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A semi-artificial rearing system for the specialist predatory ladybird *Cryptolaemus montrouzieri*

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Abstract In the present study a semi-artificial rearing system for the Australian ladybird *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), a specialist predator of mealybugs, was developed. In a first step, a rearing system using eggs of the Mediterranean flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) as a food and synthetic polyester wadding as an oviposition substrate was compared with a natural rearing system using the citrus mealybug, *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae), as to its effects on the predator's developmental and reproductive parameters. In a second series of experiments the performance of *C. montrouzieri* on bee pollen or on a mixture of *E. kuehniella* eggs and bee pollen was assessed. *E. kuehniella* eggs proved to be a suitable food to support larval development of the predator. Ladybird larvae reared on flour moth eggs developed two days faster and weighed approximately 10 % more than their counterparts reared on mealybugs. Despite a prolongation of the preoviposition period with ca. eight days and a decrease in egg hatch by about 10 %, *C. montrouzieri* females fed moth eggs accepted the synthetic wadding as an oviposition substrate and deposited the same number of eggs their counterparts maintained on mealybugs. A mixture of *E. kuehniella* eggs with pollen yielded similar developmental and reproductive rates as *E. kuehniella* eggs alone, but a diet of bee pollen alone was not adequate for the predator. Our findings indicate the potential of a rearing system using *E. kuehniella* eggs as a factitious food and synthetic wadding as an artificial oviposition substrate for the mass production of *C. montrouzieri*.

Keywords Biological control · Rearing · Factitious food · Artificial oviposition substrate · Coleoptera · Coccinellidae

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Introduction

The mealybug destroyer, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), is native to Australia and was among the first species to be used in the (classical) biological control of mealybug pests (Pseudococcidae) (Fisher 1963; Clausen 1978). Several attempts have been made to introduce *C. montrouzieri* in different parts of the world, but particularly in moderate climates most of these attempts have failed because of the ladybird's low capacity to survive cold winters (DeBach and Hagen 1964). Therefore, the biological control of mealybugs in fruit trees and other perennial crops largely relies on the periodical release of mass cultured *C. montrouzieri* ladybirds (Fisher 1963; Hodek and Honěk 2009). Also the augmentative use of *C. montrouzieri* in protected cultivation and interior landscaping requires large numbers of commercially produced ladybirds (Chong and Oetting 2007; Muştu et al. 2008; Hodek and Honěk 2009). The relatively high cost price of commercial natural enemies is an important drawback of augmentative biological control and slows down its adoption in most agricultural systems (van Lenteren 2012). This obstacle can be in part overcome by rationalizing the mass production techniques for arthropod natural enemies thus lowering their production cost (van Lenteren and Tommasini 2003).

Given its specialist feeding habit, *C. montrouzieri* is traditionally produced using mealybugs reared on plant materials like potato sprouts (Fisher 1963) or pumpkins (Babu and Azam 1987). Not only is this natural rearing method time-consuming but the seasonal availability of these plant materials also complicates the continuous supply of adequate quantities of mealybugs. In order to improve the economy of mass production, factitious food sources have been employed for the rearing of different coleopteran predators with notable success (Hodek and Honěk 2009; Riddick and Chen 2013). For example, eggs of different Lepidoptera species have shown to be a suitable factitious food for the predatory ladybirds *Adalia bipunctata* (L.) (De Clercq et al. 2005), *Propylea japonica* (Thunberg) (Hamasaki and Matsui 2006) and *Harmonia axyridis* (Pallas) (Berkvens et al. 2008b). *C. montrouzieri* larvae can also be successfully reared on eggs of the Angoumois grain moth *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) and the Mediterranean flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) (Pilipjuk et al. 1982; Attia et al. 2011).

Previous studies indicated that *C. montrouzieri* females largely depend on mealybug wax filaments to stimulate oviposition and that contact chemical cues seem predominant in inducing the search for an oviposition site. Besides, mechanical and thigmotactic cues, such as those provided by mealybug ovisacs, must be met (Merlin et al. 1996; Finlay-Doney and Walter 2012). Therefore, the implementation of factitious prey in the mass culture of *C. montrouzieri* is only useful if an artificial oviposition substrate is available that triggers oviposition in the absence of mealybug ovisacs. To our knowledge no artificial oviposition substrate has been reported in the literature for *C. montrouzieri*.

