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Pollen as an alternative or supplementary food for the mirid predator *Macrolophus pygmaeus*

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ABSTRACT

For the mass production of the mirid predator *Macrolophus pygmaeus* eggs of the Mediterranean flour moth *Ephestia kuehniella* are routinely used as an effective but expensive factitious food. In the current study, the potential of pollen as a supplementary food for *M. pygmaeus* was investigated. In a first experiment, the minimum amount of *E. kuehniella* eggs needed for optimal development and reproduction was determined to be 40 eggs per individual per 3 days. Then, different amounts of *E. kuehniella* eggs were offered to individual nymphs, supplemented or not with frozen moist honeybee pollen. Insects reared on only 10 *E. kuehniella* eggs per 3 days suffered higher mortality, developed slower and had lower adult weights and oocyte counts than insects reared on 40 *E. kuehniella* eggs or 10 eggs supplemented with pollen. When the nymphs were fed only pollen, survival rates and oocyte production were lower than when both pollen and flour moth eggs were provided. On pollen alone, ca. 80% of the nymphs successfully reached adulthood; their adult weights and oocyte counts were, respectively, 12% and 32% lower compared with individuals fed optimal amounts of flour moth eggs. When an egg yolk-based artificial diet was supplemented with bee pollen, development and fecundity were better than on the artificial diet alone. The practical implications of pollinivory for the mass production and the use of this beneficial insect in augmentative biological control programs are discussed.

1. Introduction

Macrolophus pygmaeus (Rambur) (Hemiptera: Miridae) is a natural enemy of major economic importance for the biological control of whiteflies and other small arthropod pests in protected cultivation of temperate and Mediterranean Europe (Perdikis et al., 1999; Perdikis and Lykouressis, 2000; Margaritopoulos et al., 2003). *M. pygmaeus* is a zoophytophagous predator and is able to complete its development in the absence of prey on several crop and non crop plants such as tomato, pepper, cucumber and eggplant (Lykouressis et al., 2001; Perdikis and Lykouressis, 2004). It has been shown by Perdikis and Lykouressis (2000) that pollen can stimulate development of *M. pygmaeus* when combined with other food sources, such as eggplant leaves and aphids.

The current study investigated the effect of pollen as a sole or supplementary food on the developmental and reproductive fitness of *M. pygmaeus*. Eggs of the Mediterranean flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) are currently widely used as a factitious food for rearing *M. pygmaeus*. These eggs are highly effective in supporting nymphal and adult cultures of the predator but are also costly, with current market prices in the range of 600–800 EUR/kg. Partial replacement of *E. kuehniella* eggs in the production process of *M. pygmaeus* could lead to a cost reduction, which may further stimulate the use of the predator in biological control programs (De Clercq, 2008). In the present study, the effect of supplementing suboptimal amounts of *E. kuehniella* eggs with frozen moist honeybee pollen on the developmental and reproductive performance of *M. pygmaeus* was investigated. The potential of pollen to supplement a nutritionally suboptimal artificial diet was also tested.

2. Materials and methods

2.1. Insect colony

The test insects of *M. pygmaeus* were taken from a laboratory colony started with eggs supplied by Koppert B.V. (Berkel & Rodenrijs, The Netherlands). Nymphs were reared in plastic containers (24 × 16 × 8 cm), furnished with paper towels and covered with nylon netting. The nymphs were reared on leaves of *Nicotiana tabacum* L. cv. Xanthi and were fed frozen eggs of *E. kuehniella*, also supplied by Koppert B.V.; flour moth eggs were replenished every two days. Water was provided via a soaked

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paper plug fitted into a plastic dish (4.3 cm diameter). The insects were maintained in growth chambers at 23 ± 1 °C, 60 ± 5% RH and a 16L:8D h photoperiod for the rearing of the stock colony and for all experiments described below.

2.2. Preparation of the artificial diet

The artificial diet used in the experiments is based on the diet described by White et al. (2000). It contained 2 g Primatone®, 20 g soy flour, 10 g maltose (all from MPX Biomedicals, Asse-Relegem, Belgium), 25 g tap water and 20 g fresh hen's egg yolk. The ingredients were mixed at high speed with a Virtis mixer (SP Industries Inc., Gardiner, New York) for 2 min. The diet was stored in a refrigerator at 4 °C and new diet was prepared every week. The semi-liquid artificial diet was encapsulated using a diet encapsulation device (Analytical Research Systems, Gainesville, Florida) into 70 μL hemispherical domes, consisting of stretched Parafilm®'M' and sealed with adhesive tape (Scotch® 3 M packaging super tape).

