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Prey range of the predatory ladybird *Cryptolaemus montrouzieri*

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Abstract The prey range of *Cryptolaemus montrouzieri* was studied in the laboratory to investigate whether the mealybug destroyer can contribute to the suppression of other pest insects besides mealybugs and to assess its potential impact on non-mealybug populations as part of an environmental risk assessment for its use in biological control. Prey tested in these experiments were: tobacco aphid *Myzus persicae nicotianae* (Sulzer)(Hemiptera: Aphididae), pea aphid *Acyrthosiphon pisum* (Harris)(Hemiptera: Aphididae), tobacco whitefly *Bemisia tabaci* (Gennadius)(Hemiptera: Aleyrodidae), southern green stinkbug *Nezara viridula* (L.)(Hemiptera: Pentatomidae) eggs, western flower thrips *Frankliniella occidentalis* (Pergande)(Thysanoptera: Thripidae), two-spotted ladybird *Adalia bipunctata* (L.)(Coleoptera: Coccinellidae) eggs and eggs of the greater wax moth *Galleria mellonella* L. (Lepidoptera: Pyralidae). Larval survival was high to moderate when *C. montrouzieri* was provided with hemipteran prey and poor to zero when the ladybirds were provided with non-hemipteran prey. Females reared on *M. persicae* and *A. pisum* produced similar numbers of eggs as their counterparts fed the citrus mealybug *Planococcus citri* (Risso)(Hemiptera: Pseudococcidae), but fecundity was significantly lower when the ladybirds were reared on *B. tabaci* nymphs or on *A. bipunctata* eggs. Prey species that were found to be less suitable for immature development of *C. montrouzieri* could still be an adequate food source for reproduction and survival of adult ladybirds. For example, only 8 % of the predator larvae reached the adult stage when provided with *A. bipunctata* eggs, but females that had developed on eggs of the Mediterranean flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) and that were supplied with *A. bipunctata* eggs from adult emergence on, were only 35 % less fecund than females provided with mealybugs in their adult life. The results are discussed in relation to the development of a suitable methodology for prey/host range testing in the framework of an environmental risk assessment for arthropod natural enemies.

Keywords Biological control · Environmental risk assessment · Prey range · Non-native species · Predator · Coleoptera · Coccinellidae

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Introduction

The mealybug destroyer, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), was one of the first coccinellids used in (classical) biological control. Originating from Australia, *C. montrouzieri* was introduced around the world to control the citrus mealybug *Planococcus citri* (Risso)(Hemiptera: Pseudococcidae)(Fisher 1963; Clausen 1978). More recently, mass produced ladybirds are intensively used for augmentation biological control in protected cultivation and interior landscaping (Chong and Oetting 2007; Muştu et al. 2008; Hodek and Honěk 2009; Roy and Migeon 2010). Although *C. montrouzieri* is mainly released against mealybug pests, it has also been reported to feed on a wide range of other hemipterans in the field, including aphids, scale insects and whiteflies (Malais and Ravensberg 2002; Ślipiński 2007; Finlay-Doney and Walter 2012b; Kairo et al. 2012; Cock 2013). Its potential to develop and reproduce when offered these alternative prey, however, has hardly been investigated (Finlay-Doney and Walter 2012b).

The potential to feed on different prey species can be an advantageous trait for biological control agents (van Lenteren and Woets 1988; Albajes and Alomar 1999). Not only can polyphagous natural enemies sustain themselves on non-target foods when the target prey is absent in the agricultural ecosystem, their generalist feeding habit can also be an asset increasing their market value as a management tool against different pest species (De Clercq 2002; Berkvens et al. 2009). Polyphagy of a non-native biological control agent can, however, also be disadvantageous when it spreads to natural ecosystems after being released and affects the abundance of non-target species, thus threatening local biodiversity (van Lenteren et al. 2003; Loomans and van Lenteren 2005; De Clercq et al. 2011). For this reason, the release of polyphagous biological control agents is not always considered to be environmentally safe and, therefore, an appropriate regulation concerning the import and use of natural enemies is required to prevent these undesired side-effects (van Lenteren et al. 2006a; Bale 2011; Ehlers 2011). A scientific risk assessment methodology, integrating information on the potential of a biological control agent to establish (overwinter), its ability to disperse, its prey (or host) range, and its direct and indirect effects on non-target organisms, should form the basis of any regulation for biological control agents (van Lenteren et al. 2003; van Lenteren and Loomans 2006; Ehlers 2011).

The prey range of a candidate biological control agent is a key element in the risk assessment process, because a lack of prey specificity might lead to unacceptable risk if the agent establishes and disperses widely, whereas a highly specific species is not expected to create serious risk even when it establishes as in classical biological control. However, the development of a concrete experimental methodology to assess the prey range of arthropod natural enemies is still an ongoing process and several factors complicate this assessment, including the number of non-target species to be tested, the criteria for their selection, the need for reliable methods for determining prey acceptance and the discrepancy between prey ranges observed in the field and in the lab (Babendreier et al. 2005; Kuhlmann et al. 2006; van Lenteren et al. 2006b; Hatherly et al. 2009). In weed biological control, a survey across all potential host plant species in the native range of a herbivore is considered to be a good start in quantifying its degree of polyphagy (Manners et al. 2011).

