J. Appl. Entomol.

Effects of food and temperature on development, fecundity and life-table parameters of *Adalia bipunctata* (Coleoptera: Coccinellidae)

M. A. Jalali, L. Tirry & P. De Clercq

Laboratory of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

Keywords

Acyrthosiphon pisum, *Myzus persicae*, alternative vs. essential foods, biological control, factitious food, two-spot ladybird

Correspondence

Patrick De Clercq (corresponding author), Laboratory of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Coupure links 653, B-9000 Ghent, Belgium.
E-mail: patrick.declercq@ugent.be

Received: September 16, 2008; accepted: March 22, 2009.

doi: 10.1111/j.1439-0418.2009.01408.x

Abstract

Development, reproduction and life tables of *Adalia bipunctata* (L.) were studied at three temperatures (19, 23 and 27°C) on a mixture of frozen pollen and *Ephestia kuehniella* Zeller eggs as a factitious food and on the aphids *Myzus persicae* (Sulzer) and *Acyrthosiphon pisum* (Harris) as natural foods. Development time of *A. bipunctata* on all tested diets decreased with increasing temperature. Mortality was lowest at 23°C, averaging 44.5%, 42.6% and 24.3% on factitious food, *A. pisum* and *M. persicae* respectively. The shortest developmental time from egg to adult at this temperature was observed on factitious food (18.55 days). However, the factitious food was inferior to the aphid diets in terms of reproduction, yielding the longest pre-oviposition period, shortest oviposition period and lowest fecundity. The mean oviposition rate at 23°C varied from 19.94 to 25.03 eggs day^{-1} on factitious food and *M. persicae* respectively. The intrinsic rate of increase (r_m) on different foods increased with increasing temperature and ranged from a minimum of 0.08 females/female/day on factitious food (19°C) to a maximum of 0.18 females/female/day on *A. pisum* (27°C). The results suggest that a mixture of *E. kuehniella* eggs and pollen fully support development of *A. bipunctata* larvae and can be used as an alternative to live aphids in the mass rearing of the pre-imaginal stages of the predator. However, reproductive performance of a laboratory population may be better on aphids than on the factitious food.

Introduction

With growing concerns about unwanted environmental impacts of non-indigenous biocontrol agents (Simberloff and Stiling 1996; Thomas and Willis 1998; van Lenteren et al. 2006), the use of indigenous species for biological pest control receives increasing attention. The two-spot ladybird *Adalia bipunctata* (L.) is a polyphagous predator (Hodek 1973; Omkar and Pervez 2005), occurring in arboreal habitats of Europe, Central Asia and North America (Majerus 1994) and is one of the most common coccinellids in orchards (Putman 1964; Hodek and Honek 1996). *Adalia bipunctata* is a potential natural enemy of aphid pests in augmentative biological control in Europe (De Clercq et al. 2005). At present, it only has a 6% share of the total world market for aphidophagous agents, and in western Europe, about 75% of the total production of this predator is commercialized for aphid control in avenue trees (R. Timmer, Koppert BV, the Netherlands, personal communication). It has been suggested for aphid biocontrol in greenhouse crops (Hämäläinen 1977, 1980), but it is currently not widely used in greenhouses and is probably too expensive for use there. The implementation of augmentative biological control using *A. bipunctata*, and many other aphidophagous biocontrol agents, is strongly limited by the necessity to produce large numbers of aphids and to maintain the first trophic level (the prey's food plant), resulting in a high production price for the predator.

The use of factitious foods or artificial diets may make the production of beneficial arthropods for biological control more cost effective (De Clercq 2004). They may reduce problems associated with the space and manpower required for mass rearing of a biocontrol agent, enhance mechanization of rearing procedures and thus lower production costs (Waage et al. 1985; De Clercq 2002; De Clercq et al. 2005). Factitious (or unnatural) prey or hosts are organisms that are not normally attacked by the beneficial because they do not occur in its natural habitat, but that do sustain its development and/or reproduction (De Clercq 2004). Eggs of the lepidopterans *Ephestia kuehniella* Zeller and *Sitotroga cerealella* (Olivier) have been routinely used as factitious foods for the laboratory production of several insect predators. For instance, a number of coccinellids have been successfully reared on *E. kuehniella* eggs (Iperti et al. 1972; Schanderl et al. 1988; Kato et al. 1999; Specty et al. 2003; Hamasaki and Matsui 2006; Berkvens et al. 2008). De Clercq et al. (2005) found that a mixture of frozen moist bee pollen and *E. kuehniella* eggs is a suitable factitious food to sustain development and reproduction of *A. bipunctata*.

