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Development and reproduction of *Adalia bipunctata* on factitious and artificial foods

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Abstract The native coccinellid *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae) may be an alternative to exotic species like *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) for the biological control of aphid pests in Europe. The availability of adequate factitious or artificial foods may help optimize its mass production. This study examines the nutritional value of *Ephestia kuehniella* Zeller (Lepidoptera: Phycitidae) eggs plus bee pollen, pea aphids *Acyrthosiphum pisum* (Harris) (Hemiptera: Aphididae) and mixtures of bee pollen and cysts of *Artemia franciscana* Kellogg (Branchiopoda: Artemiidae) and/or a lyophilized artificial diet based on bovine meat and liver. Reproducing adults of *A. bipunctata* were obtained on all diets tested, but immature survival, adult weights, development times and reproductive rates differed among diets. Over 84% of first instars fed on *E. kuehniella* eggs plus pollen or aphids survived to adulthood. Feeding predator larvae on pollen combined only with *A. franciscana* cysts or artificial diet yielded 40–55% immature survival, but survival increased to 74% when all of these components were mixed. Adult weights of *A. bipunctata* on the mixtures of pollen, *A. franciscana* cysts and/or artificial diet were 55–75% of those on aphids or on *E. kuehniella* eggs plus pollen. Lifetime fecundity was superior on *E. kuehniella* eggs plus pollen (1,864 eggs) to that on the other diets (264–889 eggs). The use of mixtures of plant and animal foods for *A. bipunctata* and other predators may contribute to increasing the cost-effectiveness of commercial mass production by reducing inputs of natural prey like aphids, or of nutritious but expensive factitious foods like lepidopteran eggs.

Keywords *Adalia bipunctata* · *Acyrtosiphon pisum* · *Ephestia kuehniella* · *Artemia franciscana* · Pollen · Mass rearing

Introduction

The two-spotted lady beetle *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae) is a polyphagous coccinellid native to the Palearctic and Nearctic regions. Since the use of the non-indigenous multi-coloured Asian lady beetle *Harmonia axyridis* (Pallas)

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(Coleoptera: Coccinellidae) as a biological control agent has been compromised (e.g., Brown et al. 2008; van Lenteren et al. 2008), there is growing interest in commercializing *A. bipunctata* for biological control of aphids in Europe. The two-spotted lady beetle feeds on a broad spectrum of aphid species but can also use coccids, diaspidids and lepidopteran eggs as alternative prey (De Clercq et al. 2005b; Omkar and Pervez 2005). In times of prey scarcity *A. bipunctata* has been observed feeding on pollen (Hemptinne and Desprets 1987).

Mass rearing of *A. bipunctata* is mostly done on live aphids, requiring a tritrophic system to produce the predators, prey and the prey's host plants. Problems of discontinuity occurring at one of these levels, the intensive labour and the large greenhouse surfaces needed to maintain such tritrophic rearing systems lead to high market prices for the produced natural enemies. In search of an alternative rearing system, De Clercq et al. (2005b) reported that the two-spotted lady beetle can be reared on a mixture of bee pollen and eggs of the Mediterranean flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). However, the cost of these lepidopteran eggs is high and there is an ever increasing demand for this factitious food as it is extensively used in the production of other natural enemies, including trichogrammatids and a number of predatory heteropterans (De Clercq 2008). Therefore, the development of a nutritionally adequate artificial diet could lower the dependency on *E. kuehniella* eggs and as such decrease the production cost for *A. bipunctata* and enhance its implementation as a biological control agent.

