

## Pollen as an alternative food for *Harmonia axyridis*

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**Abstract** The current study examines the potential of the multicoloured Asian lady beetle *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) to use pollen as a food to sustain development and reproduction in the absence of insect prey. Three populations of *H. axyridis* were used in this study: a long-term laboratory population (since 1998) and a melanic and non-melanic population originating from field collected individuals in Belgium. The insects were allowed to develop and reproduce on frozen eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Phycitidae), frozen moist bee pollen or an even mixture of the two. Females of the field population offered the mixed diet initiated oviposition sooner than those fed only *E. kuehniella* eggs, but other developmental and reproductive traits were similar on these diets. A diet of pollen alone allowed 35–48% of the larvae of the field population of *H. axyridis* to successfully reach adulthood. However, developmental time for these individuals was prolonged by 31–49% and adult body weight was reduced by 37–68%, compared to individuals offered the diets containing *E. kuehniella* eggs. When fed exclusively on pollen in their larval and adult life, about 40% of the adult females of

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either field population were able to produce a small number of viable eggs. The laboratory and field strains differed in their response to diet for a number of developmental and reproductive traits. The exploitation of pollen and other plant foods at times when insect prey is scarce, may offer a further competitive advantage to the non-indigenous coccinellid *H. axyridis* over native European predatory lady beetles that share the same niche and are less capable of using pollen as an alternative food.

**Keywords** Coccinellidae · Coleoptera · Development · *Ephestia kuehniella* · *Harmonia axyridis* · Invasive species · Morph type · Pollen · Reproduction

## Introduction

*Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) is an aphidophagous coccinellid, indigenous to central and eastern Asia (Coderre et al. 1995; Nalepa et al. 1996; Koch 2003). The species was used as an augmentative biological control agent of aphid pests in orchards, field crops and greenhouses in both North America and Western Europe. Most probably as a result of these augmentative releases *H. axyridis* established in both continents (Koch 2003; Coutanceau 2006; Brown et al. 2007). Accompanying the large-scale colonisation in North America, several harmful side-effects were recorded: a shift in the coccinellid communities due to the dominance of the invader along with a decline in population numbers of some indigenous lady beetle species, nuisance to humans during its dormancy phase and secondary damage to crops (Koch 2003; Kenis et al. 2007; Pell et al. 2007). The species is at present exponentially expanding its geographical range in Europe (Brown et al. 2007) and could in its aftermath have a similar impact as that in North America. The mechanisms driving the successful colonisation and the ecological consequences for Europe are, however, still not entirely clear.

The present direction in scientific research concerning invasive species is in understanding the mechanisms determining the success of invasions and identifying the traits of successful invaders and the conditions under which these traits promote invasion (Holway 1999; Byers 2000). An important factor in the establishment of an invading species is the food range (van Lenteren et al. 2003). The exploitation of available resources and the nature of mutual competitive interactions with native ecological analogs is an important determinant of the invasion success of a species (Byers 2000). *Harmonia axyridis* is a generalist predator attacking Tetranychidae, Psyllidae, Coccoidea and immature stages of Chrysomelidae, Curculionidae and Lepidoptera, but the main food source for adults consists of several aphid species (Teddners and Schaefer 1994; Hodek and Honek 1996; Koch 2003). Many predaceous arthropods, including coccinellids, are also facultative plant feeders and additionally feed on a wide variety of non-prey foods such as pollen, nectar, fungal spores, plant wound exudates and phloem sap (Gilbert 1981; Hodek and Honek 1996; Lamana and Miller 1996; Patt et al. 2003; Koch et al. 2004; Lundgren et al. 2004). The supplementary nutrients found in these foods can either sustain the predators in periods where nutritionally optimal prey is scarce or complement the nutrients obtained from suboptimal prey (Kiman and Yeorgan 1985; Hodek and Honek 1996; Jørgenson and Toft 1997; Patt et al. 2003).