The ecology of the two-spot ladybird *A. bipunctata* has been reviewed by Omkar and Pervez (2005), whereas aspects of its predator–prey dynamics were treated by Dixon (2000). Although there have been several earlier studies on the development and reproduction of *A. bipunctata* at different temperatures (e.g. Ellingsen 1969a; Obrycki and Tauber 1981; Schüder et al. 2004) or on different prey (e.g. Blackman 1965, 1967; Kalushkov 1998; Ozder and Saglam 2003), only a few studies have constructed life tables for this coccinellid species (Pelicano and Folcia 2003; Lanzoni et al. 2004) and there have been no detailed reports of temperature and food effects on life-table parameters of *A. bipunctata*. The objective of the current study was to assess survival, development, reproduction and population growth potential of *A. bipunctata* fed on two natural prey species, the green peach aphid, *Myzus persicae* (Sulzer) and the pea aphid, *Acyrthosiphon pisum* (Harris) or on a mixture of bee pollen and *E. kuehniella* eggs at three different temperatures ranging from 19 to 27°C. The results may contribute to the improvement of rearing methods for *A. bipunctata* and of our understanding of its potential as a biocontrol agent of different aphid species.

Materials and Methods

Predator culture

Insects were taken from a laboratory stock colony at Ghent University, started in September 2004 with eggs purchased from Biobest NV (Westerlo, Belgium). In the laboratories of Biobest NV, the ladybirds had originally been fed with live pea aphids, but in the stock colony at Ghent University larvae and adults were fed on an *ad libitum* supply of a 50–50 mixture of frozen *E. kuehniella* eggs and fresh bee pollen (De Clercq et al. 2005). Frozen eggs of *E. kuehniella* and the pollen, consisting of pollen pellets collected by honeybees, used in our study were supplied by Koppert BV (Berkel en Rodenrijs, the Netherlands) and stored for no longer than 1 month at −18°C. The insects were maintained in plastic containers (29 × 21 × 9 cm), with ventilation holes in the lid screened with fine nylon mesh. A soaked paper plug fitted into a plastic dish served as a source of water for the insects. The stock colony of the predator was maintained in a growth chamber at 23 ± 1°C, 65 ± 5% RH and a 16 : 8 h (L : D) photoperiod.

Experimental conditions

The experiments were done at three constant temperatures (19, 23 and 27 ± 1°C), under the same conditions of relative humidity and photoperiod as for the stock colony and using three diets: a mixture of frozen bee pollen and eggs of *E. kuehniella* as a factitious food and two aphid prey, *M. persicae* and *A. pisum*, as natural foods. The former aphid was reared on broad bean, *Vicia faba* L. var. *thalia* at 26 ± 2°C, 60 ± 20% RH and a 16 : 8 h (L : D) photoperiod, whereas the latter was supplied from a mass colony on broad bean shoots by Biobest NV. For the experiments, a mixture of different nymphal stages of either aphid was provided.

Development

For the experiment on factitious food, clutches of *A. bipunctata* eggs (<24 h old) were collected from the stock colony at 23°C and were randomly allocated to the three temperature treatments. At each temperature, 1 day after egg hatching, at least 50 first instar larvae were placed in individual 9-cm Petri dishes, containing two plastic cups (3.0 × 0.5 cm), one with an *ad libitum* supply of factitious food and another one with a piece of soaked paper as a source of water. Foods were replenished every 2 days at 19 and 23°C and every day at 27°C.

For the experiments with either aphid, the predator was reared on *A. pisum* or *M. persicae* for one generation at 23°C. Eggs from first generation females were placed in incubators set at 19, 23 or 27°C. Upon egg hatch, at least 50 first instars (<24 h old) were selected randomly and placed in individual 14-cm Petri dishes. Each Petri dish was lined with filter paper and contained a two leaves seedling of broad bean infested with *A. pisum* or *M. persicae*. The stalks of the broad bean seedlings were inserted in an Eppendorf tube containing water.

In each treatment, development and survival of larval and pupal stages was monitored on a daily basis. One day after emergence, adults were weighed on a Sartorius Genius ME215P balance (±/0.01 mg; Sartorius, Göttingen, Germany). Sex ratio was determined at the time of death by dissecting the adults.

Reproduction

At each temperature, newly emerged adults (<24 h old) of *A. bipunctata* from the above mentioned experiments were paired and transferred to individual 14-cm Petri dishes. Dishes were lined with filter paper and either contained two plastic cups (with factitious food and water) or a broad bean seedling infested with pea aphids or green peach aphids. If the number of adults obtained in the developmental tests was not sufficient to produce the number of pairs required for the reproduction assays, additional newly emerged adults that had developed together in a rearing container (29 × 21 × 9 cm) under the same conditions, were used in the respective experiments. Pairs that successfully mated and oviposited during the first 2 weeks after adult emergence were selected for oviposition experiments. Reproductive parameters of adults fed on the factitious food were measured using 14 (19 and 27°C) and 23 pairs (23°C). For oviposition tests with aphids as food, only 11 pairs were studied because of aphid culture limitations. Males which died before their female partners were replaced by males reared under the same conditions. Female survival and oviposition were monitored daily. At 10-day intervals, all eggs collected from five randomly selected pairs were kept to determine egg hatch.