In the present study, we investigated the prey range of *C. montrouzieri* in the laboratory. The first objective was to determine whether the mealybug destroyer can contribute to the biological control of other hemipteran pests besides mealybugs and whether the prey range of the ladybird is limited to the order Hemiptera or includes species from other insect orders. The second objective was to gain insight in its potential impact on populations of non-target species as part of an environmental risk assessment. We compared the development and reproduction of *C. montrouzieri* when offered different alternative prey species in a no-choice design. In augmentative biological control, *C. montrouzieri* is mostly released as adult. Therefore, we also assessed the reproductive capacity of ladybirds that received one of the candidate prey during their adult life, but had been reared on a nutritionally suitable food source (eggs of the flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae)) during their larval stages (Attia et al. 2011; Maes et al. 2014).

Materials and methods

Selection of candidate prey species

As it is well documented that several members of the Pseudococcidae family are suitable prey for *C. montrouzieri* (Malais and Ravensberg 2002; Ślipiński 2007; Finlay-Doney and Walter 2012b; Kaur and Virk 2012), species from other families within the Hemiptera were considered for testing according to their descending degree of taxonomic relatedness with Pseudococcidae (Bourgoin and Campbell 2002). The nearest families tested were the Aphididae and Aleyrodidae. The tobacco aphid, *Myzus persicae nicotianae* (Sulzer)(Hemiptera: Aphididae), the pea aphid, *Acyrthosiphon pisum* (Harris)(Hemiptera: Aphididae), and the tobacco whitefly, *Bemisia tabaci* (Gennadius)(Hemiptera: Aleyrodidae), were chosen as representatives for these families because they are known to cause economic crop damage. The southern green stinkbug, *Nezara viridula* (L.)(Hemiptera: Pentatomidae), was chosen as a representative for the Pentatomidae family, which is more distantly related to the Pseudococcidae but still belongs to the Hemiptera. Furthermore, the western flower thrips, *Frankliniella occidentalis* (Pergande)(Thysanoptera: Thripidae), was selected as another economically important hemimetabolous pest insect. Because eggs of the flour moth *E. kuehniella* constitute an adequate factitious food source for *C. montrouzieri* (Attia et al. 2011; Maes et al. 2014), we also tested the predator's ability to develop and reproduce on eggs of two other species in the framework of its risk assessment. The greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae), was selected based on its taxonomic relatedness to *E. kuehniella*. Furthermore, as egg cannibalism is not infrequent in *C. montrouzieri,* we selected eggs of another ladybird, the two-spotted ladybird, *Adalia bipunctata* (L.)(Coleoptera: Coccinellidae). This species was also chosen because of its ecological value as native aphid predator in the study area (Western Europe).

Insect cultures

Cryptolaemus montrouzieri

A laboratory colony of *C. montrouzieri* was established in 2010 with larvae acquired from Katz Biotech AG (Baruth, Germany) and maintained in a climatic chamber set at 25 ± 1 °C, a relative humidity (RH) of 75 ± 5 % and a 16:8 (L:D)h photoperiod. The ladybirds were reared on frozen *E. kuehniella* eggs. Water was provided by way of a moist piece of cotton wadding fitted into a 1.5 cm plastic dish. A larger piece of dry cotton wadding (5 × 5 cm) was offered to adult beetles and served as an oviposition substrate for females (Maes et al. 2014). Individuals of the 12th and 13th generations were used in the experiments.

Prey species

The citrus mealybug, *P. citri,* was cultured on potato sprouts. In the feeding experiments, a mixture of all nymphal stages of *P. citri* was offered to *C. montrouzieri*. Sprouts contaminated with mealybugs were brushed above Petri dishes containing ladybirds. A colony of *M. persicae nicotianae* was maintained on sharp pepper plants, *Capsicum annuum* L. Ladybirds were provided with leaves containing nymphs and adults of the peach aphid. The pea aphid *A. pisum* was fed on faba bean *Vicia faba* L. Leaves infected with a mixture of all stages (nymphs and adults) were offered to the ladybird for testing. Tobacco whiteflies, *B. tabaci,* were reared on tobacco plants, *Nicotiana tabacum* L. Ladybirds were provided with leaves containing second and third instar nymphs. The stinkbug *N. viridula* was fed on pods of green bean, *Phaseolus vulgaris* L., and on seed kernels of sunflower, *Helianthus annuus* L. In the experiments, only stinkbug eggs, harvested on a piece of household paper, were offered to *C. montrouzieri*. *Frankliniella occidentalis* thrips were cultured in plastic boxes containing vermiculite and green bean pods (*P. vulgaris*). The second instar of the western flower thrips was tested as a prey for *C. montrouzieri*. Beans contaminated with thrips were brushed above Petri dishes containing ladybirds. The ladybird *A. bipunctata* was fed on a mixture of frozen *E. kuehniella* eggs and bee pollen, as described by De Clercq et al. (2005). The eggs of *A. bipunctata*, laid on a piece of household paper, were offered to *C. montrouzieri* for testing. A laboratory culture of *G. mellonella* was reared on an artificial diet described by Vanhaecke and Degheele (1980). In the feeding experiments, ladybirds were provided with *G. mellonella* eggs harvested on a piece of paper.