This study investigated whether pollen can be exploited by *H. axyridis* as an alternative food source. This was done by assessing the effect of pollen on the developmental and reproductive performance of melanic and non-melanic morphs of a European field population of *H. axyridis*. The results are discussed in view of the significance of non-prey food

for the establishment potential of this species. Additionally, a long-term laboratory population originating from a commercial culture that was one of the sources of biological control releases in the area since 1997 (Brown et al. 2007) was included in the study to compare its performance with that of the field population.

## Materials and methods

### Populations of *H. axyridis*

All experiments were conducted in the laboratory using three populations: a laboratory population and two field populations.

Larvae acquired from Biobest NV (Westerlo, Belgium) were used to start the laboratory population in September 1998 at the Laboratory of Agrozoology of Ghent University. The laboratory colony was repeatedly infused with new adults obtained from the same source up to August 2000. The commercial supplier did not reveal the origin of its culture or the methods used for rearing. The current experiments were conducted using individuals of the 55th generation of rearing at Ghent University. At the time of this study, the laboratory population consisted of more than 99% melanic *spectabilis* and *conspicua* morphs.

Using visual, sweeping and beating techniques (Elliott et al. 1991) individuals from an established wild population were collected in October 2005 in a wild open space in Ghent, Belgium. Two populations were initiated using the collected individuals; these populations will be referred to as 'field populations'. The first field population consisted of non-melanic *succinea* individuals (the 'red field population') and the second of melanic *spectabilis* and *conspicua* individuals (the 'black field population'). Individuals of the 5th generation of these populations were used for the experiments.

All populations were reared on frozen *Ephestia kuehniella* Zeller (Lepidoptera: Phycitidae) eggs, as described by Ongagna et al. (1993) and De Clercq et al. (2003).

### Experimental design

Experiments were conducted in Petri dishes (diameter 9 cm, height 2 cm) that were kept in incubators held at  $23 \pm 1^\circ\text{C}$ ,  $65 \pm 5\%$  RH and a 16:08 h (L:D) photoperiod. Three diets were tested: frozen *E. kuehniella* eggs, frozen moist pollen collected by honeybees (i.e. fresh, non-dried pollen placed in the deepfreeze immediately upon collection) and a mixture of frozen bee pollen and *E. kuehniella* eggs in equal weight proportions (referred to as the mixed diet). Both frozen bee pollen and *E. kuehniella* eggs were obtained from Koppert BV (Berkel en Rodenrijs, The Netherlands). The composition of the pollen was determined after Erdtman's acetolysis and mounting on microscope slides in glycerol (Erdtman 1960). Identifications of the pollen were based on Moore et al. (1991) and Beug (2004) and a reference pollen collection. The honeybee collected pollen consisted mainly of pollen from Brassicaceae (74.9–79.2%) and *Verbascum* spp. (Scrophulariaceae) (9.7–16.2%). Additionally small percentages of pollen of *Cornus sanguinea* L., *Trifolium repens* L., *Ranunculus acris* L. and of *Prunus* and *Vitis* sp. were found. Pollen pellets were thawed and finely pulverized using a Nova type 20 grinder before being offered to the insects or being mixed with the *E. kuehniella* eggs.

Twenty pairs of adults (3–5 days old) were randomly taken from each population. The adult pairs were placed in individual Petri dishes and left to mate and reproduce for one week. During this period they were fed a mixture of *E. kuehniella* eggs and pollen to prevent food source conditioning. After this week an accordion-pleated carton board strip was placed in each Petri dish to serve as an oviposition substrate and all eggs deposited during the following day were collected. Upon hatching, nine larvae were randomly collected per adult pair and placed individually in a Petri dish. Three larvae from each female were allotted to each of the diets tested. In this manner each diet treatment started off with 60 first-instar larvae that were then left to develop to adulthood. Sex of the resulting adults was determined (McCornack et al. 2007) and twenty to thirty pairs were formed from individuals that had experienced the same diet. When composing adult pairs, the pairing of brothers and sisters was carefully avoided. Each pair was then placed in a Petri dish, subjected to the same food regimen as during immature development and left to reproduce. During the entire experiment the foods were offered ad libitum and were replaced every other day; water was provided by way of a moist paper plug fitted into a 1.5-cm plastic dish.