Data analysis

Data were submitted to two-way ANOVA at $\alpha = 0.05$ (SPSS 2006) for the significance of the main effects (food, temperature) and interactions. Percentages of egg hatch were transformed using a square-root transformation before analysis. Mean values were separated using the Tukey-Kramer honestly significant difference (HSD) test. Life and fertility table parameters were calculated using developmental and reproduction data obtained from the experiment described above. A VBA-macro for the Jackknife method to calculate life-table parameters described by Vantornhout et al. (2005) was used to calculate net reproductive rate (R_0), intrinsic rate of population increase (r_m), finite rate of increase (λ), mean generation length (T) and doubling time (DT).

Results

Developmental time, adult weight and sex ratio

Development time of *A. bipunctata* decreased with increasing temperature from 19 to 27°C (table 1). An increase of 4°C in temperature (19–23°C and 23–27°C) shortened the total development time of the predator by 60–80%, independently of the food. The effect of food on the duration of the different developmental stages (egg, L1–L4 and pupa) at 19 and 23°C, and on the developmental duration of egg, first and second instars and pupal stage at 27°C was significant ($P < 0.001$). The relative duration of the larval instars (as a proportion of total developmental duration) was similar in different treatments of temperature and diet, averaging 20–34%. A two-way ANOVA for the duration of total development with food and temperature as factors revealed a significant interaction of the two factors ($F = 85.63$; df = 4,343; $P < 0.001$). At 19 and 23°C, diet had a significant effect on the total developmental time of the predator ($F = 34.98$; df = 2,91; $P < 0.001$ at 19°C and $F = 189.89$; df = 2,149; $P < 0.0001$ at 23°C), with a shorter developmental time on *A. pisum* at 19°C and on factitious food at 23°C. However at 27°C, developmental time was not affected by diet ($F = 0.14$; df = 2,116; $P = 0.865$).

Two-way ANOVA showed a significant effect of food (males: $F = 11.01$; df = 2,175; $P < 0.001$; females: $F = 21.57$; df = 2,215; $P < 0.001$) and temperature (males: $F = 60.63$; df = 2,175; $P < 0.001$; females: $F = 48.67$; df = 2,15; $P < 0.001$) on body weight of adults. The interaction of the two factors for adult weight was significant for males but not so for

Table 1 Development time (days) for egg, larval (L1–L4), pupal and total immature stage, sex ratio and adult weight of *Adalia bipunctata* at different temperatures, fed on a factitious food (mixture of pollen and *Ephestia kuehniella* eggs) and two natural prey, *Acyrthosiphon pisum* and *Myzus persicae*

Temperature (°C)	Food	Developmental time[1]							Sex ratio (%)	Adult weight[1] (mg)	
		Egg	L1	L2	L3	L4	Pupa	Total		Female	Male
19	Factitious food	5.75 ± 0.04c (228)	4.06 ± 0.11b (60)	3.01 ± 0.15a (56)	2.98 ± 0.10a (56)	5.79 ± 0.11b (56)	9.54 ± 0.14ab (56)	31.09 ± 0.20b (54)	54 (54)	12.14 ± 0.24a (29)	9.80 ± 0.19a (25)
	A. pisum	5.40 ± 0.03b (200)	3.49 ± 0.09a (53)	2.69 ± 0.09a (50)	3.11 ± 0.07a (48)	5.09 ± 0.11a (48)	9.03 ± 0.09a (47)	28.94 ± 0.22a (45)	60 (45)	13.16 ± 0.25b (27)	10.01 ± 0.19a (18)
	M. persicae	5.02 ± 0.02a (264)	5.00 ± 0.15c (67)	3.70 ± 0.13b (63)	3.88 ± 0.16b (58)	6.22 ± 0.18b (44)	9.90 ± 0.14b (42)	33.48 ± 0.36c (38)	63 (38)	13.16 ± 0.42b (24)	10.03 ± 0.27a (14)
23	Factitious food	3.00 ± 0.03a (270)	2.16 ± 0.04a (71)	1.89 ± 0.08a (68)	1.91 ± 0.03a (68)	3.26 ± 0.04a (66)	6.44 ± 0.05a (63)	18.55 ± 0.07a (57)	58 (57)	12.22 ± 0.31a (33)	9.12 ± 0.24a (24)
	A. pisum	3.60 ± 0.04b (288)	3.13 ± 0.13b (50)	2.25 ± 0.09ab (47)	2.72 ± 0.09b (45)	3.76 ± 0.11b (42)	6.51 ± 0.10a (40)	22.08 ± 0.29b (38)	55 (38)	13.21 ± 0.23b (21)	10.39 ± 0.13b (17)
	M. persicae	3.78 ± 0.02c (208)	2.98 ± 0.10b (65)	2.30 ± 0.07b (62)	2.14 ± 0.07ab (61)	4.03 ± 0.09b (61)	6.07 ± 0.07a (60)	21.32 ± 0.14b (58)	53 (58)	13.35 ± 0.35b (31)	10.19 ± 0.17b (27)
27	Factitious food	2.81 ± 0.03b (241)	1.99 ± 0.05a (55)	1.61 ± 0.05a (54)	1.40 ± 0.10a (53)	2.50 ± 0.13a (51)	4.60 ± 0.14a (41)	14.99 ± 0.29a (37)	54 (37)	11.21 ± 0.16a (20)	8.85 ± 0.22a (17)
	A. pisum	2.57 ± 0.04a (262)	1.89 ± 0.05a (64)	1.72 ± 0.06a (60)	1.62 ± 0.07a (58)	2.87 ± 0.07a (51)	4.54 ± 0.08a (46)	15.12 ± 0.11a (41)	49 (41)	11.51 ± 0.34a (20)	8.74 ± 0.27a (21)
	M. persicae	2.50 ± 0.03a (236)	2.05 ± 0.04a (70)	1.60 ± 0.07a (65)	1.63 ± 0.07a (57)	2.61 ± 0.07a (54)	4.55 ± 0.07a (46)	15.01 ± 0.14a (40)	48 (40)	11.76 ± 0.23a (19)	8.91 ± 0.22a (21)