In the present study a semi-artificial rearing system using a factitious prey and an artificial oviposition substrate for *C. montrouzieri* was evaluated. Developmental and reproductive performance of the predator reared on *E. kuehniella* eggs as a food source and synthetic wadding as an oviposition substrate was compared to that in a natural rearing method based on the citrus mealybug *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) as prey and *P. citri* ovisacs as a substrate for oviposition. Because of the relatively high cost price of *E. kuehniella* eggs, we also investigated the nutritional value of frozen bee pollen to sustain the development and reproduction of *C. montrouzieri* and whether the production cost of the predator can be lowered further by mixing the flour moth eggs with bee pollen.

Materials and methods

Insect cultures

Laboratory colonies of *C. montrouzieri* and *P. citri* were established in 2010 with larvae acquired from Katz Biotech AG (Baruth, Germany) and Koppert B.V. (Berkel and Rodenrijs, The Netherlands), respectively. The mealybugs were cultured on potato sprouts and kept in a dark room at ambient conditions. Potatoes infested with mealybugs and covered with ovisacs were transferred to the stock colony of *C. montrouzieri* maintained at 25 ± 1 °C, 75 ± 5 % relative humidity (RH) and a 16:8 h (L:D) photoperiod.

Experiments on oviposition substrate and diet

In a first laboratory experiment, the potential of *E. kuehniella* eggs as a food source and the suitability of Rolta®Soft synthetic polyester wadding (1 × 1 cm) as an artificial oviposition substrate for *C. montrouzieri* (semi-artificial rearing method 1) was tested. *E. kuehniella* eggs were offered to larvae and adults in a

1.5 cm (Ø) plastic dish. Developmental parameters (immature survival, developmental time, adult body weight), reproductive traits (preoviposition period, total oviposition, egg hatch), sex ratio and longevity of predators reared under these conditions were compared to those of a control group offered *P. citri* nymphs as a diet and mealybug ovisacs as an oviposition substrate (natural rearing method). Because oviposition may be triggered by the presence of food for the developing larvae in the oviposition substrate, a treatment in which the females were offered polyester wadding sprinkled with *E. kuehniella* eggs in addition to the moth eggs supplied in a plastic dish was also included (semi-artificial rearing method 2).

In a second experiment, four food sources were tested. The value of frozen moist bee pollen as a (supplemental) food source to sustain the development and reproduction of *C. montrouzieri* was assessed. The performance of the ladybird on a mixture of *E. kuehniella* eggs and bee pollen (1:1 proportion) (food source 1) and on bee pollen alone (food source 2) was compared with that on *P. citri* nymphs (food source 3) or *E. kuehniella* eggs (food source 4). Both flour moth eggs and bee pollen were supplied by Koppert B.V. (Berkel and Rodenrijs, The Netherlands). Polyester wadding served as an oviposition substrate in all treatments, including the mealybug treatment.

In all treatments of both experiments, newly emerged first instar larvae (±50 larvae in both experiments depending on the availability of ladybird larvae and mealybugs) were taken out of the stock colony and placed individually in polystyrene Petri dishes (diameter 9 cm, height 2 cm). All foods used in both experiments were offered ad libitum and replenished every other day. Water was provided to larvae and adults by way of a moist wadding plug fitted into a 1.5 cm (Ø) plastic dish. Survival and development of *C. montrouzieri* larvae was monitored daily. Newly emerged adults were sexed and weighed using a Sartorius Genius ME215P balance, after which they were randomly paired. Adults received the same food as during their larval stage. Oviposition substrates were checked daily for eggs to determine the preoviposition period. Once the first egg was laid, substrates were replaced three times a week for a time period of one month (second experiment) or until the female died (first experiment). Oviposition rate and egg hatch were monitored during the first 30 days of egg laying (second experiment) or during the entire lifetime of the female (first experiment). Longevity of both males and females was determined. All experiments were conducted in a climatic chamber set at 25 ± 1 °C, a 75 ± 5 % RH and a 16:8 h (L:D) photoperiod.

