

# Evaluation of the quality of *Aphis fabae*, *Myzus persicae* (Homoptera: Aphididae) and *Aleyrodes proletella* (Homoptera: Aleyrodidae) as prey for *Coccinella undecimpunctata* (Coleoptera: Coccinellidae)

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## Introduction:

The study of population parameters related with the predator and prey interaction (such as growth rate and voracity) is essential to evaluate the predator's potential as a biological control agent (Tsaganou et al., 2004).

Quantitative data on main developmental parameters (such as developmental rates, survival, and reproductive capacity) indicate whether the prey is essential or alternative (Hodek & Honěk, 1996). Essential foods show varying degrees of favourability, enabling different development rates, fecundity and survival. Alternative foods may range from highly toxic to quite suitable, enabling survival in periods of scarcity of essential food (Hodek & Honěk, 1996, Evans et al., 1999). In spite of their considerable polyphagy, coccinellids are very specific as far as essential food is concerned (Soares et al., 2004; Soares et al., 2005). Thus to access the possible impact of a coccinellid and its potential as biological control agent against any given pest, it is necessary to test range of their essential preys (Hodek & Honěk, 1996; Obrycki, 1990, Tsaganou, 2004, Dixon, 2000).

*Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae) is a worldwide distributed aphidophagous predator (Frazer, 1988) also found in Azores (Raimundo & Alves, 1986). According to Soares et al. (2003), this specie is, potentially, one of the best biological control agents in the Azores.

In this work we evaluated the quality of three prey species as food sources for *C. undecimpunctata*: *Aphis fabae* Scopoli, *Myzus persicae* Sulzer (two aphid species with a wide and cosmopolite distribution causing large damages in several crops) and *A. proletella*, a whitefly frequently found on greenhouses. Prey quality was evaluated by studying the impact of prey consumption on the predator's population growth parameters (mortality and development time of larvae and, longevity and reproductive performance of adults) and feeding parameters (voracity, daily biomass consumption daily weight gain and feeding efficiency).

## Material and Methods

### Biological material:

*C. undecimpunctata* adults were collected in Sta. Maria Island, Azores, Portugal, earlier summer before experiments took place. Individuals were reared at  $22\pm 1^\circ\text{C}$ ,  $75\pm 5\%$  RH and a photoperiod of 16L:8D. To avoid food adaptation, ladybeetles were fed *ad libitum* on a mixed diet (Rana et al., 2002) of the aphids *Aphis fabae* Scopoli and *Myzus persicae* (Sulzer) and eggs of *Ephestia kuehniella* Zeller.

### Impact of the *A. fabae*, *M. persicae* and *A. proletella* on the population growth parameters of *C. undecimpunctata*:

The suitability of *A. fabae*, *M. persicae* and *A. proletella*, as food sources for *C. undecimpunctata* was evaluated by measuring mortality and development time of larvae and, longevity and reproductive performance of adults. The data obtained was used to construct life tables. The age-specific survival rate and age-specific fecundity were calculated. The net reproductive rate ( $R_0$ ), intrinsic rate of natural increase ( $r_m$ ), finite rate of increase ( $\lambda$ ), mean generation time (T) and doubling time (DT) were estimated. Voracity, daily biomass consumption, daily weight gain and efficiency of food utilisation were measured to all developmental stages of the predator.

All experiments were performed at  $25\pm 1^\circ\text{C}$ , 75%RH, a photoperiod of 16L : 8D under fluorescent lamps (Philips ref.: TDL 23W/54 e TDL 18W/54).

### Development and survival of immature stages

We evaluated the preimaginal developmental time and mortality rate by observing the individuals (isolated in plastic boxes Ø: 3 cm, height: 1 cm) twice a day from the egg to the emergence of the adult. The number of replicates was > 30.

### Longevity and reproductive performance

To evaluate longevity and reproductive performance of the adults, individuals were sexed and paired ( $n > 10$  pairs) inside a 60 ml Petri dish (Ø: 5 cm, height: 3 cm). The number of provided preys exceeded the daily consumption values. Each couple was observed daily to estimate the longevity. Egg clusters were removed from twice a day.

Pre-oviposition period, total oviposition time, total fecundity and fertility (including sibling cannibalism) and percentage of egg hatching were compared.

### **Voracity and daily biomass consumption.**

The number of apterous females of *A. fabae* and *M. persicae* and larvae of 3<sup>rd</sup> instar of *A. proletella* eaten in 24 h by larvae and adults (in sexual maturation period) of *C. undecimpunctata*, were evaluated. Weight of prey provided and weight of predators before and after prey consumption was recorded.

Survival in the control treatments was 87%, 88,15% and 97,5% for *A. fabae*, *M. persicae* and *A. proletella* respectively. Voracity ( $V_0$ ) was calculated according to Soares et al. (2003).