Feeding experiments

The potential of *C. montrouzieri* to develop and reproduce on the different test species was investigated by offering the ladybirds the same candidate prey during their larval and adult stages. Prey species

that proved to be less suitable to support the development of *C. montrouzieri* (based on immature survival rate, developmental time and adult body weight of the predator) were subjected to a further laboratory experiment, in which their potential for the reproduction of *C. montrouzieri* was assessed. In the latter experiment, ladybird larvae were reared to adulthood on a diet of *E. kuehniella* eggs, which was found to be an adequate food source for *C. montrouzieri* larvae (Attia et al. 2011; Maes et al. 2014), and switched to one of the candidate prey species once they reached the adult stage. This was done for all candidate prey species, except for the aphids *M. persicae* and *A. pisum*.

In both experiments, approximately 60 first instar *C. montrouzieri* larvae (< 24 h) per prey species (depending on availability of both ladybirds and prey) were taken from the stock colony and placed individually in plastic Petri dishes (diameter 9 cm, height 2 cm). Water was provided by way of a moist wadding plug fitted into a 1.5 cm plastic dish. All foods were offered ad libitum and replenished every day, except for *E. kuehniella* eggs, which were replenished every two days. Survival and development of *C. montrouzieri* larvae was monitored daily. Newly emerged adults were sexed and weighed using a Sartorius Genius ME215P balance. Males and females were paired. The oviposition substrate (a piece of synthetic wadding (1 × 1 cm)) was checked daily for eggs to determine the preoviposition period. Once the first egg was laid, substrates were replaced three times a week for a total period of one month. All eggs were effectively laid in the artificial substrate. Oviposition rate and egg hatch were monitored during the first 30 days of egg laying. When offered *A. bipunctata* eggs during their larval development, only two females reached the adult stage. These were paired with newly emerged males from the stock colony in order to study their reproductive potential. In both series of experiments, a positive control treatment consisting of *P. citri* mealybugs and a negative control treatment consisting of water only were included. All experiments were conducted in a climatic chamber set at 25 ± 1 °C, 75 ± 5 % RH, and a 16:8(L:D)h photoperiod.

Statistical analysis

All data were analyzed using SPSS 21.0 (SPSS Inc. 2009). Survival rates, egg hatch and sex ratio of the predators 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.

A Kolmogorov–Smirnov test indicated that male and female body weight of the predator were normally distributed and therefore analyzed using a one-way analysis of variance (ANOVA). As a Levene test indicated homoscedasticity, the means were separated using Tukey tests. According to a Kolmogorov–Smirnov test, male and female developmental times were not normally distributed, therefore, we used the non-parametric Kruskal–Wallis H test and Bonferroni-corrected Mann–Whitney U tests to evaluate differences in developmental time among treatments.

In all cases, total fecundity (i.e., the number of deposited eggs during a 30-day period) was found to be normally distributed (Kolmogorov–Smirnov test) and thus analyzed using ANOVA. For the experiment in which both larvae and adults were presented with the test prey species, the ANOVA indicated no differences among treatments. In contrast, for the experiment in which only adults were presented with the test species and larvae were reared on *E. kuehniella* eggs, the ANOVA indicated differences among treatments. As Levene's test indicated homoscedasticity, the means were separated using Tukey post-hoc tests. The parameter preoviposition period was not normally distributed and thus analyzed using a non-parametric Kruskal–Wallis H test followed by Bonferroni-corrected Mann–Whitney U tests (SPSS Inc. 2009).

Results

Immature survival was significantly influenced by prey species ($\chi^2 = 147.25$, $df = 5$, $P < 0.001$) (Table 1). Whereas survival was high when *C. montrouzieri* was fed on *P. citri* and *M. persicae*, low on *A. pisum* and *B. tabaci*, and very poor on *A. bipunctata* and *G. mellonella* eggs, none of the larvae succeeded in reaching the adult stage when offered *F. occidentalis* nymphs or *N. viridula* eggs. For candidate prey species where *C. montrouzieri* survival was poor or zero, mortality of the predator occurred during the first and second instars (Fig. 1). Although total survival on *A. pisum* and *B. tabaci* was similar, most larvae died early in their development (L1–L2) when offered *A.*