### Development and reproduction

The influence of the tested diets on the developmental and reproductive performance of the three *H. axyridis* populations was assessed.

Developmental duration and survival of the immature stages were monitored on a daily basis. The presence of exuviae and colour patterns (Koch 2003) were used to identify larval instars. The total developmental period was defined as the period from oviposition to adult emergence.

Fresh body weight was determined at adult emergence. Adults were starved for one day and weighed on a semi-microbalance Sartorius Genius ME215P (Sartorius AG, Goettingen, Germany) ( $\pm 0.01$  mg). In addition, body length of adults was measured at the time of death. Using an ocular micrometer the distance between the anterior margin of the pronotum and the posterior margin of the elytra was measured.

Reproduction was characterised using four parameters: pre-oviposition period, number of oviposition days, egg batch size and hatching rate of eggs. The values of pre-oviposition period and number of oviposition days were determined by checking the Petri dishes daily for oviposited eggs. Egg batch size and hatching rate were determined on a weekly basis. In addition, longevity of female adults was determined on each diet.

### Data analysis

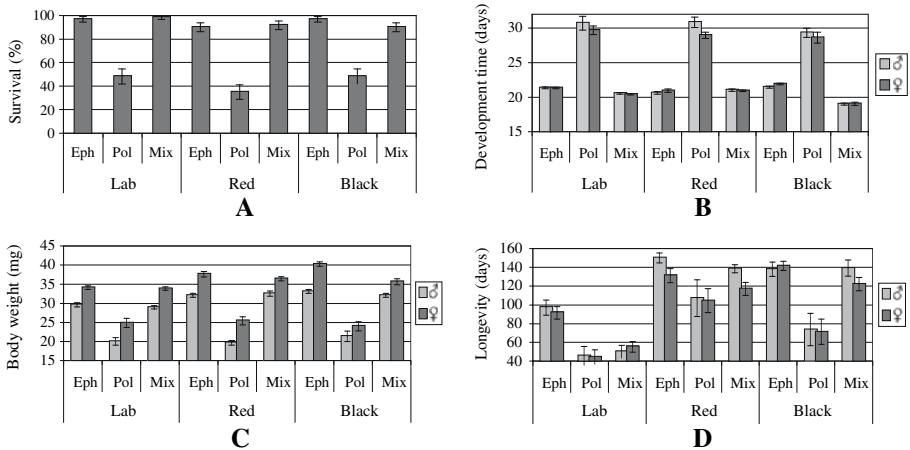
Stata/SE 9.2 was used to perform the statistical data analysis (StataCorp 2005). A generalised linear model was used with the link function and error distribution depending on the nature of the data (Poisson distribution for count data and negative binomial distribution in case of over dispersion). This generalised linear model is an extension of the general linear model and allows the use of error distributions other than the normal distribution. The model uses the principle of regression analysis and thus each time tests the difference between the average of the reference group and the average of the group in question. 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). If none of the parametric models could be used, a bootstrapped median regression was applied. Analysis started with a saturated model and interactions and non-significant main factors were dropped at a significance level of 0.05. Each saturated model contained three factors: population (laboratory, red field or black field), diet (*E. kuehniella* eggs, pollen or the mixed diet) and sex (male or female). The most parsimonious model is reported, using likelihood ratios to assure model fit.