Values within parentheses are replications.

[1] Mean ± SEM within the same temperature and column followed by the same letter are statistically equivalent (Tukey-Kramer HSD test, $\alpha = 0.05$).

females (males: $F = 7.58$; $df = 4{,}175$; $P < 0.001$; females: $F = 1.27$; $df = 4{,}215$; $P = 0.289$). At 19°C, adult weight of females and at 23°C, that of both sexes was significantly affected by diet (females 19°C: $F = 8.71$; $df = 2{,}79$; $P < 0.001$; females 23°C: $F = 16.85$; $df = 2{,}82$; $P < 0.001$; males 23°C: $F = 11.79$; $df = 2{,}34$; $P < 0.001$), with individuals fed on aphids being heavier than those fed on factitious food (table 1). The percentage of females in the different treatments ranged between 48% and 63%, indicating an essentially 50 : 50 sex ratio.

Egg hatch

Egg hatch was lower on factitious food than on aphid diets (table 2). Eggs were two to three times less likely to hatch on factitious food than on *M. persicae* and *A. pisum* at the higher temperature. The highest percentage of hatched eggs (up to 93%) was observed at an intermediate temperature of 23°C on *M. persicae*. At different temperatures, egg hatch decreased gradually towards the end of the oviposition period. For example, at 23°C, it reached 0% *c.* 60, 70 and 90 days after the start of oviposition on factitious food, *A. pisum* and *M. persicae*, respectively (fig. 1). Percentage of hatched eggs in the first month of oviposition was significantly affected by food at all temperatures ($P < 0.001$). A two-way ANOVA revealed a non-significant interaction of the two factors at 19°C ($F = 1.14$; $df = 4{,}27$; $P = 0.368$), 23°C ($F = 0.32$; $df = 4{,}27$; $P = 0.860$) and 27°C ($F = 0.31$; $df = 4{,}27$; $P = 0.870$). Although at 19 and 23°C significantly higher egg viability was observed on *M. persicae*, at 27°C it was significantly higher on *A. pisum* in the first month of oviposition.

Larval and pupal mortality

Survivorship of first instars increased with increasing temperature from 19 to 27°C on the different diets (table 2), whereas mortality of late larval stages and pupal stage on all diets was highest at 27°C. Mortality of total larval and pupal stages was somewhat higher on aphid diets than on factitious food. The minimum and maximum total larval and pupal mortality of *A. bipunctata* on factitious food and *M. persicae* was observed at 23 and 27°C and on *A. pisum* at 19 and 27°C, respectively.

Longevity and oviposition

Temperature and food mainly influenced longevity and thus total fecundity of female adults of

Table 2 Age-specific mortality (%) of egg, larval (L1–L4), pupal and total immature stage of *Adalia bipunctata* at different temperatures, fed on a factitious food and two natural prey, *Acyrthosiphon pisum* and *Myzus persicae*

	Factitious food			*Acyrthosiphon pisum*			*Myzus persicae*		
Stage	19°C	23°C	27°C	19°C	23°C	27°C	19°C	23°C	27°C
n[1]	500	518	230	635	677	709	768	678	640
Egg	41.20	36.29	63.06	35.75	26.29	21.16	19.66	16.52	23.59
L1	6.67	4.21	1.82	5.66	6.00	3.12	5.95	4.62	4.29
L2	0.00	0.00	1.85	4.00	4.25	3.33	2.94	0.00	10.77
L3	0.00	0.00	3.77	0.00	6.67	10.34	12.07	0.00	3.51
L4	0.00	0.00	15.69	2.08	2.38	9.80	4.54	1.64	14.81
Pupa	3.57	9.09	9.76	4.25	5.00	10.86	9.52	3.33	13.04
Total immature stage	47.10	44.52	73.93	45.44	42.57	46.79	44.29	24.30	53.35

[1] Numbers of initially tested individuals.