Table 1 Development of *C. montrouzieri* fed different candidate prey species

Prey	N	Developmental time (days)		Adult weight (mg)		Sex ratio (% females)
		Females	Males	Females	Males	
P. citri nymphs	45	24.1 ± 0.2a	24.5 ± 0.3a	10.6 ± 0.2a	9.3 ± 0.2a	48.9 ± 7.5b
M. persicae nymphs	59	26.3 ± 0.3b	27.1 ± 0.4b	7.7 ± 0.2b	7.0 ± 0.2b	43.8 ± 7.2b
A. pisum nymphs	59	31.0 ± 1.5c	30.2 ± 0.5c	7.4 ± 0.7b	7.3 ± 0.5b	40.0 ± 16.3b
B. tabaci nymphs	58	40.0 ± 3.5c	39.4 ± 1.6d	4.6 ± 0.4c	4.3 ± 0.3c	36.4 ± 15.2b
A. bipunctata eggs**	64	31.0 ± 1.0c	–	6.2 ± 0.9bc	–	100 ± 0.0a
G. mellonella eggs***	60	–	43	–	4.3	0.0
F. occidentalis nymphs	60	–	–	–	–	–
N. viridula eggs	60	–	–	–	–	–
Water only	54	–	–	–	–	–

Mean ± SE within a column followed by the same letter are not significantly different ($P > 0.05$; Probit analysis (Wald-χ^2) (sex ratio), Mann–Whitney U test (developmental time) or Tukey test (adult weight))

** only two females reached the adult stage

*** only one male reached the adult stage

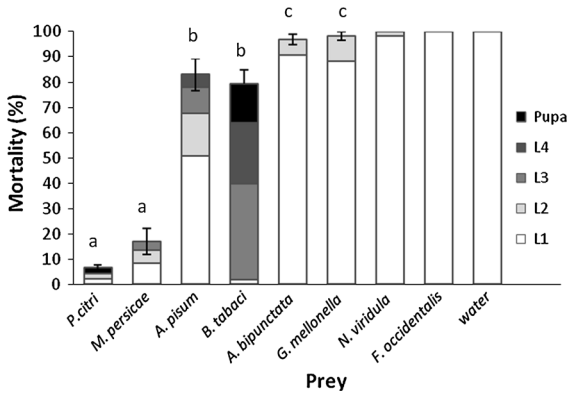

Fig. 1 Total mortality (mean ± SE) shown per consecutive developmental stage (L1-Pupa) of *C. montrouzieri* fed different prey species. Bars with the same letter are not significantly different ($P > 0.05$; Probit analysis (Wald-χ^2))

pisum but late in their development (L3–L4) when provided with *B. tabaci*. Only two individuals reached adulthood on *A. bipunctata* eggs and only one individual reached the adult stage on *G. mellonella* eggs.

Prey had a significant effect on developmental time of both males ($\chi^2 = 42.35$, df = 3, $P < 0.001$) and females ($\chi^2 = 39.48$, df = 4, $P < 0.001$) (Table 1). When *C. montrouzieri* larvae were reared on *P. citri*, development was approximately two days faster than on *M. persicae*, six days faster than on *A. pisum* and *A. bipunctata,* and sixteen days faster than on *B. tabaci*. Also adult body weight was influenced by prey ($F = 62.15$, df = 3, 60, $P < 0.001$ for males; $F = 65.78$, df = 4, 51, $P < 0.001$ for females) (Table 1). Predators provided with *B. tabaci* weighed approximately 40 % less than those provided with *M. persicae* or *A. pisum* and 60 % less than those provided with *P. citri*.

The preoviposition period was affected by prey species ($\chi^2 = 28.29$, df = 2, $P < 0.001$) (Table 2). Egg laying was postponed by six and nine days when the ladybirds were fed with *M. persicae* and *A. pisum,* respectively, instead of *P. citri*. No significant differences in total numbers of deposited eggs were found between ladybirds fed mealybugs or aphids ($F = 1.65$, df = 2, 46, $P = 0.20$) (Table 2). Prey species also influenced the hatching rate of the eggs ($\chi^2 = 111.90$, df = 4, $P < 0.001$) (Table 2). A single female succeeded in producing viable eggs on *B. tabaci* and another female produced viable eggs when presented with *A. bipunctata* eggs. The egg hatch rate observed on *B. tabaci* was, however, substantially higher than on *A. bipunctata* and equalled that of females fed *P. citri* or *M. persicae*.

No egg laying was observed for adults offered *G. mellonella*, *F. occidentalis, N. viridula* or water alone. In these treatments all females died within 15, 9, 11 and ten days, respectively (Table 3). The preoviposition period on the remaining diets was affected by prey

Table 2 Reproduction of *C. montrouzieri* fed different candidate prey species

Prey	N	Preoviposition period (days)	No. of eggs laid per female in 30 days	Egg hatch (%)
P. citri nymphs	21	4.1 ± 0.2a	154.3 ± 17.5a	67.5 ± 0.8a
M. persicae nymphs	20	10.4 ± 0.9b	200.2 ± 19.3a	62.9 ± 0.8b
A. pisum nymphs	4	13.5 ± 1.7b	161.5 ± 29.0a	54.6 ± 2.0c
B. tabaci nymphs	3	24**	51	72.6 ± 6.3ab
A. bipunctata eggs	2	8**	69	8.7 ± 3.4d

All predators were offered the same prey during their larval and adult stages (experiment 1)

Mean ± SE within a column followed by the same letter are not significantly different ($P > 0.05$; Mann–Whitney U test (preoviposition period), Tukey test (No. of oviposited eggs) or Probit analysis (Wald-χ^2)(egg hatch))