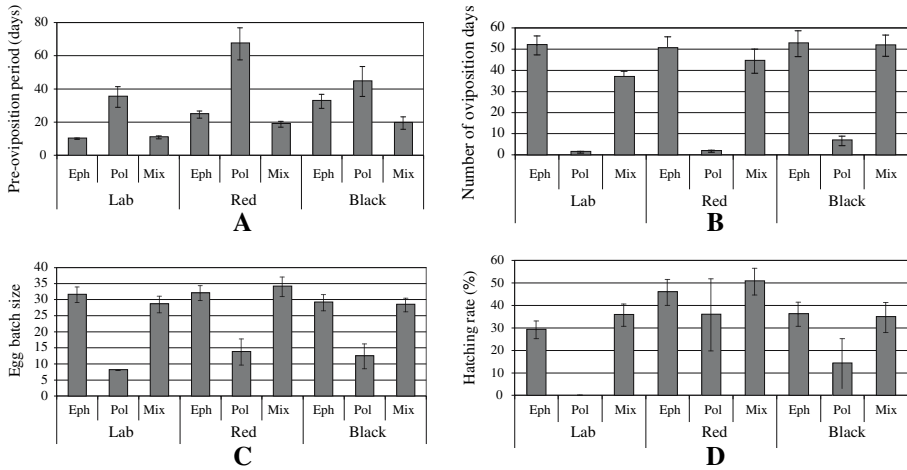
**Results**

Figures 1 and 2 show the values of the fitness parameters found during this study for the three populations reared on the three diets. Figure 1a–d present immature survival, developmental time, adult body weight and female longevity, respectively, while Fig. 2a–d present the reproductive parameters pre-oviposition period, number of oviposition days, egg batch size and egg hatch, respectively. There was a strong significant correlation between body weight of adults at emergence and body length of adults at the time of death ( $r = 0.85, P < 0.001$ ). For this reason only data on body weight are presented.

When reared individually, a diet of moist pollen alone allowed 35–48% of the larvae to successfully reach adulthood, whereas this was 90–98% for larvae offered *E. kuehniella* eggs or a mixture of *E. kuehniella* eggs and pollen. About 40–43% of the females from the field populations maintained on pollen were able to produce a small number of viable eggs, whereas only 5% of the females from the laboratory population laid eggs, none of which hatched. On the other hand, beetles reared on pollen took about twice as long to develop ( $P < 0.001$ ) and had considerably lower body weights ( $P < 0.001$ ) than those reared on *E. kuehniella* eggs or the mixed diet. Overall, when compared with females fed *E. kuehniella* eggs or the mixed diet, females of the field populations maintained on pollen alone took longer to initiate oviposition ( $P = 0.015$  for the black field population fed



**Fig. 1** Immature survival (means ± SE,%) (A), development time (means ± SE, days) (B), body weight (means ± SE, mg) (C), and longevity (means ± SE, days) (D) of a laboratory population (Lab) and a red (Red) and black (Black) field population of *H. axyridis* on frozen *E. kuehniella* eggs (Eph), frozen moist honeybee pollen (Pol), or a mixture of frozen moist bee pollen and frozen *E. kuehniella* eggs in equal weight proportions (Mix)



**Fig. 2** Pre-oviposition period (means  $\pm$  SE, days) (**A**), number of oviposition days (means  $\pm$  SE) (**B**), egg batch size (means  $\pm$  SE) (**C**), and egg hatch (means  $\pm$  SE, %) (**D**) of a laboratory population (Lab) and a red (Red) and black (Black) field population of *H. axyridis* on frozen *E. kuehniella* eggs (Eph), frozen moist honeybee pollen (Pol), or a mixture of frozen moist bee pollen and frozen *E. kuehniella* eggs in equal weight proportions (Mix)

*E. kuehniella* and  $P < 0.001$  in the other cases) and produced fewer eggs ( $P < 0.001$  for number of oviposition days and egg batch size). Eggs laid by females of the field populations fed pollen had lower hatching rates than those laid by females maintained on *E. kuehniella* eggs and the mixed diet ( $P = 0.045$  and  $0.014$ , respectively).

Adding pollen to a diet of *E. kuehniella* eggs overall yielded similar developmental and reproductive rates compared to a diet of *E. kuehniella* eggs alone, but pre-oviposition period of females from the field populations was shorter when reared on the mixed diet ( $P < 0.001$ ).