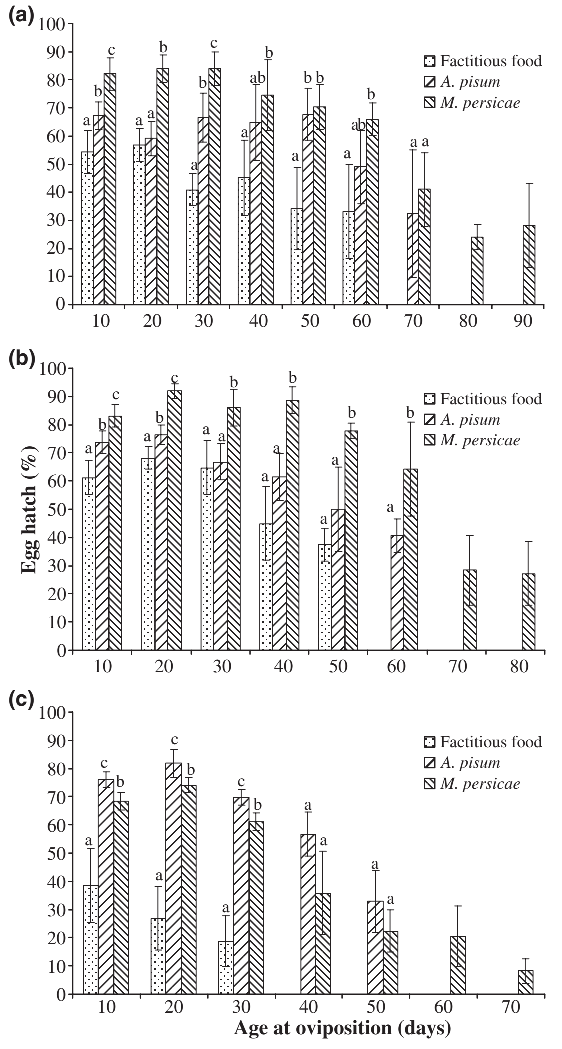

Fig. 1 Percentage of hatched eggs of *Adalia bipunctata* on different diets at 19°C (a), 23°C (b) and 27°C (c). Bars with the same letter are not significantly different according to Tukey's test (P > 0.05).

A. bipunctata. Two-way ANOVA revealed significant effects of temperature ($F = 46.28$; $df = 2,108$; $P < 0.001$) and food ($F = 13.32$; $df = 2,108$; $P < 0.001$), and no significant interaction between temperature and food ($F = 1.65$; $df = 4,108$; $P = 0.166$) on the longevity of female adults. Also, there was a significant effect of temperature ($F = 4.13$; $df = 2,108$; $P = 0.019$) and food ($F = 29.43$; $df = 2,108$; $P < 0.001$) on total fecundity and no significant temperature–diet interaction ($F = 1.92$; $df = 4,108$; $P = 0.113$).

When temperature increased from 23 to 27°C, longevity and total fecundity of *A. bipunctata* decreased sharply on factitious food (table 3), whereas these parameters did not change significantly ($P > 0.05$) on either aphid diet. In the range of the tested temperatures, female adults of *A. bipunctata* had greater fecundities on aphids than on the factitious diet. Females only lived longer ($P < 0.05$) on either aphid than on the factitious food at 27°C.

Two-way ANOVA further showed that temperature ($F = 35.10$; $df = 2,108$; $P < 0.001$) and food ($F = 16.26$; $df = 2,108$; $P < 0.001$) significantly affected the oviposition period of *A. bipunctata* (table 3). The interaction between food and temperature was not significant ($F = 1.08$; $df = 4,108$; $P = 0.365$). Where differences were found, *A. bipunctata* had a longer oviposition period on the aphid diets than on factitious food. Pre-oviposition period of the predator decreased with increasing temperature on the different diets. At 19 and 23°C, pre-oviposition period was 3–4 days longer on factitious food than on aphids but this time lag was not observed at 27°C.

Daily oviposition rates of female adults were superior on aphids than on the factitious food at 19°C

Table 3 Reproduction and longevity of *Adalia bipunctata* at different temperatures, fed on a factitious food and two natural prey, *Acyrthosiphon pisum* and *Myzus persicae*

