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Development and reproduction of *Adalia bipunctata* (Coleoptera: Coccinellidae) on eggs of *Ephestia kuehniella* (Lepidoptera: Phycitidae) and pollen

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Abstract: Due to growing criticism over the use of non-indigenous coccinellids, the two-spot ladybird, *Adalia bipunctata* (L.), has enjoyed increasing attention for aphid biocontrol in Europe. In the current study, eggs of the Mediterranean flour moth, *Ephestia kuehniella* Zeller, whether or not supplemented with bee pollen, were evaluated as a factitious food for larvae and adults of *A. bipunctata*. The predator showed slower larval development and lower survival when reared on live pea aphids, *Acyrthosiphon pisum* (Harris), than on *E. kuehniella* eggs. Survival on gamma-irradiated eggs of *E. kuehniella* was superior to that on frozen flour moth eggs, but other developmental characteristics were similar. Adults of *A. bipunctata* reared on *Ac. pisum* were only half as fecund as those offered irradiated or frozen *E. kuehniella* eggs, but egg hatch was markedly better on live aphids than on flour moth eggs (61 *versus* 20–27%, respectively). However, when a diet of flour moth eggs was supplemented with frozen moist bee pollen, egg hatch of *A. bipunctata* was equally as good as on live aphids. Supplementing flour moth eggs with dry pollen did not yield satisfactory results. Only 10% of larvae reached adulthood on moist bee pollen alone and resulting adults weighed less than half as much as those obtained on flour moth eggs. Our findings indicate that *A. bipunctata* is able to compensate for a suboptimal diet of animal prey by supplementary feeding on flower pollen. It is concluded that pollinivory may be a crucial trait for both the rearing of this natural enemy and its use in biological control programmes.

Keywords: *Adalia bipunctata*; rearing; factitious food; *Ephestia kuehniella*; pollinivory; biological control

1 INTRODUCTION

The use of non-indigenous coccinellids, such as *Harmonia axyridis* (Pallas) and *Hippodamia convergens* Guérin-Méneville, for biological control of aphid pests in European protected cultivation has met growing criticism because of possible environmental risks.[1,2] As a result, attention has shifted towards the potential of native coccinellids. The two-spot ladybird *Adalia bipunctata* (L.) has been commercialized for aphid biocontrol in Europe since 1999 (J-L Hemptinne, pers. comm.). Rearing of the predator is usually done on live aphids, including *Acyrthosiphon pisum* (Harris) and *Myzus persicae* Sulzer.[3–6] However, the necessity of maintaining three trophic levels (predator, prey and prey's food plant) may cause problems of discontinuity, and the high costs for rearing facilities and labour lead to high market prices for the predator. Production costs may be reduced when natural prey can be replaced partly or fully by factitious foods.[7] The polyphagous Asian multi-coloured ladybird *H. axyridis* shows successful development and reproduction on eggs of the Mediterranean flour moth *Ephestia kuehniella* Zeller and this food has routinely been used for mass production of the coccinellid[8,9] and many other insect predators.[7] The current paper investigates the value of *E. kuehniella* eggs as a factitious food for *A. bipunctata*, whether or not supplemented with pollen.

2 MATERIALS AND METHODS

A colony of *Adalia bipunctata* was established in August 2002 with specimens purchased from Biobest NV (Westerlo, Belgium). Since establishment, the colony has been augmented on several occasions with individuals from the same source. The predators

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were originally fed with live pea aphids, *Acyrthosiphon pisum*, purchased weekly from Biobest NV; later on, a mixture of pea aphids and frozen *Ephestia kuehniella* eggs was offered as food. Colonies were maintained and experiments performed in climatic chambers at 23 (±1)°C, 65 (±5)% RH and a 16:8 h light:dark photoperiodic regime.