** only one female oviposited

Table 3 Reproduction of *C. montrouzieri* fed *E. kuehniella* eggs as larvae and different candidate prey species as adults (experiment 2)

Prey	N	Preoviposition period (days)	No. of eggs laid per female in 30 days	Egg hatch (%)
P. citri nymphs	25	10.4 ± 0.9a	179.4 ± 17.0a	69.3 ± 0.7a
B. tabaci nymphs	25	15.4 ± 0.6b	40.6 ± 16.5c	46.8 ± 2.9b
A. bipunctata eggs	21	15.8 ± 0.9b	116.4 ± 16.7b	33.6 ± 1.0c
G. mellonella eggs	25	–	0.0	–
F. occidentalis nymphs	25	–	0.0	–
N. viridula eggs	25	–	0.0	–
water	25	–	0.0	–

Mean ± SE within a column followed by the same letter are not significantly different ($P > 0.05$; Mann–Whitney U test (preoviposition period), Tukey test (No. of oviposited eggs) or Probit analysis (Wald-χ^2)(egg hatch))

($\chi^2 = 14.03$, df = 2, $P = 0.001$) and was nearly five days longer on *B. tabaci* and *A. bipunctata* than on *P. citri*. Total fecundity ($F = 9.87$, df = 2, 52, $P < 0.001$) and egg hatch ($\chi^2 = 816.44$, df = 2, $P < 0.001$) were also influenced by diet (Table 3). Females fed *A. bipunctata* laid significantly more eggs than those fed *B. tabaci*, but were less fecund than those reared on *P. citri*. Egg hatch, on the other hand, was higher for females fed *B. tabaci* nymphs than for those given *A. bipunctata* eggs, but was still lower than for females fed *P. citri* nymphs.

Discussion

Prey specificity is a key element in the risk assessment of a candidate biological control agent. A critical step in determining the prey range of a natural enemy in the laboratory is the selection of the non-target species to be tested. van Lenteren et al. (2003) proposed a selection procedure for non-target species based on the phylogenetic centrifugal method used for the evaluation of weed biocontrol agents. This procedure starts with testing non-target species that are closely related to the target and then progresses to species that are more distantly related to the target organism. If none of the non-target species is attacked, one can stop testing (Wapshere 1974; Londsdale et al. 2001; van Lenteren et al. 2006b). In the present study, survival was high to moderate when *C. montrouzieri* was provided with prey species that are closely related to the mealybug target prey (*M. persicae*, *A. pisum*, *B. tabaci*) and overall poor to zero when the ladybird was provided with prey species that belong to a different insect order than the Hemiptera (*F. occidentalis*, *A. bipunctata*, *G. mellonella*) or even hemipteran prey from a different suborder (*N. viridula*). Also the reproductive capacity of *C. montrouzieri* ladybirds decreased when they were provided with more distantly related prey species. While ladybirds reared on aphids during their development and adult life deposited similar numbers of eggs as their

counterparts fed on *P. citri*, fecundity was markedly lower when the ladybirds were presented with whitefly and ladybird prey, and no eggs were laid in the presence of the other prey species.

However, predicting a predator's prey range solely based on phylogenetic relatedness to the target prey may not be straightforward and the outcome may depend on the species selected for testing. Eggs of *G. mellonella* were not a suitable food source for *C. montrouzieri*: only 2 % of the larvae reached the adult stage when fed *G. mellonella* eggs and not a single female produced eggs when provided with this food source. In contrast, the eggs of another member of the Pyralidae family (*E. kuehniella*) were found to be a suitable factitious food source for both development and reproduction of this ladybird (Attia et al. 2011; Maes et al. 2014). Although *M. persicae* and *A. pisum* belong to the same family of Aphididae, *C. montrouzieri* performed differently on these aphid species. Whereas reproductive capacity and adult body weight were not affected by the aphid prey species, development was three days shorter and survival was four times higher when the predator was provided with *M. persicae* instead of *A. pisum*. These findings indicate that taxonomic relatedness *in se* may not necessarily be a sufficiently reliable criterion for determining prey ranges and even closely related prey may substantially differ in their suitability to support immature development and/or reproduction of a natural enemy. Furthermore, our study provides support for the hypothesis that in addition to non-target species that can easily be tested in a laboratory setting, prey range testing should give additional attention to economically important species, threatened or valued species and native natural enemies (Sands and van Driesche 2000; Babendreier et al. 2005; van Lenteren et al. 2006b).