Temperature (°C)	Food	n[1]	Pre-oviposition period (days)	Oviposition period (days)	Female longevity (days)	Total no. eggs	Eggs/female/day
19	Factitious food	14	12.51 ± 0.62b (10–15)	47.00 ± 5.71a (28–66)	60.84 ± 5.63a (40–78)	601.81 ± 125.31a (178–1162)	12.32 ± 1.71a (5–19)
	A. pisum	11	9.07 ± 0.25a (8–12)	58.43 ± 3.59ab (42–90)	69.64 ± 3.68a (53–101)	1144.43 ± 79.75b (737–1829)	19.63 ± 0.71b (16–24)
	M. persicae	11	8.45 ± 0.25a (8–10)	66.18 ± 6.91b (27–93)	78.27 ± 7.40a (38–108)	1187.82 ± 120.00b (471–1809)	18.24 ± 0.82b (14–24)
23	Factitious food	23	9.08 ± 0.26b (6–12)	28.34 ± 4.91a (4–59)	40.22 ± 6.13a (11–64)	598.00 ± 151.72a (235–1480)	19.94 ± 3.58a (6–39)
	A. pisum	11	5.46 ± 0.14a (5–6)	37.23 ± 3.81ab (21–62)	44.46 ± 3.87ab (28–70)	824.69 ± 92.21b (480–1525)	22.53 ± 1.38a (15–34)
	M. persicae	11	6.09 ± 0.28a (5–7)	44.81 ± 5.24b (21–79)	53.18 ± 5.33b (28–88)	1081.54 ± 108.35b (545–1673)	25.03 ± 1.48a (16–31)
27	Factitious food	14	5.10 ± 0.19ab (4–7)	14.05 ± 1.84a (3–39)	21.40 ± 1.90a (9–44)	302.75 ± 37.27a (74–632)	22.84 ± 1.92a (7–45)
	A. pisum	11	4.45 ± 0.25a (3–6)	36.64 ± 3.40b (23–54)	44.00 ± 3.74b (28–62)	1008.82 ± 125.46b (491–1763)	26.91 ± 1.30a (20–33)
	M. persicae	11	5.73 ± 0.14b (5–6)	36.54 ± 5.41b (13–60)	44.45 ± 5.60b (21–67)	966.18 ± 140.34b (327–1726)	28.00 ± 2.69a (18–47)

Values within parentheses are minimum–maximum. Mean ± SEM within the same temperature and column followed by the same letter are statistically equivalent (Tukey–Kramer HSD test, $\alpha = 0.05$).

[1] Number of replications.

($F = 18.15$; df = 2,35; $P < 0.001$), but no differences were found ($P > 0.05$) at the other temperatures.

Life tables

The life-table analysis suggested that the factitious food had the lowest nutritional quality among the tested diets (table 4). At 19 and 23°C, R_0 was higher on *M. persicae* than on *A. pisum* but at 27°C this was the reverse. At 19 and 27°C, r_m and λ were higher on *A. pisum*, while DT was higher on *M. persicae*. However, mean generation time (T) was always longer on *M. persicae*. With increasing temperature from 19 to 23°C, a large increase of r_m was observed on all diets. However, at 27°C a substantial increase in r_m was only observed on *A. pisum*. The two-way ANOVA revealed that r_m was significantly affected by temperature ($F = 31729.89$; df = 2,108; $P < 0.001$) and food ($F = 6604.35$; df = 2,108; $P < 0.001$). Interaction between these two factors was also significant for this parameter ($F = 1140.03$; df = 4,108; $P < 0.001$). Further, the effect of the studied factors and their interaction was significant for the parameters R_0, λ, T and DT ($P < 0.001$).

Discussion

At the temperatures examined here, *A. bipunctata* successfully completed its immature development on the tested factitious food (a mixture of frozen *E. kuehniella* eggs and pollen) as well as on live nymphs of *M. persicae* and *A. pisum*. Our data indicate the existence of rate isomorphy (i.e. the constant allocation of relative times to different stages of development, see Jarosik et al. 2002) over different temperatures and diets in our laboratory population of *A. bipunctata*. Development time decreased with increasing temperature, whereas total immature mortality increased with temperature on all tested diets. At 23 and 27°C, total developmental time of the immature stages was shorter on factitious food than on aphids. A similar result was noted by De Clercq et al. (2005). They observed that at 23°C, development time (first instar–adult) of *A. bipunctata* on a mixture of frozen *E. kuehniella* eggs and frozen pollen was 16.3 days, being 1 day shorter than on live *A. pisum*. Our data for larval development time of *A. bipunctata* on both aphid species at the range of 23–27°C (8–12 days) are in line with the observations of earlier authors (8.4–14.2 days: Smith 1965; Montes 1970; Obrycki and Tauber 1981; Lanzoni et al. 2004). Although our record for the larval development time of the predator on *A. pisum* at

Table 4 Life-table parameters of female *Adalia bipunctata* at different temperatures, fed on a factitious food and two natural prey, *Acyrthosiphon pisum* and *Myzus persicae*