Statistical analysis

All data were analysed using SPSS 21.0 (SPSS Inc. 2009). In both experiments, survival rates, egg hatch and sex ratios were compared by means of a logistic regression. This regression is a generalized linear model using a probit (log odds) link and a binomial error function (McCullagh and Nelder 1989). *P*-values below 0.05 were considered significant.

For the first experiment, the developmental parameters of predators reared according to the two semi-artificial systems were pooled as the larvae received the same treatment. A Kolmogorov–Smirnov test indicated that developmental time and adult body weight of naturally and semi-artificially reared individuals were normally distributed. These parameters were analysed using Student's homo- or heteroscedastic *t*-tests when the Levene test indicated equal or unequal variances, respectively. The preoviposition period and longevity parameters were also normally distributed and analysed using a one-way analysis of variance (ANOVA). Means were separated using Tukey's test as a Levene test proved homoscedasticity. A non-parametric Kruskal–Wallis test was used to evaluate differences in the number of deposited eggs among treatments.

For the second experiment, a Kolmogorov–Smirnov test again indicated that adult body weight and total number of deposited eggs were normally distributed. These parameters were therefore analysed using a one-way ANOVA and means were separated using Tukey's test, after homoscedasticity was confirmed using a Levene test. Developmental times and preoviposition periods were not normally distributed and thus analysed using a Kruskal–Wallis test followed by Mann–Whitney U tests (SPSS Inc. 2009).

Results

Predator larvae reared in the first experiment on *E. kuehniella* eggs or *P. citri* mealybugs developed successfully with survival rates above 90 % (Table 1). Developmental time ($t = 10.035$, $df = 25.887$,

Table 1 Survival, developmental time, adult body weight and sex ratio of *C. montrouzieri* ladybirds when reared according to a semi-artificial rearing system (with *E. kuehniella* eggs) or a natural rearing system (with *P. citri* mealybugs)

Rearing method	N	Immature survival (%)	Developmental time (days)		Adult weight (mg)		Sex ratio (% females)
			♀	♂	♀	♂	
Semi-artificial	95	93.7 ± 2.4a*	22.4 ± 0.1a	22.5 ± 0.1a	10.6 ± 0.1a	9.6 ± 0.1a	50.6 ± 5.3a
Natural	43	90.7 ± 4.5a	24.6 ± 0.2b	25.1 ± 0.3b	9.7 ± 0.3b	8.9 ± 0.2b	48.7 ± 8.1a

* Mean ± SE within a column followed by the same letter are not significantly different ($P > 0.05$; Probit (survival, sex ratio) or Student's *t* test (developmental time and adult weight))

Table 2 Reproduction and female longevity of *C. montrouzieri* reared according to a natural rearing system (food: mealybugs, oviposition substrate: mealybug ovisacs), semi-artificial rearing system 1 (food: *E. kuehniella* eggs, oviposition substrate: polyester wadding) or semi-artificial rearing system 2 (food: *E. kuehniella* eggs, oviposition substrate: polyester wadding with imbedded *E. kuehniella* eggs)

Rearing method	N	Preoviposition period (days)	No. of deposited eggs (eggs per female)	Egg hatch (%)	Longevity (days)
Natural	19	7.5 ± 1.1a*	737.7 ± 111.8a	71.9 ± 1.7a	207.1 ± 20.2a
Semi-artificial 1	21	15.0 ± 1.6b	746.0 ± 130.4a	60.9 ± 1.8b	223.3 ± 15.8a
Semi-artificial 2	23	15.7 ± 1.0b	468.1 ± 73.8a	59.8 ± 2.3b	229.6 ± 14.2a

* Mean ± SE within a column followed by the same letter are not significantly different ($P > 0.05$; Tukey's test (preoviposition period, longevity), Mann–Whitney U test (No. of deposited eggs) or Probit (egg hatch))

$P < 0.001$ for males and $t = 10.828$, $df = 62$, $P < 0.001$ for females) and adult body weight ($t = -2.542$, $df = 62$, $P = 0.014$ for males, $t = -3.038$, $df = 62$, $P = 0.003$ for females) were affected by diet. Ladybirds fed *E. kuehniella* eggs developed nearly two days faster and weighed approximately 10 % more than those fed mealybugs. Sex ratios were proportionally divided in both groups ($\chi^2 = 0.037$, $df = 1$, $P = 0.848$).