A major practical concern in the evaluation of the prey range of a candidate biological control agent is the number of non-target species that needs to be tested. Eventually, this will determine the practical feasibility of the proposed risk assessment procedure to be followed by commercial biocontrol producers who wish to place a new species on the market. Kuhlmann et al. (2006) suggested to design an initial test list with 50 non-target species and reduce this list to 10–20 species by the application of criteria filters such as ecological similarity and phenological overlap with the target prey. Based on the findings of the present study, it should be possible to perform a quick scan with a limited number of non-target prey to highlight those species that are potentially at risk and deserve the focus of the prey range testing. Our results indicate that the focus of prey range tests for *C. montrouzieri* should be on small, less mobile and soft-bodied prey species. Despite several feeding attempts on eggs of the stinkbug *N. viridula*, most eggs were not consumed. This might indicate that *C. montrouzieri* has difficulty handling prey materials characterized with a rigid body texture. The mobility of thrips larvae was deemed responsible for the low predation rate of *C. montrouzieri* on *F. occidentalis*, suggesting that the predator is adapted to less mobile prey such as mealybugs. Also the body size of a test species might determine its suitability as prey for a predator. Over 80 % of the *C. montrouzieri* larvae reared on *M. persicae* aphids successfully completed development, whereas only 20 % of the larvae reared on *A. pisum* aphids reached the adult stage, with the highest mortality being observed during the first and second instar. Similar survival rates during the third and fourth instar and similar reproductive capacity might indicate that the smaller body size of *M. persicae* is responsible for the better performance of *C. montrouzieri* on *M. persicae* than on *A. pisum,* rather than their respective biochemical compositions. However, it cannot be excluded that tritrophic effects caused by the different host plant-prey associations and experimental conditions may have affected the outcome of the experiments. For instance, whereas some prey were offered on paper or without a substrate, others were presented on plant materials. Besides the indirect effects of the host plant on the predator through prey quality, predation capacity and fitness of the predator may also have been directly affected by the presence or absence of plant materials in the test arenas. In addition, it is worth noting that small scale laboratory experiments do not take into account prey location cues used by the predator in the field (Finlay-Doney and Walter 2012a).

The selection of life history parameters to quantify the suitability of a non-target prey is another important aspect of a prey range testing procedure for candidate biological control agents (van Lenteren et al. 2003). In the present study, the parameters proposed by van Driesche and Murray (2004a) were monitored: larval development, adult survival and oviposition. The investigation of multiple parameters in our

experiments was critical as each parameter revealed additional information. For instance, prey species that were found to be less suitable for development and reproduction of *C. montrouzieri* could still be an adequate food source to sustain adult survival (85 % of the adults provided with *A. bipunctata* eggs was still alive after 65 days). This is probably due to the predator's different nutritional requirements during its larval stages and adult life (Michaud 2005). Besides, our experiments indicate that it is worth investigating a predator's reproductive capacity on a certain candidate prey even when the larvae had difficulty to complete their development on this prey. Despite the fact that only 8 % of the *C. montrouzieri* larvae reached the adult stage when provided with *A. bipunctata* eggs, females that had developed on *E. kuehniella* eggs and were supplied with *A. bipunctata* eggs from the adult stage on, were able to produce an average of 116 eggs in 30 days. This is 35 % less than females provided with *P. citri* mealybugs, but is 35 % more than females supplied with *B. tabaci* larvae, which proved to be a more suitable prey for larval development of *C. montrouzieri*. Finally, the relationship between development and survival on the one hand and reproduction on the other was not always straightforward. Whereas survival rates of *C. montrouzieri* on *A. pisum* and *B. tabaci* were similar, adult females laid 70 % more eggs on *A. pisum* than on *B. tabaci*.

Arguably, laboratory experiments exploring the prey range of a predator like those conducted in the present study have their limitations. First, long-term rearing of natural enemies could induce selective adaptation to the food source offered in the laboratory and could result in natural enemies that have lost their ability to feed on some of their natural prey (Grenier and De Clercq 2003), which might lead to an underestimation of the prey range. Also, the potential of *C. montrouzieri* to reproduce in the absence of mealybugs might have been overestimated due to the experimental methods used. Adult ladybirds were always provided with a polyester wadding as an (artificial) oviposition substrate, which mimics the physical properties of mealybug egg masses and fulfils the requirements to trigger egg laying in *C. montrouzieri* (Maes et al. 2014). Furthermore, no-choice experiments present a worst-case scenario as a positive response to a non-target prey can be artificially induced by confinement and lack of choice (van Driesche and Murray 2004b). Conducting more realistic experiments, in which two or more prey species are presented to the predator (choice test) or host plants are included in the experimental set-up (semi-field test), might yield a more reliable estimation of a predator's prey range (van Lenteren et al. 2003; van Driesche and Murray 2004b; Babendreier et al. 2005). Thus, test species that showed to be suitable prey for *C. montrouzieri* in the present no-choice Petri dish experiments do not necessarily have to be at risk in a natural situation. On the other hand, negative results observed in the current study indicate that *C. montrouzieri* is not likely to use these species as a field prey. However, it cannot be excluded that the predator may be able to use this prey as part of a mixed diet, as many predators appear to benefit from mixed diets as compared to certain single-species diets (Lefcheck et al. 2013). van Driesche and Murray (2004b) noted that a predator may in fact not have a choice of prey species if it expands geographically beyond the range of its target pest, if it invades habitats not occupied by the target pest, if the predator is partially out of synchrony with its target pest, or if the target pest is absent for any other reason (including biological control itself and chemical control). In our no-choice reproduction experiments, a single female each time was able to produce viable eggs on *B. tabaci* nymphs or *A. bipunctata* eggs. It should be noted that when confronted with a lack of choice, a strong selection in favour of the few females able to reproduce on alternative prey could occur. Conducting multiple generation experiments on candidate prey can help to understand this mechanism.