Riddick (2009) reviewed the artificial rearing of predatory beetles, lacewings and heteropterans. For aphidophagous coccinellids several attempts have been made to develop artificial diets, with variable success. Because it was once believed to hold promise as a biological control agent of aphid and coccid pests in several parts of the world, a number of studies were done to rationalize the rearing of *H. axyridis* using factitious foods and artificial diets. The factitious foods developed for *H. axyridis* consisted of such varied components as drone honeybee brood (Okada 1970), eggs from several species of lepidopterans (Schanderl et al. 1988; Abdel-Salam and Abdel-Baky 2001) and also *Artemia* cysts (Branchiopoda: Artemiidae) (Hongo and Obayashi 1997). Brine shrimp of the genus *Artemia* are a widely used feed in the larviculture of fish and shellfish. The nauplii are nutritionally and physiologically suitable prey for numerous marine and freshwater organisms (Lavens and Sorgeloos 2000). Hongo and Obayashi (1997) were able to rear *H. axyridis* on diapausing eggs (i.e. cysts) of *Artemia salina* (L.). Cysts of *Artemia franciscana* Kellogg were successfully used as a factitious food for *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) (Arijs and De Clercq 2001) and *Macrolophus* spp. (Hemiptera: Miridae) (Castañé et al. 2006; Vandekerkhove et al. 2009).

Artificial diets tested for *H. axyridis* contained mostly chicken or pork liver (Hongo and Obayashi 1997; Dong et al. 2001; Sighinolfi et al. 2008) and whole hen's eggs or egg yolk (Dong et al. 2001). Artificial diets with meat products as the main component have also been developed for other predatory coccinellids, including *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae) (Smith 1966; Racioppi et al. 1981) and *Coleomegilla maculata* De Geer (Coleoptera: Coccinellidae) (Atallah and Newsom 1966). Kariluoto et al. (1976) were the only workers who attempted designing an artificial diet for *A. bipunctata* based on whole eggs and fresh beef liver. However, development on this artificial diet was strongly delayed and the weight of attained adults was substantially lower than that of those fed aphids. Moreover, no eggs were laid even though adults survived for six months.

The current study compared the nutritional value of different alternative foods to sustain development and reproduction of the two-spotted lady beetle *A. bipunctata*. Both insect and non-insect animal foods, as well as plant foods were tested.

Materials and methods

Predator culture

A colony of *A. bipunctata* was started in August 2002 with insects acquired from Biobest NV (Westerlo, Belgium) and has repeatedly been infused with material from the same source (last in February 2006). At the time of the experiments, the predator was in its 29th generation of rearing since February 2006. Adults and larvae were fed on a 50:50 (w/w) mixture of *E. kuehniella* eggs and frozen honeybee pollen (De Clercq et al. 2005b). Colonies were maintained and experiments performed in incubators set at 23 ± 1°C, 65 ± 5% RH and a 16 h photoperiod.

Experimental design

In the present experiment, five diets were tested: live *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae), *E. kuehniella* eggs supplemented with pollen, *A. franciscana* cysts supplemented with pollen, a lyophilized meat and liver based diet supplemented with pollen and a mixture of lyophilized diet, *A. franciscana* cysts and pollen. Preliminary experiments had shown that larvae did not reach adulthood when offered only dehydrated or hydrated decapsulated cysts of *A. franciscana*, or only the lyophilized meat and liver diet.

Live pea aphids, *A. pisum* (late instars and adults) reared on *Vicia faba* L., were purchased on a weekly basis from Biobest NV. The frozen eggs of *E. kuehniella* and honeybee pollen were supplied by Koppert BV (Berkel en Rodenrijs, The Netherlands) and stored in a freezer at −18°C. The frozen pollen used in this study mainly consisted of pollen grains from Brassicaceae and Anacardiaceae and contained about 50% water. The decapsulated cysts of *A. franciscana*, originating from the Great Salt Lake (USA) were supplied by the *Artemia* Reference Center (Ghent University, Ghent, Belgium) and were not hydrated before use. The artificial diet was the meat and liver diet designed for *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae) by De Clercq et al. (1998). This diet contained 100 g ground beef, 100 g beef liver, 10 g egg yolk and 12 ml of sucrose solution (5%). For the experiment, this diet was frozen at −18°C, lyophilized with a Thermo Heto PowerDry 3000 (Thermo Scientific Cooperation, Massachusetts, USA) and ground. In the mixed diets, all components were present in a 1:1 or 1:1:1 weight ratio. All foods were provided ad libitum and replenished on Mondays, Wednesdays and Fridays except for the live pea aphids which were replaced every day.