Fresh gamma-irradiated eggs of *E. kuehniella* (4 days old or less) were supplied by Koppert BV (Berkel en Rodenrijs, The Netherlands) and stored for not more than 2 weeks in a refrigerator at 4 °C. Frozen flour moth eggs were obtained from the same supplier and kept in a deep freeze at −18 °C for a maximum of 2 months. The pollen used in our study consisted of pollen pellets collected by honey bees. Both dry and frozen moist pollen pellets were tested as foods for *A. bipunctata*. Dry bee pollen used in our study was a commercial product from Weyn's Honingbedrijf (Sint-Niklaas, Belgium); fresh, moist bee pollen was obtained from Koppert BV and stored in a deep freeze. Composition of the pollen pellets was determined after Erdtman's acetolysis[10] and mounting on microscopic slides in glycerol. In the feeding experiments, pollen pellets were pulverized to facilitate mixing with flour moth eggs.

Because of the discontinuous availability of some of the foods tested, a series of three experiments was carried out in order to compare the nutritional value of the different foods for *A. bipunctata*.

In a first experiment, development and reproduction of the coccinellid were assessed on three diets: gamma-irradiated *E. kuehniella* eggs, frozen *E. kuehniella* eggs and live pea aphids (late instars and apterous adults). Eggs were taken from stock cultures and allowed to hatch. Hatchlings were left on the egg batches for one day and then transferred to individual plastic Petri dishes (9 cm diameter) lined with absorbent paper. Water was provided by way of a moist paper plug fitted into a 1.5-cm plastic dish. Forty replicates were used for each diet. Development and survival of immatures were monitored daily and food was replenished every other day. Newly emerged adults were weighed on a Sartorius Genius ME215P balance (Sartorius, Göttingen, Germany). To study reproduction, 15 first mating adult couples were taken from stock cultures and isolated in plastic Petri dishes (14 cm diameter) lined with absorbent paper. Food and water were provided as above. Oviposition on each diet was monitored daily for a 30-day period. Oviposition rate was calculated by dividing the total number of eggs laid by the oviposition period. Hatching rate of eggs deposited during the experimental period was also determined. Females that died within the first 5 days were excluded from analysis.

In a second experiment, development and reproduction of *A. bipunctata* were evaluated on the following diets: irradiated *E. kuehniella* eggs, irradiated *E. kuehniella* eggs + dry pollen, and irradiated *E. kuehniella* eggs + frozen pollen. As in the first experiment, 40 one-day-old larvae were taken from stock colonies to monitor immature development. Adult pairs used in the reproduction tests, however, were not taken from stock colonies but originated from the development tests. Newly emerged adults were placed together in rearing containers and first mating pairs were transferred to individual Petri dishes (14 cm diameter) for testing. The insects were thus allowed to develop on the food that they would be receiving in the adult stage. As a result, the number of pairs tested varied as a function of the developmental success and mating propensity on the different diets. Furthermore, oviposition was monitored only for 18 days because irradiated flour moth eggs could no longer be supplied. Other methods were similar to those used in experiment 1.

In a third experiment, the coccinellid was offered either frozen *E. kuehniella* eggs, frozen *E. kuehniella* eggs + frozen pollen or only frozen pollen. Methods used here were the same as those in experiment 2, but oviposition was monitored for 30 days. Reproduction on frozen bee pollen alone was not tested because of the high immature mortality on that diet.

Data were subjected to one-way analysis of variance (ANOVA) followed by a Tukey test ($P = 0.05$) or, in case of non-normality or heteroscedasticity, by a non-parametric Kruskal–Wallis ANOVA and multiple comparisons ($P = 0.05$).[11]

3 RESULTS AND DISCUSSION

Adalia bipunctata larvae showed slower development and lower survival when reared on live pea aphids than on irradiated *E. kuehniella* eggs (Table 1, experiment 1). When fed aphids, only 40% of first instars successfully reached adulthood, with mortality evenly distributed over the different instars. Other workers have reported better immature survival (70–90%) for *A. bipunctata* fed on *Ac. pisum*.[3,4,12] Survival on irradiated eggs of *E. kuehniella* was superior to that on frozen eggs (87.5 *versus* 55%, respectively). Fresh weights of newly emerged adults did not differ among diets and were similar to those reported by Blackman[3] but lower than the values noted by Olszak.[12] Adults reared on *Ac. pisum* were only half as fecund as those offered flour moth eggs, and 27% of the tested females fed on aphids died within the first 5 days. However, egg hatch was markedly better on live food than on *E. kuehniella* eggs: over 60% of *A. bipunctata* eggs hatched successfully on aphids *versus* only 20–27% on irradiated or frozen flour moth eggs.