Considering that *A. fabae* and *M. persicae* body weights are different and coccinellid satiation could be reached at a different number of preys, therefore, daily biomass consumption (*DBC*) was evaluated using the methodology of Schanderl (1987).

### **Daily weight gain and feeding efficiency.**

We evaluated the daily weight gain (*DWG*) and calculated the feeding efficiency (*FE*) (Soares et al, 2005) of larvae and adults in sexual maturation period, after being fed with *A. fabae*, *M. persicae* or *A. proletella*.

### **Statistical analysis**

Samples were first described with regular average and standard errors. Analyses of variance (ANOVA) were conducted on all data, except life-tables. Where statistical differences existed between data sets ( $P < 0.05$ ), Fisher's Least Significant Difference tests (LSD) were used to separate the differing means (Zar, 1996).

To reduce variance differences, data concerning the percentage of hatching were transformed by  $\arcsin\sqrt{x}$  and, the development time, mortality rate, time of sexual maturation, total oviposition time, fecundity, fertility, voracity and biomass consumption were transformed by  $\sqrt{x+0.5}$  (Zar, 1996). All the analyses were performed using SPSS 12.0.1 Windows (SPSS Inc., 2004).

## **Results**

### **Development and survival of immature stages**

With the exception of the prepupa and pupa, the development times of the immature stages of *C. undecimpunctata* fed on *A. proletella* was significantly longer than the individuals fed on *A. fabae* or *M. persicae* (Table 1). Consequently, the total development time of the immature stages displayed statistically significant differences among aphid prey species and whiteflies, where the total development time almost duplicates (Table 1).

Table 1. Pre-imaginal development times (days  $\pm$  SE) of *C. undecimpunctata* fed on single diets of *A. fabae*, *M. persicae* or *A. proletella*.

Developmental stage	Prey			Anova
	<i>A.fabae</i>	<i>A.proletella</i>	<i>M. persicae</i>	
<b>Egg</b>	2,93 $\pm$ 0,08a	-----	2,33 $\pm$ 0,05b	F= 43,70; n= 62; df=1; p $\leq$ 0,0001
<b>1<sup>st</sup> instar</b>	2,13 $\pm$ 0,10a	3,44 $\pm$ 0,11b	1,91 $\pm$ 0,13a	F= 55,07; n= 117; df=2; p $\leq$ 0,0001
<b>2<sup>nd</sup> instar</b>	1,25 $\pm$ 0,06a	3,12 $\pm$ 0,22b	1,39 $\pm$ 0,11a	F= 47,00; n= 102; df=2; p $\leq$ 0,0001
<b>3<sup>rd</sup> instar</b>	1,59 $\pm$ 0,07a	4,63 $\pm$ 0,34b	1,67 $\pm$ 0,09a	F= 74,09; n= 70; df=2; p $\leq$ 0,0001
<b>4<sup>th</sup> instar</b>	2,90 $\pm$ 0,86a	7,88 $\pm$ 0,38b	2,33 $\pm$ 0,18c	F= 115,38; n= 36; df=2; p $\leq$ 0,0001
<b>Pré-pupa + Pupa</b>	3,00 $\pm$ 0,11a	3,00 $\pm$ 0,00a	3,50 $\pm$ 0,22a	F= 2,90; n= 29; df=2; p=0,073
<b>Total</b>	10,82 $\pm$ 0,18a	21,5 $\pm$ 1,73b	10,62 $\pm$ 0,32a	F= 97,23; n= 33; df=2; p $\leq$ 0,0001

\* Different letters within a row indicate significant differences (Fisher's Protected LSD test;  $P < 0,05$ ).

The egg development time was significantly longer when the predator was fed with *A. fabae* compared to *M. persicae*.

The numbers of individuals that were able to complete their life cycle when fed with *M. persicae* (40%) or *A. fabae* (50%) were considerably higher than when fed on *A. proletella* (4%) (Table 2). Furthermore, the vulnerability of each preimaginal stage varied with the prey offered: for example, the mortality rates of 4<sup>th</sup> instar larvae were very high when individuals are fed with *A. proletella* (near to 90%) but decreased to 25% when fed with *A. fabae* (Table 2).

Table 2. Life table of *C. undecimpunctata* preimaginal stages when fed on single diets of *A. fabae*, *M. persicae* or *A. proletella*. Legend: L<sub>1</sub>, L<sub>2</sub>, L<sub>3</sub> and L<sub>4</sub> - first, second, third and fourth instar respectively; PP+P- pre-pupa and pupa; Ad-adults.