For each diet 60 hatchlings (<24 h old) were individually placed in Petri dishes (9 cm diameter; 2 cm high) lined with absorbent paper and furnished with a moist paper plug fitted in a small plastic dish as a source of free water. Larval development and survival were monitored daily. Newly emerged adults were starved for 24 h before being weighed on a Sartorius Genius ME215 balance (Sartorius, Goettingen, Germany) (±0.01 mg). Adults that emerged on the same day were placed together in a rearing container (5.5 × 14.5 × 20 cm) and mating pairs were transferred into individual 9 cm diameter Petri dishes. Thus, the number of pairs used for the assessment of reproductive potential depended on the developmental success and mating propensity of the adults obtained on the different diets. Adult couples were given the same diet as in their larval stages. The pre-oviposition period, number of oviposition days and number of oviposited eggs were determined by daily checking each pair for oviposited eggs. Eggs were collected and placed under the same conditions as those experienced by the mothers and hatching rates were determined. Longevity of the female adults was recorded. All adults obtained on the different diets were dissected upon death to determine their gender.

Statistical analysis

All data except survival rates were subjected to one-way analysis of variance (ANOVA) followed by a Tukey test or, in case of heteroscedasticity, by a Tamhane test (SPSS Inc 2003). Percentages of egg hatch were transformed using arcsine transformation before analysis. Survival rates had a binomial distribution and were compared by means of a logistic regression (StataCorp 2005). The model uses the principle of the regression analysis and thus each time tests the difference between the average of the reference group and the average of the group in question. Thus, each test consists of a regression coefficient being calculated and tested for being significantly different from zero, for which P-values are presented (McCullagh and Nelder 1989). Analysis of covariance (ANCOVA) was used to assess variation in the number of eggs laid over an 80 day period on each diet, using time as the covariate. For all tests, the significance level was set at $P = 0.05$.

Results

Immature survival on *E. kuehniella* eggs and pollen was similar to that on live aphids but superior to that on

Table 1 Survival, developmental time and adult weight of *A. bipunctata* fed on various foods[a]

Diet	Survival (%)	Developmental time (days)			Adult weight (mg)
		Larva	Pupa	Total	
A. pisum	84.5 ± 4.8ab	9.8 ± 0.1b	7.1 ± 0.05b	16.9 ± 0.1b	12.6 ± 0.3a
E. kuehniella + pollen	94.9 ± 2.9a	8.7 ± 0.08a	6.7 ± 0.07a	15.3 ± .0.1a	12.1 ± 0.3a
A. franciscana + pollen	55.2 ± 6.6c	15.1 ± 0.4c	6.9 ± 0.1ab	22.0 ± 0.3d	8.2 ± 0.3b
Lyophilized artificial diet + pollen	40.4 ± 6.6c	18.1 ± 0.6d	7.0 ± 0.7b	25.1 ± 0.5e	6.9 ± 0.2c
A. franciscana + lyophilized artificial diet + pollen	74.1 ± 5.8b	13.8 ± 0.3c	7.0 ± 0.7b	20.7 ± 0.3c	9.1 ± 0.3b

[a] Means ± standard error; means within a column followed by the same letter are not significantly different ($P > 0.05$; logistic regression (survival) or Tamhane test (other parameters))

Table 2 Reproduction and longevity of *A. bipunctata* fed on various diets[a]

Diet	Preoviposition period (days)	Fecundity (eggs/♀)	Oviposition rate (eggs/♀/day)	Fertility (larvae/♀/day)	Egg hatch (%)[b]	Longevity (days)
A. pisum	5.6 ± 0.3a	796.1 ± 101.1b	28.1 ± 2.2a	12.6 ± 1.9a	43.9 ± 5.1a	40.7 ± 6.2b
E. kuehniella + pollen	6.3 ± 0.2ab	1864.1 ± 193.3a	33.3 ± 2.0a	12.7 ± 2.4a	35.1 ± 5.4ab	75.0 ± 11.7ab
A. franciscana + pollen	7.6 ± 0.6bc	889.9 ± 215.4b	11.3 ± 1.3b	1.7 ± 0.5b	13.9 ± 3.9b	108.1 ± 18.9a
Lyophilized artificial diet + pollen	8.0 ± 0.4c	264.7 ± 80.4b	6.3 ± 1.0b	1.3 ± 0.5b	18.6 ± 7.0b	62.3 ± 14.6ab
A. franciscana + lyophilized artificial diet + pollen	8.5 ± 0.4c	467.6 ± 68.6b	10.8 ± 1.1b	2.9 ± 0.5b	25.6 ± 4.6ab	82.4 ± 12.4ab