			Life-table parameter[2]				
Temperature (°C)	Diet	n[1]	Net reproductive rate, R_0 (females/female)	Intrinsic rate of increase, r_m (females/female/day)	Finite rate of increase, λ (females/female/day)	Mean generation time, T (days)	Doubling time, DT (days)
19	Factitious food	14	141.56a (±4.67)	0.0862a (±0.0026)	1.0901a (±0.0011)	58.26b (±0.25)	8.148c (±0.062)
	A. pisum	11	337.77b (±1.33)	0.1077c (±0.0001)	1.1137c (±0.0001)	54.07a (±0.04)	6.437a (±0.006)
	M. persicae	11	344.11b (±2.90)	0.0967b (±0.0001)	1.1015b (±0.0001)	60.39c (±0.08)	7.167b (±0.008)
23	Factitious food	23	133.86a (±3.84)	0.1303a (±0.0014)	1.1394a (±0.0010)	38.10a (±0.11)	5.391c (±0.022)
	A. pisum	11	221.70b (±1.30)	0.1425b (±0.0002)	1.1531b (±0.0002)	37.91a (±0.06)	4.866b (±0.006)
	M. persicae	11	355.79c (±2.91)	0.1516c (±0.0002)	1.1637c (±0.0002)	38.75b (±0.05)	4.573a (±0.005)
27	Factitious food	14	31.05a (±1.54)	0.1369a (±0.0004)	1.1468a (±0.0003)	25.50a (±0.01)	5.141c (±0.007)
	A. pisum	11	227.71c (±2.35)	0.1895c (±0.0004)	1.2087c (±0.0005)	28.63b (±0.05)	3.657a (±0.008)
	M. persicae	11	159.66b (±1.95)	0.1641b (±0.0003)	1.1784b (±0.0004)	30.90c (±0.07)	4.224b (±0.008)

[1] Number of replications.

[2] Means ± SEM within the same temperature and column followed by the same letter are statistically equivalent (Tukey-Kramer HSD test, $\alpha = 0.05$).

19°C (14.5 days) lies within the range of earlier reports (10.8–16.3 days: Blackman 1965; Obrycki and Tauber 1981), the larval development time on *M. persicae* at 19°C (18.5 days) was much longer than earlier records (10.5–13.5 days: Blackman 1965; Ellingsen 1969a; Francis et al. 2001). Some discrepancies in experimental methods including different rearing conditions, food maintenance and food supply, may explain this contrast.

On all diets, mortality of older larvae and of pupae was higher at 27°C than at the lower temperatures, suggesting that a temperature of 27°C would be suboptimal for advanced immature stages. This is, however, in contrast with the findings of Obrycki and Tauber (1981) who showed that *A. bipunctata* had optimal pre-imaginal development and survival between 26.7 and 29.4°C. Further, at 23°C total larval and pupal mortality of the predator was two times higher on *A. pisum* than on *M. persicae*. This may be a result of differences in nutritional content, energetic values or costs associated with the capture and ingestion of prey (Hodek and Honek 1996; Roger et al. 2000; Soares et al. 2004). Atwal and Sethi (1963) found that *Coccinella septempunctata* L. developed better on *Lipathis erysimi* Kalt. than on other tested aphid species because it had a higher protein content. Many studies have indicated that first instar coccinellids have difficulty feeding on active and large prey (Majerus 1994; Phoofolo and Obrycki 1997; Dixon 2000). In our study, its larger body size may have rendered *A. pisum* more difficult to be captured by early instars of *A. bipunctata*. The costs associated with feeding on active prey may also partly explain the somewhat higher survival of *A. bipunctata* larvae on the inanimate factitious food vs. that on the aphid diets.

In our study food and temperature affected body weight, longevity and fecundity of *A. bipunctata*. Newly emerged adults reared on aphids were heavier than those provided factitious food at 19–23°C, but adults that had developed on either aphid diet had similar body weights. In contrast, Blackman (1965) produced heavier adults of *A. bipunctata* on *A. pisum* than on *M. persicae*. Furthermore, De Clercq et al. (2005) obtained heavier adults, with a higher oviposition rate, when larvae were fed on a mixture of *E. kuehniella* eggs and pollen than on *A. pisum*. Discrepancies between our study and that by De Clercq et al. (2005) may be related to the variable quality of the insect materials (pea aphids, *E. kuehniella* eggs) supplied from commercial sources.

Females reared on aphids had a shorter pre-oviposition period (except at 27°C) and a greater fecundity than those provided factitious food. It has been shown that the diet of immature predators can have a significant influence on adult life-history characteristics and a decrease in body weight (as a result of larval diet) may result in an eventual decrease in reproductive output (O'Neil and Wiedenmann 1990; Mohaghegh et al. 1999). However, Ware et al. (2008) found that larval diets did not affect adult longevity, ovipositional lag, proportion of eggs laid in clutches or ovariole number in *A. bipunctata* and *Harmonia axyridis* (Pallas), but some variation in the maximum clutch size and oviposition rate was seen. A wide range of adult longevity and fecundity rates has been recorded for *A. bipunctata* in the literature. Whereas Lanzoni et al. (2004) noted a longevity of

30.7 days and a total fecundity of 537 eggs at 25°C on *M. persicae* and *Pisum sativum* L. plants, Ellingsen (1969b) reported a mean longevity of 135 days and fecundity of 1466 eggs, when individuals developed on the same prey but on *Brassica napus* (L.) plants at 22°C.