Prey	Stage (X)	Age specific survivorship (I <sub>x</sub> )	Age specific mortality (d <sub>x</sub> )	Mortality rate per age interval (100 q <sub>x</sub> )	Accumulated mortality rate
<i>M.persicae</i>	L <sub>1</sub>	35	4	11	0.11
	L <sub>2</sub>	31	4	13	0.23
	L <sub>3</sub>	27	5	19	0.37
	L <sub>4</sub>	22	2	9	0.43
	PP+P	20	6	30	0.60
	Ad	14			
<i>A.fabae</i>	L <sub>1</sub>	30	1	3	0.03
	L <sub>2</sub>	29	7	24	0.27
	L <sub>3</sub>	22	2	9	0.33
	L <sub>4</sub>	20	5	25	0.50
	PP+P	15	0	0	0.50
	Ad	15			
<i>A.proletella</i>	L <sub>1</sub>	75	17	23	0.23
	L <sub>2</sub>	58	13	22	0.40
	L <sub>3</sub>	45	16	36	0.61
	L <sub>4</sub>	29	26	90	0.96
	PP+P	3	0	0	0.96
	Ad	3			

### Longevity and reproductive capacity

Concerning the adults' longevity, we found that there were significant differences between the three diets, being longer with *M. persicae* and shortened with *A. proletella*. These differences were accentuated on males (Table 3)

Table 3. Longevity, pré-oviposition and oviposition period (days ± SE) and reproductive parameters [fecundity (number of eggs ± SE); fertility (number of hatched eggs ± SE); percentage of hatching (percentage of hatched larvae ± SE)] of *C. undecimpunctata*, when fed on single diets of *A. fabae*, *M. persicae* or *A. proletella*.

Biologic Parameters	Prey			Anova
	<i>A. fabae</i>	<i>A. proletella</i>	<i>M. persicae</i>	
Longevity				
Female	30,36 ± 4,93a	14,88 ± 2,09b	30.10 ± 2.87a	F= 9.51; n= 37; df=2; p=0.001
Male	25.73 ± 2.84a	12.25 ± 0.94b	44.90 ± 7.35c	F= 19.46; n= 37; df=2; p<0.0001
Adults	28.05 ± 2.82a	13.56 ± 1.15b	37.50 ± 4.20c	F= 26.58; n= 74; df=2; p<0.0001
Pré-oviposition period	7.0 ± 1.04a	-----	4.40 ± 0.27b	t= 1.04; n= 21; df=12.48; p=0.025
Oviposition period	20.9 ± 4.78a	-----	24.6 ± 3.04a	t= 0.46; n= 21; df=14.98; p=0.315
Fecundity (Fec)				
Total	194 ± 49.86a	0.00 ± 0.00b	596.20 ± 118.52c	F= 64.95; n= 36; df=2; p<0.0001
Fec <sub>(oviposition period/2)</sub>	71.27 ± 16.60a	0.00 ± 0.00b	308.30 ± 57.67c	F= 85.99; n= 36; df=2; p<0.0001
Fertility (Fer)				
Total	151.00 ± 37.66a	-----	353.20 ± 70.23b	t= 3.44; n= 21; df=19; p=0.003
Fer <sub>(oviposition period/2)</sub>	58.14 ± 13.80a	-----	213.65 ± 53.96b	t= 3.40; n= 21; df=19; p=0.003
Percentage of hatching (%)				
Total	78.42 ± 2.6a	-----	68.00 ± 10.48a	t= -0.69; n= 21; df=10.16; p=0.503
% <sub>(oviposition period/2)</sub>	78.48 ± 4.67a	-----	71.58 ± 10.31a	t= -0.42; n= 21; df=12.63; p=0.679

\*Different letters within a row indicate significant differences (Fisher's Protected LSD test; P<0.05)

The pre-oviposition time was significantly higher when *C. undecimpunctata* feed on *A. fabae*, but the oviposition period had no differences. Concerning the reproductive performance we observed that both *A. fabae* and *M. persicae* allowed the oviposition with high values of fecundity. However fecundity and fertility was significantly higher when *C. undecimpunctata* was fed with *M. persicae*, but there was no significant difference on the percentage of hatching between both aphid species (Table 3). *C. undecimpunctata* females fed on *A. prolella* did not oviposit. (Table 3).