[a] Means ± standard error; means within a column followed by the same letter are not significantly different ($P > 0.05$; Tukey test or Tamhane test)

[b] Original data, transformed for analysis using arcsin

the other diets (Table 1). Survival on the mixture of lyophilized diet, *A. franciscana* cysts and pollen did not differ from that on aphids. Larval development ($F = 211.18$; $df = 4, 194$; $P < 0.001$) was shortest for larvae fed *E. kuehniella* and pollen and was more than twice as long on the lyophilized artificial diet supplemented with pollen. Pupal developmental time ($F = 5.74$; $df = 4, 194$; $P < 0.001$) was significantly shorter for larvae offered *E. kuehniella* and pollen than for those fed live aphids, *A. franciscana* cysts mixed with pollen and artificial diet mixed with pollen. Diet also affected total larval and pupal period ($F = 233.21$; $df = 4, 194$; $P < 0.001$), which was ca. ten days shorter on *E. kuehniella* eggs and pollen than on artificial diet supplemented with pollen. Adult weights ($F = 68.29$; $df = 4, 194$; $P < 0.001$) on moth eggs or aphids were almost twice those on the lyophilized artificial diet and pollen. Sex ratios of adults obtained on the different diets were essentially 1:1.

Diets affected all tested reproductive parameters, i.e. preoviposition period ($F = 9.686$; $df = 4, 57$; $P < 0.001$), lifetime oviposition ($F = 18.61$; $df = 4, 67$; $P < 0.001$), oviposition period ($F = 4.248$; $df = 4, 67$, $P = 0.004$), number of eggs per female per day ($F = 41.278$; $df = 4, 67$; $P < 0.001$), number of larvae per female per day ($F = 10.262$; $df = 4, 66$; $P < 0.001$), egg hatch ($F = 4.076$; $df = 4, 64$; $P = 0.005$) and longevity ($F = 3.604$; $df = 4, 67$; $P = 0.01$) (Table 2). Preoviposition period of females fed aphids was 2–3 days shorter than that of females fed brine shrimp cysts or artificial diet supplemented with pollen. Fecundity was characterized by high variability with coefficients of variation ranging from 45 to 80%. The number of eggs laid on *E. kuehniella* eggs plus pollen (>1,800 eggs per

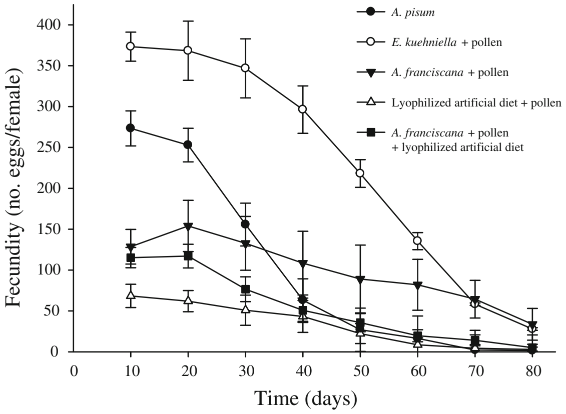

Fig. 1 Age-dependent fecundity (mean ± SE) of *A. bipunctata* reared on several foods

female) was superior to that on the other diets but no significant differences were found among aphids and the other unnatural diets tested, with mean values ranging from 265 to 890 eggs per female. The number of larvae produced per day by females fed aphids or lepidopteran eggs and pollen greatly exceeded that of females reared on the other unnatural diets. Whereas ca. 44% of the eggs produced over the insect's lifetime hatched on *E. kuehniella* eggs and pollen, egg hatch dropped to ca. 14% on *A. franciscana* and pollen. Females reared on *A. franciscana* cysts and pollen were longer lived than those offered live aphids.