In experiment 2, a diet of irradiated *E. kuehniella* eggs was supplemented with dried or frozen moist pollen. Here, developmental time was not affected by diet, but survival and adult weight were somewhat higher on *E. kuehniella* eggs plus frozen pollen than on *E. kuehniella* eggs plus dry pollen or on *E. kuehniella* eggs alone. Adult sex ratios were not influenced by diet, ranging from 0.61:1 to 0.81:1 (male:female). On a diet of irradiated *E. kuehniella* eggs alone, a smaller number of mating pairs could be collected than in the

Table 1. Development from first instar to adult, oviposition rate and egg hatch of *Adalia bipunctata* on various diets

Diet	Developmental time (days) (±SE)[a]	Survival (%)[b]	Adult weight (mg) (±SE)[a]	Oviposition rate (eggs/female/day) (±SE)[a]	Egg hatch (%) (±SE)[a]
Experiment 1					
Irradiated *E. kuehniella* eggs	16.7 (±0.2) a (35)	87.5	12.5 (±0.3) a (35)	28.8 (±1.9) a (14)	20.2 (±4.9) b (14)
Frozen *E. kuehniella* eggs	17.1 (±0.3) ab (22)	55.0	12.3 (±0.5) a (22)	22.6 (±2.1) a (14)	27.4 (±5.2) b (14)
Live *Ac. pisum*	17.5 (±0.3) b (16)	40.0	11.4 (±0.4) a (16)	11.6 (±1.4) b (11)	61.5 (±8.5) a (11)
Experiment 2					
Irradiated *E. kuehniella* eggs	17.0 (±0.3) a (24)	60.0	11.4 (±0.3) b (24)	30.6 (±2.4) a (7)	37.7 (±11.7) b (7)
Irradiated *E. kuehniella* eggs + dry pollen	17.0 (±0.4) a (28)	70.0	11.8 (±0.4) b (28)	35.0 (±3.8) a (11)	44.0 (±9.8) b (11)
Irradiated *E. kuehniella* eggs + frozen pollen	17.4 (±0.2) a (33)	82.5	12.7 (±0.3) a (33)	36.4 (±2.4) a (13)	70.1 (±6.6) a (13)
Experiment 3					
Frozen *E. kuehniella* eggs	17.2 (±0.2) b (31)	77.5	13.8 (±0.3) a (31)	34.3 (±1.7) b (9)	45.3 (±8.5) b (9)
Frozen *E. kuehniella* eggs + frozen pollen	16.3 (±0.1) a (31)	77.5	14.5 (±0.4) a (31)	43.8 (±2.6) a (12)	65.9 (±6.3) a (12)
Frozen pollen	31.0 (±1.8) c (4)	10.0	6.7 (±0.4) b (4)	—	—

[a] Means within a column and an experiment followed by the same letter are not significantly different ($P > 0.05$, Kruskal–Wallis or Tukey test); numbers of observations are indicated in parentheses.
[b] The initial number of individuals in each development test was 40.

other treatments (7 *versus* 11–13 pairs), due to the lower survival in the development test and the lower mating propensity of the resulting adults. Oviposition rates were similar among diets (31–36 eggs/female day^{-1}), but egg hatch was significantly improved when irradiated flour moth eggs were supplemented with frozen pollen (70% *versus* 38–44% on the other diets).