In the current study, melanic and non-melanic morphs responded differentially to diet for a number of traits. Immatures of the red field population developed faster on *E. kuehniella* eggs than on the mixed diet ( $P = 0.009$ ). Individuals of the red field population also developed faster than those of the black field population on *E. kuehniella* eggs ( $P < 0.001$ ). In contrast, immatures of the black field population developed faster on the mixed diet than on *E. kuehniella* alone ( $P < 0.001$ ) and total developmental time of the black morphs was shorter than that of the red morphs when fed the mixed diet ( $P < 0.001$ ). On a diet of pollen alone, the black morphs laid eggs on more days than the red ones ( $P < 0.001$ ), whereas the latter were longer lived ( $P = 0.012$ ).

Several biological differences were found between individuals from the long-term laboratory population and the two field populations. Overall, individuals of the laboratory population were smaller than those of the field populations when fed *E. kuehniella* eggs ( $P < 0.001$ , both sexes pooled) and the mixed diet ( $P = 0.013$  compared to the black field population and  $P < 0.001$  compared to the red field population, both sexes pooled). Further, females of the laboratory population were shorter lived ( $P < 0.001$ ) and started ovipositing sooner ( $P = 0.002$  compared to the red field population when fed pollen,  $P < 0.001$  in all other cases), except when compared to those of the black field population reared on pollen alone.

## Discussion

*Harmonia axyridis* demonstrated a lower developmental and reproductive fitness when fed on pollen than when offered diets containing *E. kuehniella* eggs. This may indicate a lack or shortage of certain nutritional components in the pollen used in the current study, which are essential for optimal development and reproduction of *H. axyridis*. The composition of pollen varies among plant species, but in general it contains high levels of proteins, amino acids and starch and to a lesser extent lipids, sugars and traces of vitamins, flavonoids, carotenoids, and minerals (Patt et al. 2003; Atrouse et al. 2004; Lundgren and Wiedemann 2004). According to Hodek and Honek (1996) ‘essential’ foods support full development and reproduction of a predaceous coccinellid, whereas ‘alternative’ foods serve simply as a source of energy and thus increase survival of the predator. As pollen allows full development, albeit at lower rates, and the production of a small number of viable eggs in a proportion of *H. axyridis* females, it is difficult to categorize pollen as an essential or alternative food for this species based on the above definitions. However, we consider this food to be ‘alternative’ in a broader sense, as it is expected to be a temporary food source that will be used primarily in the absence of nutritionally superior insect prey.

Hodek and Honek (1996) 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. Besides the provision of supplementary nutrients, pollen may contain phagostimulating compounds that can influence food uptake. De Clercq et al. (2005) found that individuals of *Adalia bipunctata* (L.) fed on a mixed diet of frozen bee pollen and *E. kuehniella* eggs had a faster development, higher oviposition rate and egg hatch than on a diet of only *E. kuehniella* eggs. Likewise, Smith (1965a, b) found that *Coleomegilla maculata* (DeGeer) developed better on a mixture of aphids and corn pollen than on aphids or pollen alone. In the current study, however, supplementing a diet of *E. kuehniella* eggs with bee pollen only shortened the pre-oviposition period of *H. axyridis*, but had no effect on other developmental and reproductive parameters.

The availability and suitability of food is fundamental for the establishment of an exotic species in a new area (Byers 2000; Bartel and Nair 2003; Barlow and Kean 2004; Boivin et al. 2006). As a polyphagous predator, *H. axyridis* can feed on an extensive range of arthropod species (Teddars and Schaefer 1994; Hodek and Honek 1996; Koch 2003). Our findings indicate that the use of plant foods such as pollen may further sustain populations of the predator when insect prey are scarce or absent, albeit with reduced developmental and reproductive rates. Field research by Lundgren et al. (2004) demonstrated that even at times when aphids were at hand, *H. axyridis* larvae fed on pollen of corn (*Zea mays* L.). *Harmonia axyridis* has been reported to use other types of plant foods besides pollen. In autumn the species shows a tendency to aggregate on late season fruits, probably aiming to obtain carbohydrates to boost their reserves for winter (Koch et al. 2004).