ANCOVA revealed that there was a negative trend (common $\text{slope} = -1.55$; $t = 14.872$; $P < 0.001$) for the number of eggs laid over time. During the first 80 days of oviposition, the number of eggs laid per female was significantly influenced by diet ($F = 74.001$; $df = 4, 574$; $P < 0.001$) (Fig. 1). Fecundity on *E. kuehniella* eggs and pollen was superior to that on the other diets up to day 60. Females had greater fecundity on live aphids than on the diets containing pollen plus brine shrimp cysts or artificial diet only during the first 20 days.

Discussion

Larvae of *A. bipunctata* were unable to reach adulthood when reared solely on hydrated or dry decapsulated cysts of the brine shrimp *A. franciscana* or on the lyophilized meat and liver diet. This implies that these foods are nutritionally inadequate or its nutrients at least in part physiologically inaccessible. For instance, De Clercq et al. 2005a noted that dry cysts of *A. franciscana* contain only 3% water and lyophilized foods contain no accessible water at all (Cohen 2004). When *Artemia* cysts are hydrated, a slushy mass is produced. However, the cysts in a feed portion tend to lump together and as they dry out, the mass hardens and becomes difficult to process by the coccinellid larvae. Younger coccinellid larvae consistently use extra-oral digestion for prey consumption and for this process the availability of water is essential (Hodek 1973). Continuous loss of water for extra-oral digestion is energy consuming and may eventually cause mortality. Apparently, the presence of an external source of free water could not compensate for the lack of water within these foods. When bee pollen were added to dry *Artemia* cysts or lyophilized diet larval survival increased up to 40–55%. The pollen used in our study initially contained about 50% water, but due to its hygroscopic nature, pollen may absorb extra water from a humid environment. According to Diehl et al. (2001) some pollens can absorb up to 250% of their initial mass of water. Lyophilized biological products are also hygroscopic but when exposed to a relative humidity of 65% the resulting moisture content is expected to increase with about 10–20% only (Constantino et al. 1998; Wang 2004). Mixing pollen with dry brine shrimp cysts or lyophilized artificial diet ensures a continued hydration of latter materials, making them easier to be processed by the predator.

Besides this hydrating effect, pollen may also provide essential nutrients for the predator. The nutritional value of pollen for insects varies greatly among and even within plant species (Lundgren 2009), but particularly zoophilous pollen is known to contain high amounts of proteins, amino acids, starch, lipids, and sugars and traces of vitamins and minerals (Richards and Schmidt 1996; Patt et al. 2003; Atrouse et al. 2004; Lundgren 2009). Besides supplying the predator with extra nutrients, mixing pollen with the unnatural foods tested in our study may also have stimulated the ingestion of those foods. Developmental performance of *A. bipunctata* was improved when *A. franciscana* cysts, lyophilized artificial diet and pollen were mixed together as compared to combining pollen with cysts or artificial diet alone. The three-component mix probably provides the predators with a sufficiently broad range of physiologically accessible nutrients to complete development. Hodek and Honek (1996) suggested that many polyphagous coccinellids may have a ‘mixed’ feeding habit, allowing the coccinellids to select a favourable balance of important nutrients from plant and animal sources. Evans et al. (1999) found that egg production of two aphidophagous lady beetles, *Coccinella septempuncata* L. and *Coccinella transversoguttata* Brown increased when they were offered a mixture of an essential food (pea aphids) and an alternative food (alfalfa weevil larvae). The tendency to feed on mixed diets may allow these generalist predators to grow and reproduce in situations with uncertain availability of food resources.

Developmental parameters of *A. bipunctata* reared on *A. pisum* and *E. kuehniella* eggs mixed with pollen were broadly similar to those reported by De Clercq et al. (2005b) and Jalali et al. (2009). Only survival rates on *A. pisum* were higher than those found in the study of De Clercq et al. (2005b), but comparable with those reported by Obrycki and Tauber (1981).

All unnatural diets tested in this study supported development and reproduction of *A. bipunctata* to some extent. Kariluoto et al. (1976) were the only workers who tested a number of oligidic artificial diets based on whole hen’s eggs and beef liver for *A. bipunctata*. They reported similar developmental results as in our study with an average development time of 21.7 d, immature survival of 80% and adult weight of 7.0 mg. However, latter authors failed to obtain reproduction on any of the diets tested.