In conclusion, our laboratory study indicates that the prey range of *C. montrouzieri* is not limited to the Pseudococcidae, but includes other small, soft-bodied and sedentary hemipterans. To a lesser extent, also eggs of coleopterans and lepidopterans supported survival, larval development and/or reproduction of the ladybird. Whereas there were only scattered reports of the feeding on non-mealybug prey by *C. montrouzieri* in the literature (Kairo et al. 2012; Finlay-Doney and Walter 2012b), the present study compared the effects of non-mealybug prey from different insect orders on the developmental and reproductive performance of the predator. Although we observed a reduced fitness of the predator when offered non-mealybug prey species, our data indicate that it may be able to sustain itself in a crop on

alternative prey when mealybugs are absent or mealybug populations are low. Considering the ladybird's potential to develop and reproduce on *M. persicae*, it may to some extent contribute to the suppression of this aphid pest, but this needs to be confirmed in the field. On the negative side, the non-specific feeding habit of *C. montrouzieri* increases the risk that the predator will attack non-target prey. In areas where the predator cannot establish because of its limited cold tolerance, the effect of its oligophagous feeding behaviour on populations of non-target organisms is expected to be transient. However, in warmer climates its non-specific feeding behaviour may affect the local distribution of non-target prey in both agricultural and natural ecosystems.

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References

Albajes R, Alomar O (1999) Current and potential use of polyphagous predators. In: Albajes R, Lodovica Gullino M, van Lenteren JC, Elad Y (eds) Integrated pest and disease management in greenhouse crops. Kluwer Acad Publ, Dordrecht, The Netherlands, pp 265–275

Attia AR, Afifi AI, El-Arnaouty SA, Alla AEA (2011) Feeding potential of the predator, *Cryptolaemus montrouzieri* Mulsant on eggs, nymphs and adults of *Planococcus citri* and *Ephestia kuehniella* eggs. Egypt J Biol Pest Control 21:291–296

Babendreier D, Bigler F, Kuhlmann U (2005) Methods used to assess non-target effects of invertebrate biological control agents of arthropod pests. BioControl 50:821–870

Bale JS (2011) Harmonisation of regulations for invertebrate biocontrol agents in Europe: progress, problems and solutions. In: Ehlers RU (ed) Regulation of biological control agents in Europe. Springer Science and Business Media B.V, The Netherlands, pp 323–374

Berkvens N, Baverstock J, De Clercq P, Majerus MEN, Pell JK, Roy HE, Tirry L, Wells P (2009) Good and bad traits of *Harmonia axyridis*-From biological control to invasion. In: Mason PG, Gillespie DR, Vincent C (eds) Proceedings of 3rd international symposium on biological control for arthropods, pp 394–402

Bourgoin T, Campbell BC (2002) Inferring a phylogeny for hemiptera: falling into the 'Autapomorphic Trap'. Denisia 4:67–82

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

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

Cock MJW (2013) Risks of non-target impact versus stakeholder benefits in classical biological control of arthropods: selected case studies from developing countries. In: van Driesche RG (ed) Proceedings of the international symposium on biological control of arthropods, Honolulu, Hawaii, United States Department of Agriculture, Forest Service, Morgantown, WV, USA, 14–18 Jan 2002, FHTET-2003–05, pp 25–33

De Clercq P (2002) Dark clouds and their silver linings: exotic generalist predators in augmentative biological control. Neotrop Entomol 31:169–176

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, Mason PG, Babendreier D (2011) Benefits and risks of exotic biological control agents. Biocontrol 56:681–698

Ehlers RU (2011) Regulation of biocontrol agents in Europe. Springer, Dordrecht, The Netherlands

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

Finlay-Doney M, Walter GH (2012b) The conceptual and practical implications of interpreting diet breath mechanistically in generalist predatory insects. Biol J Linn Soc 107:737–763

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

Grenier S, De Clercq P (2003) Comparison of artificially versus naturally reared natural enemies and their potential for use in biological control. In: van Lenteren JC (ed) Quality control and production of biological control agents: theory and testing procedures. CABI Publishing, Wallingford, UK, pp 115–131

Hatherly IS, Pedersen BP, Bale JS (2009) Effect of host plant, prey species and intergenerational changes on the prey preferences of the predatory mirid *Macrolophus caliginosus*. BioControl 54:35–45

Hodek I, Honěk A (1996) Ecology of Coccinellidae. Kluwer, Dordrecht, The Netherlands

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

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

Kairo MTK, Paraiso O, Gautam RD, Peterkin DD (2012) *Cryptolaemus montrouzieri* (Mulsant) (Coccinellidae: Scymninae): a review of biology, ecology, and use in biological control with particular reference to potential impact on non-target organisms. CAB Rev 8:1–20

Kaur H, Virk J (2012) Feeding potential of *Cryptolaemus montrouzieri* against the mealybug *Phenacoccus solenopsis*. Phytoparasitica 40:131–136

