIGHINOLFI L., 2005.- Valutazione e miglioramento delle caratteristiche biologiche e biochimiche di insetti predatori allevati su diete artificiali per un loro utilizzo in lotta biologica [Evaluation et amélioration des caractéristiques biologiques et biochimiques d'insectes prédateurs élevés sur des aliments artificiels pour une utilisation en lutte biologique], 161 pp. *Thèse de doctorat en co-tutelle Italie-France*, Università di Bologna & Institut National des Sciences Appliquées de Lyon (n° 05 ISAL 0026).

SIGHINOLFI L., FEBVAY G., DINDO M. L., REY M., PAGEAUX J. F., BARONIO P., GRENIER S., 2008.- Biological and biochemical characteristics for quality control of *Harmonia axyridis* (Pallas) (Coleoptera, Coccinellidae) reared on liver-based diet.- *Archives of Insect Biochemistry and Physiology*, 68: 6-39.

SIVAPALAN P., GNANAPRAGASAM N. C., 1979.- The influence of linoleic and linolenic acid on adult moth emergence of *Homona coffearia* from meridic diets in vitro.- *Journal of Insect Physiology*, 25: 393-398.

SOARES A. O., CODERRE D., SCHANDERL H., 2004.- Dietary self-selection behaviour by the adults of the aphidophagous ladybeetle *Harmonia axyridis* (Coleoptera: Coccinellidae).- *Journal of Animal Ecology*, 73: 478-486.

SPECTY O., 2002.- Elaboration d'aliments artificiels pour l'élevage de la coccinelle prédatrice *Harmonia axyridis* (Col.: Coccinellidae): analyse des besoins nutritionnels, contrôle des caractéristiques biologiques et biochimiques desinsectes produits. 174 pp., *Thèse de Doctorat*, Institut National des Sciences Appliquées de Lyon (n° 02 ISAL 0042).

SPECTY O., FEBVAY G., GRENIER S., DELOBEL B., PIOTTE C., PAGEAUX J. F., FERRAN A., GUILLAUD J., 2003.- Nutritional plasticity of the predatory ladybeetle *Harmonia axyridis* (Coleoptera: Coccinellidae): comparison between natural and substitution prey.- *Archives of Insect Biochemistry and Physiology*, 52: 81-91.

STATSOFT, 2001.- *STATISTICA data analysis software system*, Version 6.- StatSoft Inc., Tulsa, OK, USA.

TOURNIAIRE R., FERRAN A., GAMBIER J., GIUGE L., BOUFFAULT F., 2000.- Locomotory behavior of flightless *Harmonia axyridis* Pallas (Col., Coccinellidae).- *Journal of Insect Physiology*, 46: 721-726.

VAN LENTEREN J. C., LOOMANS A. J. M., BABENDREIER D., BIGLER F., 2008.- *Harmonia axyridis*: an environmental risk assessment for Northwest Europe, pp. 37-54. In: *From biological control to invasion: the ladybird* Harmonia axyridis *as a model species* (ROY H., WAJNBERG E., Eds).- Springer, Netherlands.

WANG S., ZHANG R. Z., ZHANG F., 2005.- Study of multicolored Asia ladybird: impact as a biological control agent and an invasive species. *The fifth international conference on biodiversity conservation and utilization. Beijing* (CHEN L., YANSHOU J., Eds). Beijing Science and Technology Press, Beijing, China.

ZAPATA R., SPECTY O., GRENIER S., FEBVAY G., PAGEAUX J. F., DELOBEL B., CASTANE C., 2005.- Carcass analysis allowing to improve a meat-based diet for the artificial rearing of the predatory mirid bug *Dicyphus tamaninii*.- *Archives of Insect Biochemistry and Physiology*, 60: 84-92.

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Received April 24, 2013. Accepted September 16, 2013.