This and an earlier study (De Clercq et al. 2005b) indicate that *A. franciscana* cysts, lyophilized artificial diet and pollen alone or in a mixture are not as effective as foods for *A. bipunctata* as *E. kuehniella* eggs or live pea aphids. When supplemented with pollen, brine shrimp cysts or the meat and liver diet could, however, be used in part of the rearing process for the insect or in combination with a nutritionally optimal food. Offering mixed foods may contribute to increasing the cost-effectiveness of mass production by reducing the input of natural prey like aphids, or of nutritionally superior but highly expensive unnatural foods like lepidopteran eggs. In order to rationalize the use of such mixed foods the minimal amount of the optimal food should be determined and the ideal proportion of the different components assessed. Moreover, using several components in an insect diet makes the rearing process less dependent on a single food source. Availability and cost of different insect foods is often subject to fluctuations in production or harvest yields and market demand. Finally, experiments were currently done using isolated predators, excluding the risk of cannibalism. To fully appreciate the value of the studied diets for rearing *A. bipunctata*, future experiments should assess their effect on the fitness of the predator in communal rearing systems over multiple generations.

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References

Abdel-Salam AH, Abdel-Baky NF (2001) Life table and biological studies of *Harmonia axyridis* Pallas (Col.: Coccinellidae) reared on the grain moth eggs of *Sitotroga cerealella* Olivier (Lep.: Gelechiidae). J Appl Entomol 125:445–462

Arijs Y, De Clercq P (2001) Rearing *Orius laevigatus* on cysts of the brine shrimp *Artemia franciscana*. Biol Control 21:79–83

Atallah Y, Newsom LD (1966) Ecological and nutritional studies on *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae). I. The development of an artificial diet and a laboratory rearing technique. J Econ Entomol 59:1173–1179

Atrouse OM, Oran OM, Al-Abbadi SY (2004) Chemical analysis and identification of pollen grains from different Jordanian honey samples. Int J Food Sci Technol 39: 413–417

Brown PMJ, Adriaens T, Bathon H, Cuppen J, Goldarazena A, Hägg T, Kenis M, Klausnitzer BEM, Kovář I, Loomans AMJ, Majerus MEN, Nedved O, Pedersen J, Rabitsch W, Roy HE, Ternois V, Zakharov IA, Roy DB (2008) *Harmonia axyridis* in Europe: spread and distribution of a non-native coccinellid. BioControl 53:5–21

Castañé C, Quero R, Riudavets J (2006) The brine shrimp *Artemia* sp. as alternative prey for rearing the predatory bug *Macrolophus caliginosus*. Biol Control 38:405–412

Cohen AC (2004) Insect diets science and technology. CRC Press, Boca Raton

Constantino HR, Curley JG, Wu S, Hsu CC (1998) Water sorption behavior of lyophilized protein-sugar systems and implications for solid-state interactions. Int J Pharm 166:211–221

De Clercq P (2008) Culture of natural enemies on factitious foods and artificial diets. In: Capinera JL (ed) Encyclopedia of entomology, 2nd edn. Springer, Dordrecht, pp 1133–1136

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

De Clercq P, Arijs Y, Van Meir T, Van Stappen G, Sorgeloos P, Dewettinck K, Rey M, Grenier S, Febvay G (2005a) Nutritional value of brine shrimp cysts as a factitious food for *Orius laevigatus* (Heteroptera: Anthocoridae). Biocontrol Sci Technol 15:467–479

De Clercq P, Bonte M, Speybroeck K, Bolckmans K, Deforce K (2005b) Development and reproduction of *Adalia bipunctata* (Coleoptera: Coccinellidae) on eggs of *Ephestia kuehniella* (Lepidoptera: Phycitidae) and pollen. Pest Manag Sci 61:1129–1132

Diehl K, Quick C, Matthias-Maser S, Mitra SK, Jaenicke R (2001) The ice nucleating ability of pollen. Part I: Laboratory studies in deposition and condensation freezing modes. Atmos Res 58:75–87

Dong H, Ellington JJ, Remmenga MD (2001) An artificial diet for the lady beetle *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae). Southwest Entomol 26:205–213