Kuhlmann U, Schaffner U, Mason PG (2006) Selection of non-target species for host-specificity testing. In: Bigler F, Babendreier D, Kuhlmann U (eds) Environmental impact of invertebrates for biological control of arthropods: methods and risk assessment. CABI Publishing, Wallingford, UK, pp 15–37

Lefcheck JS, Whalen MA, Davenport TM, Stone JP, Duffy JE (2013) Physiological effects of diet mixing on consumer fitness: a meta-analysis. Ecol 94:565–572

Londsdale WM, Briese DT, Cullen JM (2001) Risk analysis and weed biological control. In: Wajnberg E, Scott JK, Quimby PC (eds) Evaluating indirect ecological effects of biological control. CABI Publishing, Wallingford, UK, pp 185–210

Loomans AJM, van Lenteren JC (2005) Tools for environmental risk assessment of invertebrate biological control agents: a full and quick scan method. In: Hoddle MS (ed) Second international symposium on biological control of arthropods, Davos, Switzerland, USA, 12–16 Sept, United States Department of Agriculture, Forest Service, Washington, USA, pp 611–619

Maes S, Antoons T, Grégoire JC, De Clercq P (2014) A semi-artificial rearing system for the specialist predatory ladybird *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae). BioControl. doi:10.1007/s10526-014-9585-8

Malais MH, Ravensberg WJ (2002) Kennen en herkennen: levenswijzen van kasplagen en hun natuurlijke vijanden. Reed Business Information, Doetinchem, The Netherlands

Manners AG, Palmer WA, Burgos A, McCarthy J, Walter GH (2011) Relative host plant species use by the lantana biological control agent *Aconophora compressa* (Membracidae) across its native and introduced ranges. Biol Cont 58:262–270

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

Michaud JP (2005) On the assessment of prey suitability in aphidophagous Coccinellidae. Eur J Entomol 102:385–390

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

Roy H, Migeon A (2010) Ladybeetles (Coccinellidae). In: Roques A, Kenis M, Lees D, Lopez-Vaamonde C, Rabitsch W, Rasplus J-Y, Roy D (eds) Alien terrestrial arthropods of Europe. BioRisk 4: 293–313

Sands DPA, van Driesche RG (2000) Evaluating the host range of agents for biological control of arthropods: rationale, methodology and interpretation. In: van Driesche RG, Heard TA, McClay AS, Reardon R (eds) Host specificity testing of exotic biological control agents: the biological basis for improvement in safety. Proceedings of the international symposium on biological control of weeds, Montana, USA, 4–11 July, United States Department of Agriculture, Forest Service, Washington, USA, pp 69–83

Ślipiński SA (2007) Australian ladybird beetles (Coleoptera: Coccinellidae): their biology and classification. Australian Biological Resources Study, Canberra, Australia

van Driesche RG, Murray TJ (2004a) Parameters used in laboratory host range tests. In: van Driesche RG, Murray TJ, Reardon R (eds) Assessing host ranges of parasitoids and predators used for classical biological control: a guide to best practice. Forest Health Technology Enterprise Team, Morgantown, USA, pp 56–67

van Driesche RG, Murray TJ (2004b) Overview of testing schemes and designs used to estimate host ranges. In: van Driesche RG, Murray TJ, Reardon R (eds) Assessing host ranges of parasitoids and predators used for classical biological control: a guide to best practice. Forest Health Technology Enterprise Team, Morgantown, pp 68–89

van Lenteren JC, Loomans AJM (2006) Environmental risk assessment: methods for comprehensive evaluation and quick scan. In: Bigler F, Babendreier D, Kuhlmann U (eds) Environmental impact of invertebrates for biological control of arthropods. Methods and risk assessment. CABI Publishing, Wallingford, UK, pp 254–272

van Lenteren JC, Woets J (1988) Biological and integrated pest control in greenhouses. Annu Rev Entomol 33:239–269

van Lenteren JC, Babendreier D, Bigler F, Burgio G, Hokkanen HMT, Kuske S, Loomans AJM, Menzler-Hokkanen I, van Rijn PCJ, Thomas MB, Tommasini MG, Zeng QQ (2003) Environmental risk assessment of exotic natural enemies used in inundative biological control. BioControl 48:3–38

van Lenteren JC, Bale J, Bigler F, Hokkanen HMT, Loomans AJM (2006a) Assessing risks of releasing exotic biological control agents of arthropod pests. Annu Rev Entomol 51:609–634

van Lenteren JC, Cock MJW, Hoffmeister TS, Sands DPA (2006b) Host specificity in arthropod biological control, methods for testing and interpretation of data. In: Bigler F, Babendreier D, Kuhlmann U (eds) Environmental impact of invertebrates for biological control of arthropods. Methods and risk assessment. CABI Publishing, Wallingford, UK, pp 38–63

Vanhaecke M, Degheele D (1980) Electrophoretic characterization of the haemolymph proteins, glyco- and lipoproteins of *Galleria mellonella, Plodia interpunctella* and *Ephestia kuehniella.* Meded Fac Landbouwwet, Rijksuniv. Gent 45:1287–1297

Wapshere AJ (1974) A strategy for evaluating of organisms for biological weed control. Ann Appl Biol 77:201–211

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