Although the preoviposition period of females offered synthetic wadding as an oviposition substrate was prolonged by about eight days ($F = 12.615$, $df = 2, 62$, $P < 0.001$), they produced a similar number of eggs as naturally reared females ($\chi^2 = 3.605$, $df = 2$, $P = 0.165$). However, the hatching rate of the eggs laid in artificial substrates was significantly lower than that of eggs deposited near mealybugs ($\chi^2 = 18.920$, $df = 1$, $P < 0.001$ for semi-artificial 1 and $\chi^2 = 21.659$, $df = 1$, $P < 0.001$ for semi-artificial 2) (Table 2). No significant differences were recorded in reproduction parameters between the group that was offered clean synthetic wadding and the group that was offered wadding with imbedded *E. kuehniella* eggs (preoviposition period: $P = 0.925$ (Tukey post-hoc test); no. of deposited eggs: $\chi^2 = 3.605$, $df = 2$, $P = 0.165$; egg hatch: $\chi^2 = 0.148$, $df = 1$, $P = 0.700$; longevity: $F = 0.477$, $df = 2, 125$, $P = 0.622$).

The developmental parameters of *C. montrouzieri* fed the diets tested in experiment 2 are presented in Table 3. Larvae developed successfully on all diets (survival >90 %) except on bee pollen alone allowing only 20 % of the larvae to reach the 4th larval stage and only one male to reach the adult stage. Diet influenced both developmental time ($\chi^2 = 39.834$, $df = 2$, $P < 0.001$ for males and for $\chi^2 = 38.033$, $df = 2$, $P < 0.001$ for females) and adult body weight ($F = 5.848$, $df = 2, 70$, $P = 0.006$ for males and $F = 6.314$, $df = 2, 68$, $P = 0.003$ for females). Developmental rate (all $P < 0.001$; Mann–Whitney test) and female body weight (both $P < 0.05$; Tukey post-hoc test) of ladybirds fed *E. kuehniella* eggs or a mixture of *E. kuehniella* eggs and pollen were superior to those of their counterparts offered mealybugs. Body weight of males fed the mixture of *E. kuehniella* eggs and pollen was similar to that of males fed mealybugs ($P = 0.182$; Tukey post-hoc test). Except for pollen alone, sex ratios were essentially equal on all diets ($\chi^2 = 0.015$, $df = 2$, $P = 0.993$).

Preoviposition period was affected by diet ($\chi^2 = 8.481$, $df = 2$, $P = 0.014$) and was over four days shorter on mealybugs than on diets

Table 3 Survival, developmental time, adult body weight and sex ratio of *C. montrouzieri* fed different food sources

Food source	N	Immature survival (%)	Developmental time (days)		Adult weight (mg)		Sex ratio (% females)
			♀	♂	♀	♂	
E. kuehniella eggs	52	92.3 ± 4.1a*	22.6 ± 0.2a	22.6 ± 0.2a	11.1 ± 0.2a	9.9 ± 0.2a	50.0 ± 7.3a
E. kuehniella eggs + pollen	49	91.8 ± 4.3a	22.5 ± 0.2a	22.7 ± 0.2a	10.7 ± 0.2a	9.5 ± 0.2ab	48.9 ± 7.5a
P. citri mealybugs	51	92.1 ± 4.1a	24.4 ± 0.2b	25.1 ± 0.2b	9.8 ± 0.3b	8.9 ± 0.2b	48.9 ± 7.4a
Pollen**	47	2.1 ± 2.1b	–	60	–	3.8	0.0