Food quality can also affect the egg fertility of *A. bipunctata* (Blackman 1967; Kariluoto 1980). In the current study, egg viability was lower on factitious food indicating that this diet is nutritionally suboptimal for full egg fertility in *A. bipunctata*. In contrast, De Clercq et al. (2005) found that supplementing a diet of *E. kuehniella* eggs with frozen (moist) pollen had beneficial effect on egg hatch.

A decline in egg production and egg hatch with age was observed in females on all diets and at all temperatures. Earlier studies on *A. bipunctata* showed similar results when female adults were fed on *M. persicae* (Ellingsen 1969b; Kariluoto 1980) or *A. pisum* (El Hariri 1966). In the current study, egg hatch on *M. persicae* (23°C) remained higher than 80% during the first 6 weeks of adult life span, whereas Ellingsen (1969b) observed a decrease in egg hatch from about 75% to less than 20% during this period (22°C, on *M. persicae*). The observed decline in reproductive capacity with age may be related to the decreased levels of activity of coccinellid predators as they grow older (Frechette et al. 2004). Dixon and Agarwala (2002) showed that the decline in egg production and fertility with age in *H. axyridis* is associated with a decline in speed of locomotion, aphid consumption and assimilation.

Both aphid species yielded higher R_0 and r_m values than the factitious food, resulting in a higher number of offspring within a similar period, despite of the absence of differences between diets for the pre-imaginal development. The average r_m for *A. bipunctata* on aphid diets at 23°C in our study (0.14 females/female/day) was found to be in line with that reported by Pelicano and Folcia (2003) (0.16). The r_m recorded by Lanzoni et al. (2004) was, however, markably lower (0.08 females/female/day). Latter authors also found a lower net reproductive rate (18.49 females/female), but the reported generation time (40 days) was similar to ours. A comparison between the r_m of *A. bipunctata* and that of other Palearctic ladybirds shows that the intrinsic rate of population increase is similar for *Propylea quatuordecimpunctata* L. ($r_m = 0.15$ females/female/day, for the Palearctic population) (Obrycki et al. 1993), but greater for *C. septempunctata* ($r_m = 0.19$ females/female/day, for a population from France) (Phoofolo and Obrycki 1995) and *Hippodamia variegata* (Goeze) ($r_m = 0.178$ females/female/day) (Kontodimas and Stathas 2005). The intrinsic rate of increase of the green peach aphid ($r_m = 0.348$ females/female/day, Culliney and Pimental 1985) and pea aphid ($r_m = 0.295$ females/female/day, Walthall 1995) are markedly greater than that of *A. bipunctata* in our study, indicating that the predator will not show rapid increases in population size compared to the aphid. Thus, it appears that like in other aphid-predator systems (Edwards et al. 1979; Chiverton 1987; Madsen et al. 2004; Butler and O'Neil 2007), *A. bipunctata* may have its greatest impact prior to the exponential growth phase of its aphid prey.

Our results show that, like in other aphidophagous coccinellids (Michaud 2005), larvae and adults of *A. bipunctata* differ in nutritional requirements. For instance, the factitious food and *A. pisum* were equally suitable to support larval development at 23°C but differed in nutritional suitability to adults at this temperature. Earlier studies have also indicated that certain prey species may be suitable for larval development of a coccinellid whereas they are not a suitable food source for adult reproduction (e.g. Cabral et al. 2006). According to the definitions proposed in Hodek and Honek (1996), 'essential' foods support full development and reproduction of a predaceous coccinellid, whereas 'alternative' foods serve just as a source of energy and thus prolong survival of the predator. Essential foods may, however, show varying degrees of favourability, yielding different developmental and reproductive rates. Likewise, some alternative foods may be highly toxic whereas others may be sufficient to enable survival in periods of scarcity of essential foods. The biological parameters observed in our laboratory study suggest that *M. persicae* and *A. pisum*, as well as a factitious food composed of lepidopteran eggs and pollen can be considered essential foods for *A. bipunctata*.

In conclusion, our study shows that a factitious food consisting of *E. kuehniella* and pollen has sufficiently high nutritional value to support larval development of *A. bipunctata* but that it may not allow the coccinellid to realize its full reproductive potential. Nonetheless, we have succeeded in rearing over 35 consecutive generations of *A. bipunctata* at our facilities using this factitious food as the sole food source for larvae and adults. The population growth parameters reported here further suggest that *A. bipunctata* exhibits sufficient environmental plasticity to be used as a biological control agent against *M. persicae* in greenhouse systems and against *M. persicae* and *A. pisum* in a number of outdoor crops. However, more research needs to be undertaken to fully understand the control potential of mass-reared *A. bipunctata* in different cropping systems.

Acknowledgements

We thank the Ministry of Science, Research and Technology of Iran for financial support to M.A. Jalali (PhD grant no. 810086). We are also grateful to two anonymous reviewers for their constructive comments on the paper.

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