2.3. Determining the minimum amount of E. kuehniella eggs needed for optimal development of individual nymphs

In order to appreciate the value of pollen in supplementing suboptimal amounts of *E. kuehniella* eggs, the minimum amount of these eggs to be administered to individual nymphs for optimal development had to be determined. First instars (<24 h old) were taken out of the stock culture and were placed individually in plastic cups (4 cm diameter, 2.5 cm high) on paraffin impregnated wax paper substrates. Two individual Parafilm® domes filled with tap water were offered to the insects to serve as a moisture source. Predator nymphs were offered 5, 10, 20, 40 or 80 eggs of *E. kuehniella* (respective sample sizes: 28, 27, 28, 59, 30 and 33 first instars). The flour moth eggs and water domes were replaced every 3 days. Nymphs that died on the first day of the experiment were replaced by new ones, as it was assumed that their death was due to handling rather than to treatment effects. Nymphal development and survival were monitored on a daily basis. Nymphs that successfully reached the adult stage were sexed and weighed at emergence on a Sartorius Genius balance type 'ME215P' with 0.01 mg precision (Sartorius, Goettingen, Germany). Unmated females were kept individually for one more week on the same diet after which they were dissected; oocytes were counted following a weighted counting method described by Vandekerkhove et al. (2006): late vitellogenic to mature oocytes in ovarioles were scored 1, early to mid vitellogenic oocytes 0.5 and previtellogenic oocytes 0.25; mature oocytes present in the oviducts were also scored 1. The scores for all ovarioles were then summed yielding a weighted sum of oocytes. Escaped individuals were omitted from analysis.

2.4. Pollen as a supplement or substitute for E. kuehniella eggs

In a subsequent experiment it was investigated if supplementing pollen could compensate for a suboptimal amount of *E. kuehniella* eggs. Also developmental and reproductive fitness of *M. pygmaeus* fed solely on bee pollen was assessed. The pollen used in the experiments was frozen moist honeybee pollen (i.e. pollen placed in the freezer at the day of collection), consisting of a mixture of pollen mainly from Brassicaceae (67%) and Anacardiaceae (11%). The pollen pellets were thawed and finely ground with a Nova type 20 grinder before being offered to the insects. First instars (<24 h old) of the predator were taken out of the stock cultures and were placed in the same cups as described above on paraffin impregnated wax paper substrates. Two Parafilm® domes filled with tap water were offered to the insects to provide for moisture. Predator nymphs were fed one of the following diets: 10 *E. kuehniella* eggs, 40 *E. kuehniella* eggs, 10 *E. kuehniella* eggs supplemented with 15 mg pollen, 40 *E. kuehniella* eggs supplemented with 15 mg pollen, 80 *E. kuehniella* eggs supplemented with 15 mg pollen or 15 mg pollen only (respective sample sizes: 28, 64, 31, 26, 26 and 29 first instars). All foods and water domes were replaced every three days. Development and survival of nymphs were daily monitored and resulting adults were weighed at emergence. Unmated females were transferred to new individual cups, offered the same diet as in their nymphal life and dissected after one week of adult life to count oocytes.

2.5. Pollen as a supplement or substitute for an artificial diet

A third experiment was carried out to investigate whether a suboptimal artificial diet (White et al., 2000) could be supplemented with bee pollen to support development and reproduction of *M. pygmaeus*. The experimental setup was similar to that in the previous experiment. Besides Parafilm® domes with tap water, individual nymphs now received one of four diets: 40 eggs of *E. kuehniella*, an artificial diet dome, an artificial diet dome supplemented with 15 mg pollen or 15 mg pollen alone (respective sample sizes: 38, 36, 39 and 36 first instars). All foods and water domes were replaced on Mondays, Wednesdays and Fridays. Development and reproductive potential were assessed as described above.

2.6. Statistical analysis

One-way analysis of variance (ANOVA) was performed to determine differences in nymphal development times, adult weights, oocyte counts and predation rates among treatments. Means were separated using a Tukey test (variables with homoscedastic data) or a Tamhane test (variables with heteroscedastic data). Nymphal survival rates were compared among treatments by a two-way contingency table with Pearson's χ^2 test. All data were statistically analysed using SPSS 15.0 (SPSS Inc., 1989–2009).

3. Results

3.1. Determining the minimum amount of E. kuehniella eggs needed for optimal development of individual nymphs

Nymphal survival was significantly affected by treatment ($\chi^2 = 16.67$; $df = 5$; $P = 0.005$; Pearson's χ^2 test). Survival was high in treatments supplying at least 10 flour moth eggs to individual nymphs per 3 days, ranging from 86.4% to 96.3% (Table 1). When nymphs were fed only 5 *E. kuehniella* eggs per 3 days, survival averaged 64.3%.

There were significant differences in developmental times of predators offered different amounts of flour moth eggs ($F = 42.6$; $df = 5.172$; $P < 0.001$; ANOVA). Nymphs that were fed 20 eggs or more every 3 days developed to adults within 17.8–18.0 days. Those reared on 10 *E. kuehniella* eggs needed a significantly ($P < 0.05$) longer time (19.5 days) to complete their development, whereas those reared on 5 eggs took 23.5 days to reach adulthood.

Supplying 40 or more flour moth eggs every 3 days resulted in significantly higher adult female body weights than when 20, 10 or 5 flour moth eggs were given ($F = 65.6$; $df = 5.81$; $P < 0.001$; ANOVA). Male body weights were significantly higher on 20 eggs or more than on 10 or 5 eggs ($F = 18.3$; $df = 5.82$; $P < 0.001$; ANOVA).

Females fed 40 or more flour moth eggs every 3 days had higher oocyte counts (13.8–14.9) than those fed 20 eggs (9.6). When females only received 10 or 5 flour moth eggs, the oocyte counts dropped to 3.1 and 0.2, respectively ($F = 39.2$; $df = 5.77$; $P < 0.001$; ANOVA).