* Mean ± SE within a column followed by the same letter are not significantly different ($P > 0.05$; Probit (survival, sex ratio), Tukey's test (adult weight), Mann–Whitney U test (developmental time))

** Only one male reached the adult stage

Table 4 Reproduction (over 30 days from the onset of oviposition) of *C. montrouzieri* offered different food sources and synthetic wadding as an oviposition substrate

Food source	N	Preoviposition period (days)	No. of deposited eggs in 30 days (eggs per female)	Egg hatch (%)
P. citri mealybugs	23	10.2 ± 0.8a*	208.1 ± 19.9a	86.1 ± 6.9a
E. kuehniella eggs	24	14.8 ± 1.4b	199.2 ± 17.4a	85.3 ± 9.8a
E. kuehniella eggs + pollen	22	15.3 ± 1.5b	193.7 ± 17.4a	86.7 ± 7.0a

* Mean ± SE within a column followed by the same letter are not significantly different ($P > 0.05$; Mann–Whitney U test (preoviposition period), Tukey's test (no. of deposited eggs)), Probit (egg hatch))

containing *E. kuehniella* eggs (Table 4). The diet offered to the ladybirds, however, had no significant effect on the number of eggs deposited by the predator in the synthetic wadding over a 30-day period ($F = 0.155$, $df = 2, 68$, $P = 0.857$) and on egg hatch ($\chi^2 = 0.017$, $df = 2$, $P = 0.992$).

Discussion

The semi-artificial rearing method based on *E. kuehniella* eggs as a factitious food and synthetic wadding as an artificial oviposition substrate successfully supported development and reproduction of the specialist mealybug predator *C. montrouzieri*. *E. kuehniella* eggs appeared to be an even better food source for the developing larvae of the predator than one of *P. citri* mealybugs: larvae reared on flour moth eggs developed faster and reached a higher adult weight than their counterparts reared on mealybugs. These findings are consistent with the results of Attia et al. (2011), suggesting that *E. kuehniella* eggs may constitute a nutritionally superior food for *C. montrouzieri* larvae than its natural prey, mealybugs. Earlier studies showed that a number of other predatory insects generally perform better when reared on *E. kuehniella* eggs than when offered their natural prey, which might be attributed to the well balanced amino acid and fatty acid composition of this factitious prey (Fauvel et al. 1987; Cocuzza et al. 1997; Specty et al. 2003; De Clercq et al. 2005; Berkvens et al. 2008b; De Clercq et al. 2013).

Artificially reared females were not only capable of producing viable eggs but also deposited the same number of eggs as naturally reared females. To our knowledge, this is the first report of *C. montrouzieri* ladybirds reproducing in the absence of mealybugs. Chemical cues produced by their natural prey were always considered to be a necessity to stimulate oviposition. For example, *C. montrouzieri* ladybirds reared on eggs of the flour moth *E. kuehniella* or of the grain moth *S. cerealella* depended on mealybug egg masses to trigger oviposition (Pilipjuk et al. 1982; Attia et al. 2011). Merlin et al. (1996) reported that *C. montrouzieri* females reared on *P. citri* mealybugs and offered cotton wool pellets as an oviposition substrate refrained from ovipositing and withheld their eggs until egg masses were met. Finlay-Doney and Walter

(2012) noted that *C. montrouzieri* females were able to oviposit in the absence of ovisacs as long as mealybugs were offered as food source. In the latter study, the presence of mealybug egg masses had a significant impact on the number of deposited eggs, with significantly fewer eggs overall for females that were not provided with ovisacs but only with mealybug nymphs. Likewise, Attia et al. (2011) showed that ladybirds reared on *E. kuehniella* eggs and offered ovisacs deposited 30 % less eggs compared to females provided with mealybugs and ovisacs. In contrast to abovementioned studies, our results prove that *C. montrouzieri* can effectively be reared in the absence of mealybugs without a loss in fecundity.