The capacity of *H. axyridis* to exploit pollen when insect prey is scarce may offer this invasive species a competitive advantage over other native lady beetle species that are less capable in using this alternative food source. Blackman (1965) observed that *A. bipunctata* frequently fed on fresh hazel and willow pollen even when aphids were present. Hemptinne and Desprets (1986) and Hemptinne and Naisse (1987) reported that pollen was used as a spring food by the two-spotted ladybird. When De Clercq et al. (2005) reared *A. bipunctata* on moist bee pollen they reported that only 10% of the larvae successfully reached adulthood; the resulting adults failed to reproduce on pollen alone. Several other species of Coccinellidae have been observed to feed on pollen (Hagen 1962; Hemptinne and Desprets 1986; Hodek and Honek 1996); for these species, pollen was rarely sufficient to sustain

larval development and it did not allow the females to initiate oogenesis. Watson and Thompson (1933) and Ibrahim (1955) reported that *Harmonia conformis* (Boisduval) and *Coccinella undecimpunctata aegyptiaca* Reiche, respectively, were observed to feed on pollen and other plant foods in the field during periods of low aphid densities. Adults of both species were however reproductively inactive during these periods. According to Hagen (1962) the physiological state of the females of *Hippodamia convergens* Guérin-Méneville, *Hippodamia quinquesignata punctulata* LeConte, *Hippodamia sinuata* Mulsant and *Hippodamia parenthesis* (Say) in western North America were influenced by the quantity and quality of food. If sufficient aphids were available reproduction occurred in these species; however, if the females had to feed on non-insect foods such as pollen, honeydew, nectar, etc. these foods were converted to fat and stored, but were inadequate for oogenesis. Smith (1961) performed laboratory feeding experiments where individuals of *Cycloneda sanguinea* (L.) and *Coccinella trifasciata* L. were fed pollen of several plant species. *Cycloneda sanguinea* completed three instars when fed on pollen from hemp (*Cannabis sativa* L.), two on pollen of corn (*Z. mays*), and one on pollen of grey birch (*Betula populifolia* Marsh), hemlock (*Tsuga canadensis* L.), hickory (*Carya ovata* Mill.), hornbeam (*Carpinus caroliniana* Walt) and red oak (*Quercus rubra* L.). *Coccinella trifasciata* completed only the first instar when fed pollen of corn and butternut (*Juglans cinerea* L.), but failed to develop on the other pollen tested in the study. These findings indicate that pollen from different plant species may differ in their nutritional value for coccinellids. *Coleomegilla maculata* is the only other species of Coccinellinae that is reportedly able to fully develop and reproduce on pollen (Lundgren and Wiedenmann 2004; Michaud and Grant 2005).

Soares et al. (2001, 2005) and Berkvens et al. (2007) showed that different morph types of *H. axyridis* responded dissimilarly in terms of their developmental and reproductive performance when offered various insect foods. The current study also reveals differential responses of *H. axyridis* morph types to a plant food. When fed on pollen alone, the black morphs produced more eggs than the red ones, whereas the red morphs lived longer than the black ones. This suggests that there is an energetic trade-off between reproduction and longevity and that the morph types involved may use opposite strategies for allocating their limited resources. However, more research is needed to substantiate this hypothesis. Further, individuals from a long term laboratory population and two field populations differed in their response to diet for several biological traits. As discussed in more detail by Berkvens et al. (2007) this could either be due to inter-population variation within *H. axyridis* or to genetic and non-genetic adaptation during long term laboratory rearing.

In conclusion, the current laboratory study has demonstrated that pollen can be used by *H. axyridis* as an alternative food, yielding full development and even some reproduction in part of the population. Zoophytophagous feeding habits may contribute to the establishment potential of a non-indigenous predator by sustaining its populations in times of prey scarcity. The extent to which pollinivory determines the outcome of competition among the invasive species *H. axyridis* and native coccinellid predators in Europe requires further investigation. Gut analysis of field collected lady beetles may indicate pollen consumption and digestion by *H. axyridis* in different periods of the year.

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