In experiment 3, adding frozen moist pollen to a diet of frozen *E. kuehniella* eggs yielded faster development (16.3 *versus* 17.2 days) but survival and adult weight were not significantly affected. Sex ratios of adults obtained on frozen *E. kuehniella* eggs alone and on frozen *E. kuehniella* eggs plus frozen pollen averaged 0.69:1 and 0.64:1 (male:female), respectively. Both oviposition rate and egg hatch were significantly higher on *E. kuehniella* eggs plus frozen pollen than on *E. kuehniella* eggs alone. Frozen pollen only did not successfully support development of *A. bipunctata.* Only 10% of the larvae reached the adult stage on frozen pollen alone and the resulting adults (75% males) weighed less than half as much as those obtained on a diet of flour moth eggs.

The variable performance of the predator on irradiated *E. kuehniella* eggs in experiments 1 and 2, and on frozen eggs in experiments 1 and 3 may be explained in part by the varying quality of the subsequent batches received from the commercial supplier, due to inconsistent processing of the eggs or to suboptimal transport and storage.

From their extensive literature review, Hodek and Honek[13] concluded that *A. bipunctata* is a polyphagous predator that preys on a wide range of aphid species but also feeds on other types of arthropod prey. Burgio *et al*.[6] mentioned that they reared *A. bipunctata* larvae on *E. kuehniella* eggs (adults were fed live aphids), but did not provide detail on the nature of the eggs and the success of the method. Our findings indicate that *E. kuehniella* eggs are a suitable food for rearing larvae and adults of *A. bipunctata* provided that the diet is supplemented with moist bee pollen. Supplementing flour moth eggs with dry pollen did not yield satisfactory results. At Ghent University, over 10 continuous generations of *A. bipunctata* have been successfully reared on a mix of frozen *E. kuehniella* eggs and frozen moist bee pollen.

Hodek and Honek[13] wisely suggested that many polyphagous coccinellids may have a 'mixed' feeding habit, in which they select a favourable balance of important nutrients from various foods, including plant materials. Pollinivory has been demonstrated for several polyphagous coccinellids, including *A. bipunctata, Coleomegilla maculata* (DeGeer) and *H. axyridis*.[13,14] Blackman[15] reported that *A. bipunctata* frequently fed on fresh hazel and willow pollen even when aphids (*Ac. pisum*) were present. The use of pollen as a spring food by the two-spot ladybird has been documented by Hemptinne and Desprets[16] and Hemptinne and Naisse.[17] Both pollen mixtures used in our study originated from Southern Europe and were collected by honey bees on a variety of wild and cultivated plants. The frozen moist pollen mixture consisted mainly of pollen grains from *Phillyrea* sp. and *Olea europea* L., besides *Buxus sempervirens* L. and *Cistus* spp. The dry pollen mixture was dominated by pollen grains from *Echium* and *Cistus* spp. Differences in the nutritional value of the pollen mixtures may have contributed in part to the observed differences in performance of *A. bipunctata* in experiment 2. Also, stimulant or repellent compounds present in the consumed pollen grains may have influenced food intake by the coccinellid.

When used as a factitious food for rearing insect predators, hatching of *E. kuehniella* eggs is usually prevented by freezing or by UV- or gamma-irradiation.[7] Irradiation of *E. kuehniella* eggs was believed to be a better way of maintaining the nutritional quality of the eggs than freezing, because inappropriate freezing and thawing was expected to entail cellular damage and lead to rapid deterioration of essential nutrients. Our results show, however, that frozen *E. kuehniella* eggs are an equally suitable food for *A. bipunctata* as irradiated eggs, provided that moist pollen is offered as a supplement.

In conclusion, our study demonstrates that the polyphagous coccinellid *A. bipunctata* can compensate for a nutritionally suboptimal diet of animal food by supplementary feeding on flower pollen. Understanding the zoophytophagous habits of this and other insect predators may not only lead to more efficient production methods but may also be crucial for optimizing pest-control programmes employing these natural enemies.

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