The equally good reproductive rates observed in *C. montrouzieri* females reared according to the semi-artificial method as compared to the natural rearing system using mealybugs, can be attributed to the use of a nutritionally adequate food source for the larvae and adults of the predator and the presence of an acceptable substrate for egg laying by the adult females. The adequacy of both the factitious food and the artificial substrate for rearing the predator is confirmed by the fact that 15 consecutive generations could be produced using this semi-artificial method and in the complete absence of mealybugs in the rearing environment without evidence of loss in fitness. After 15 generations, female ladybirds had an average developmental time of 23.1 ± 0.2 days and reached an average adult body weight of 11.5 ± 0.2 mg. They laid an average of 313 eggs over a time period of 30 days. These data are comparable with (for developmental time and body weight) or even better than (for fecundity) obtained in the first generation of rearing on *E. kuehniella* eggs and synthetic wadding (Tables 3, 4).

It is unclear why the synthetic wadding used in this study was accepted as oviposition substrate while the cotton wool pellets used by Merlin et al. (1996) were refused. Latter authors asserted that thigmotactic cues are key for triggering the oviposition in *C. montrouzieri*, which stresses the importance of the physical properties of the oviposition substrate. A possible explanation for the acceptance of the polyester wadding used in the present study is that it is more successful in mimicking the filamentous structure of mealybug ovisacs than cotton wool pellets.

The semi-artificial rearing system proposed here does have its limitations. A first drawback is a prolongation in preoviposition period from seven days for the natural rearing method to 15 days for the semi-artificial method. When the ladybirds were fed mealybugs and provided with synthetic wadding an intermediate preoviposition period of ten days was found. These findings suggest that the oviposition of *C. montrouzieri* is postponed in the absence of ovisacs and potentially associated chemical cues produced by the mealybugs, but that these cues do not seem required per se if a suitable oviposition substrate is available. A significant decrease in egg hatch is the second drawback of the semi-artificial rearing system. When the ladybirds were provided with *E. kuehniella* eggs and synthetic wadding instead of mealybug ovisacs in the first experiment, egg hatch monitored over the entire lifetime of the female decreased from 72 to 60 %.

Imbedding *E. kuehniella* eggs into the oviposition substrate with the objective to accelerate reproduction, proved unsuccessful. Apparently, the availability of food for newly emerged larvae in the oviposition substrate does not trigger oviposition by the females of *C. montrouzieri*.

Ephestia kuehniella eggs were found to be an effective factitious food source to sustain the development and reproduction of *C. montrouzieri* and show promise to be implemented in a large scale production of this ladybird. As a downside *E. kuehniella* eggs are relatively expensive (De Clercq et al. 2013). The search for cheaper alternatives is recommended to lower the production cost of this and other insect predators further. Different pollens have been shown to be suitable alternative or supplementary food for the development and reproduction of various predatory arthropods (De Clercq et al. 2005; Berkvens et al. 2008a; Bonte et al. 2010; Vandekerkhove and De Clercq 2010; Nguyen et al. 2013; Vangansbeke et al. 2014). The frozen, moist bee pollen tested in the current study was not an adequate food source for immature *C. montrouzieri* as only one individual reached the adult stage, which indicates a lack or shortage of certain nutritional components essential for optimal development of *C. montrouzieri*. However, 60 % of the larvae reached the 3rd larval stage when fed pollen alone and no loss in fitness was recorded when the flour moth eggs were mixed with pollen. Dissected ladybird larvae fed the mixture of *E. kuehniella* eggs and pollen had high amounts of pollen in their gut, indicating that the larvae not only actively fed on the lepidopteran eggs but also on the pollen.

Therefore, mixing *E. kuehniella* eggs with pollen may contribute to an increase in cost-effectiveness of mass production by reducing the input of the nutritionally superior but highly expensive flour moth eggs (De Clercq et al. 2005; Berkvens et al. 2008a; Bonte et al. 2010; Lundgren and Weber 2010; Pilorget et al. 2010; Lundgren et al. 2011; Weber and Lundgren 2011).