Evans EW, Stevenson AT, Richards DR (1999) Essential versus alternative foods for insect predators: benefits of a mixed diet. Oecologia 121:107–112

Hemptinne JL, Desprets A (1987) Pollen as a spring food for *Adalia bipunctata* L. In: Hodek I (ed) Ecology of aphidophaga. Academia, Prague and Dr W Junk, Dordrecht, pp 29–35

Hodek I (1973) Biology of coccinellidae. Academia, Prague

Hodek I, Honek A (1996) Ecology of coccinellidae. Kluwer Academic Publishers, Dordrecht

Hongo T, Obayashi N (1997) Use of diapause eggs of brine shrimp, *Artemia salina* (Linné) for artificial diet of coccinellid beetle, *Harmonia axyridis* (Pallas). Jpn J Appl Entomol Zool 41:101–105

Jalali MA, Tirry L, De Clercq P (2009) Effects of food and temperature on development, fecundity and life-table parameters of *Adalia bipunctata*. J Appl Entomol 133:615–625

Kariluoto K, Junnikkalla E, Markkula M (1976) Attempts at rearing *Adalia bipunctata* L. (Col. Coccinellidae) on different artificial diets. Ann Entomol Fenn 42:91–97

Lavens P, Sorgeloos P (2000) The history, present status and prospects of the availability of *Artemia* cysts for aquaculture. Aquaculture 181:397–403

Lundgren JG (2009) The pollen feeders and adaptations to pollen feeding. In: Lundgren JC (ed) Relationships of natural enemies and non-prey foods. Progress in biological control 7. Springer Science + Business Media BV, New Jersey, pp 87–126

McCullagh P, Nelder J (1989) Generalized linear models. Chapman & Hall, London

Obrycki JJ, Tauber MJ (1981) Phenology of three coccinellid species: thermal requirements for development. Ann Entomol Soc Am 74:31–36

Okada I (1970) A new method of artificial rearing of coccinellid beetle, *Harmonia axyridis* Pallas. Heredity 24:32–35

Omkar PA, Pervez A (2005) Ecology of the two-spotted ladybird *Adalia bipunctata*: a review. J Appl Entomol 129:465–474

Patt JM, Wainright SC, Hamilton GC, Whittinghill D, Bosley K, Dietrick J, Lashomb JH (2003) Assimilation of carbon and nitrogen from pollen and nectar by a predaceous larva and its effects on growth and development. Ecol Entomol 28:717–728

Racioppi JV, Burton RL, Eikenbary R (1981) The effects of various oligidic diets on the growth of *Hippodamia convergens*. Entomol Exp Appl 30:68–72

Richards PC, Schmidt JM (1996) The effect of selected dietary supplements on survival and reproduction of *Orius insidiosus* (Say) (Hemiptera: Anthocoridae). Can Entomol 128:171–176

Riddick EW (2009) Benefits and limitations of factitious prey and artificial diets on life parameters of predatory beetles, bugs, and lacewings: a mini-review. BioControl 54: 325–339

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. Entomol Exp Appl 49:417–421

Sighinolfi L, Febvay G, Dindo ML, 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 a liver-based diet. Arch Insect Biochem 68:26–39

Smith BC (1966) Variations in weight, size and sex ratio of coccinellid adults (Col., Coccinellidae). Can Entomol 98:639–644

SPSS Inc (2003) User's guide, version 12.0. SPSS Inc., Chicago

Statacorp (2005) Stata statistical software/release 9. StataCorp LP, College Station

van Lenteren JC, Loomans AMJ, Babendreier D, Bigler F (2008) *Harmonia axyridis*: an environmental risk assessment for Northwest Europe. BioControl 53:37–54

Vandekerkhove B, Parmentier L, Van Stappen G, Grenier S, Febvay G, Rey M, De Clercq P (2009) *Artemia* cysts as an alternative food for the predatory bug *Macrolophus pygmaeus*. J Appl Entomol 133:133–142

Wang DQ (2004) Formulation characterization. In: Rey L, May JC (eds) Freeze drying/lyophilization of pharmaceutical and biological products, 2nd edn. Informa Healthcare, New York, pp 213–238