In conclusion, *C. montrouzieri* can be successfully reared on *E. kuehniella* eggs as a food source and synthetic wadding as an oviposition substrate. The major drawback of the semi-artificial rearing method is a delay in the onset of oviposition by about eight days. However, when the shorter developmental time of larvae fed *E. kuehniella* eggs is taken into consideration (two days), the semi-artificial rearing system yields a delay of only about six days compared to the traditional rearing method. Besides, the semi-artificial rearing method has an important advantage: *C. montrouzieri* can be reared without the need to maintain cultures of mealybugs and potato sprouts or other plant materials. This should make the mass rearing of *C. montrouzieri* less time-consuming, less labour-intensive and more cost-effective. A possible next step in rationalizing the rearing system for this ladybird is the development of an artificial diet (Riddick and Chen 2013). Chumakova (1962) described an artificial diet based on casein and amino acids for *C. montrouzieri* but larvae suffered from high mortality (Hodek 1967). Recent advances in the development of artificial diets for predatory arthropods (Morales-Ramos et al. 2013) may be helpful in designing an effective artificial diet for this ladybird as well.

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References

Attia AR, El-Arnaouty SA, Afifi AI, Alla AEA (2011) Development and fecundity of the Coccinellid predator, *Cryptolaemus montrouzieri* Mulsant on different types of prey. Egypt J Biol Pest Control 21:283–289

Babu R, Azam K (1987) Biology of *Cryptolaemus montrouzieri* Mulsant (Coccinellidae: Coleoptera) in relation with temperature. Entomophaga 32:381–386

Berkvens N, Bonte J, Berkvens D, Deforce K, Tirry L, De Clercq P (2008a) Pollen as an alternative food for *Harmonia axyridis*. BioControl 53:201–210

Berkvens N, Bonte J, Berkvens D, Tirry L, De Clercq P (2008b) Influence of diet and photoperiod on development and reproduction of European populations of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). BioControl 53:211–221

Bonte M, Samih MA, De Clercq P (2010) Development and reproduction of *Adalia bipunctata* on factitious and artificial foods. BioControl 55:485–491

Chong JH, Oetting RD (2007) Intraguild predation and interference by the mealybug predator *Cryptolaemus montrouzieri* on the parasitoid *Leptomastix dactylopii*. Biocontrol Sci Technol 17:933–944

Chumakova BM (1962) Opyt rozvedeniya khishchnovo zhuka kriptolemusa na isskustvennych sredakh. Biol Met Borby s Vred Selsk Kult, Moskva 1:143–146

Clausen CP (1978) Introduced parasites and predators of arthropod pests and weeds: a world review. United States Department of Agriculture, Washington, USA

Cocuzza GE, De Clercq P, Lizzio S, van de Veire M, Tirry L, Degheele D (1997) Life tables and predation activity of *Orius laevigatus* and *O. albidipennis* at three constant temperatures. Entomol Exp Appl 85:189–198

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

De Clercq P, Coudron TA, Riddick EW (2013) Production of heteropteran predators. In: Morales-Ramos JA, Guadalupe Rojas M, Shapiro-Ilan DE (eds) Mass production of beneficial organisms. Elsevier Inc, London, UK, pp 57–100

DeBach P, Hagen KS (1964) Manipulation of entomophagous species. In: DeBach P, Schlinger EI (eds) Biological control of insect pests and weeds. Chapmann and Hall, London, UK, pp 429–458

Fauvel G, Malausa JC, Kaspar B (1987) Etude en laboratoire des principales caractéristiques biologiques de *Macrolophus caliginosus* (Heteroptera: Miridae). Entomophaga 32:529–543

Finlay-Doney M, Walter GH (2012) Behavioral responses to specific prey and host plant species by a generalist predatory coccinellid (*Cryptolaemus montrouzieri* Mulsant). Biol Control 63:270–278

Fisher TW (1963) Mass culture of *Cryptolaemus* and *Leptomastix:* natural enemies of the citrus mealybug. California Agricultural experiment station, Berkeley, USA

Hamasaki K, Matsui M (2006) Development and reproduction of an aphidophagous coccinellid, *Propylea japonica* (Thunberg) (Coleoptera: Coccinellidae), reared on an alternative diet, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs. Appl Entomol Zool 41:233–237

Hodek I (1967) Bionomics and ecology of predaceous Coccinellidae. Annu Rev Entomol 12:79–104

Hodek I, Honěk A (2009) Scale insects, mealybugs, whiteflies and psyllids (Hemiptera: Sternorrhyncha) as prey for ladybirds. Biol Control 51:232–243

Lundgren JG, Weber DC (2010) Changes in digestive rate of a predatory beetle over its larval stage: implications for dietary breadth. J Insect Physiol 56:431–437

Lundgren JG, Moser SE, Hellmich RL, Seagraves MP (2011) The effects of diet on herbivory by a predaceous lady beetle. Biocontrol Sci Technol 21:71–74

McCullagh P, Nelder J (1989) Generalized linear models. Chapmann and Hall, London, UK

Merlin J, Lemaitre O, Grégoire JC (1996) Oviposition in *Cryptolaemus montrouzieri* stimulated by wax filaments of its prey. Entomol Exp Appl 79:141–146

Morales-Ramos JA, Guadalupe Rojas M, Coudron TA (2013) Artificial diet development for entomophagous arthropods. In: Morales-Ramos JA, Guadalupe Rojas M, Shapiro-Ilan DE (eds) Mass production of beneficial organisms. Elsevier Inc, London, UK, pp 203–234

Muştu M, Kilinçer N, Ulgentürk S, Kaydan MB (2008) Feeding behaviour of *Cryptolaemus montrouzieri* on mealybugs parasitized by *Anagyrus pseudococci*. Phytoparasitica 36:360–367

Nguyen DT, Vangansbeke D, Lü X, De Clercq P (2013) Development and reproduction of the predatory mite *Amblyseius swirskii* on artificial diets. BioControl 58:369–377

Pilipjuk VI, Bugaeva LN, Baklanova EV (1982) On the possibility of breeding the predatory beetle *Cryptolaemus montrouzieri* Muls. (Coleoptera: Coccinellidae) on the eggs of *Sitotroga cerealella* Ol. Entomologicheskoe Obozrenie 1:50–52

Pilorget L, Buckner J, Lundgren JG (2010) Sterol limitation in a pollen-fed omnivorous lady beetle (Coleoptera: Coccinellidae). J Insect Physiol 56:81–87

Riddick AW, Chen H (2013) Production of coleopteran predators. In: Morales-Ramos JA, Guadalupe Rojas M, Shapiro-Ilan DE (eds) Mass production of beneficial organisms. Elsevier Inc, London, UK, pp 17–55

Specty O, Febvay G, Grenier S, Delobel B, Piotte C, Pageaux JF, Ferran A, Guillaud J (2003) Nutritional plasticity of the predatory ladybeetle *Harmonia axyridis* (Coleoptera: Coccinellidae): comparison between natural and substitution prey. Arch Insect Biochem Physiol 52:81–91

SPSS Inc. (2009) Guide to data analysis. SPSS Inc, Chicago, USA

van Lenteren JC (2012) The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. BioControl 57:1–20

van Lenteren JC, Tommasini M (2003) Mass production, storage, shipment and release of natural enemies. In: van Lenteren JC (ed) Quality control and the production of biological control agents: theory and testing procedures. CABI Publishing, Wallingford, UK, pp 181–189

Vandekerkhove B, De Clercq P (2010) Pollen as an alternative or supplementary food for the mirid predator *Macrolophus pygmaeus*. Biol Control 53:238–242

Vangansbeke D, Nguyen DT, Audenaert J, Verhoeven R, Gobin B, Tirry L, De Clercq P (2014) Performance of the predatory mite *Amblydromalus limonicus* on factitious foods. BioControl 59:67–77

Weber DC, Lundgren JG (2011) Effect of prior diet on consumption and digestion of prey and non-prey food by adults of the generalist predator *Coleomegilla maculata*. Entomol Exp Appl 140:146–152

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