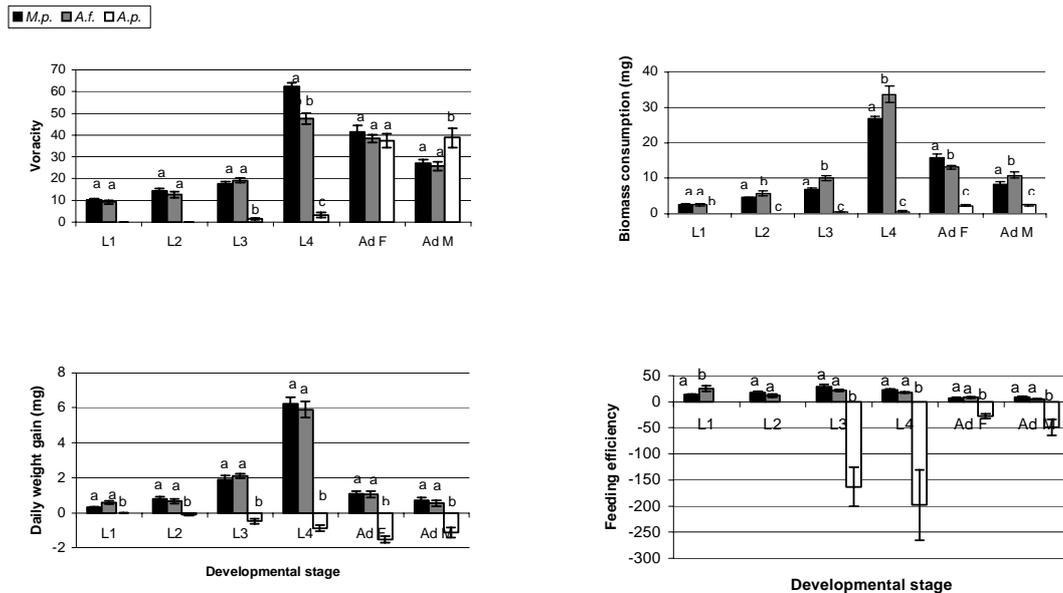
*M. persicae* showed a larger potential to increase the population of *C. undecimpunctata*, presenting a  $R_0$  two times superior than *A. fabae* and, higher  $r_m$  and  $\lambda$  values, allowing the duplication of the population in 3,6 days. On the other hand, *A. prolella* did not allow the predator sustainability, particularly, due to their incapacity to reproduce when fed on this prey (Table 4).

Table 4. Population growth parameters for *C. undecimpunctata* fed on single diets of *A. fabae*, *M. persicae* or *A. prolella*. Legend: net reproductive rate ( $R_0$ ), intrinsic rate of natural increase ( $r_m$ ), finite rate of increase ( $\lambda$ ), mean generation time (T) and doubling time (DT).

Population parameters	Prey		
	<i>A. fabae</i>	<i>A. prolella</i>	<i>M. persicae</i>
<b><math>R_0</math></b>	143.28	0	286.71
<b><math>r_m</math></b>	0.16	0	0.19
<b><math>\lambda</math></b>	1.17	0	1.21
<b>D</b>	4.33	0	3.63
<b>T</b>	31.02	0	29.64

#### ***Voracity and daily biomass consumption***

All *C. undecimpunctata* stages consume when feed in *M. persicae* and *A. fabae*, being the 4<sup>th</sup> instar the most voracious and the only with significant differences between the aphid species (Fig.1). Even though *M. persicae*, compared to *A. fabae*, has in average a smaller biomass, the number of preys consumed only differed significantly in 4<sup>th</sup> instar. Larvae of *C. undecimpunctata* consume a very low number of *A. prolella* contrarily to adults that ate almost the same quantity of whitefly comparatively to aphid prey species, however, the biomass consumption was very low.



**Figure 1:** Daily voracity (mean number of prey eaten  $\pm$  s.e.), daily biomass consumption (mg of aphids eaten  $\pm$  s.e.), daily weight gain (mg  $\pm$  s.e.), and feeding efficiency (% of ingested food to biomass per day) of larval instars (L<sub>1</sub>, L<sub>2</sub>, L<sub>3</sub>, L<sub>4</sub>) and adults (Ad M-males, Ad F-females) of *C. undecimpunctata* fed with single diets of *A. fabae* (*A.f.*), *M. persicae* (*M.p.*) or *A. proletella* (*A.p.*). Bars regarding the same developmental stage with different letters are significantly different at  $P < 0.05$  (LSD procedure within ANOVA).

### Daily weight gain and feeding efficiency

All *C. undecimpunctata* stages grow when fed in *M. persicae* and *A. fabae* (Figure 1). Although the adults ate *A. proletella* they did not increase their weight. There were no differences in the efficiency of food utilization between single diets of aphids (Figure 1).

### Discussion

It has been generally accepted that not all eaten preys are suitable food to coccinellids: thus, discrimination between the food enabling development and oviposition (essential prey) and food that is good only for survival (alternative prey) should be performed (Hodek & Honěk, 1996). Only quantitative data on predator's principal developmental parameters (at least rate of development, survival, reproductive capacity) indicate safely the adequacy of the prey (Kalushkov, 2001). Our results showed that both aphids, *A. fabae* and *M. persicae*, are equally suitable preys for *C. undecimpunctata* pre-imaginal stages, since no considerable differences were observed for the development times and survival, except on the egg development time that was longer when *A. fabae* was the food source. On the other hand, *A. proletella* showed to be an unsuitable prey to *C. undecimpunctata*, since the total development time of the predator's was increased, almost duplicated, when this prey was used as food source.

Several authors have shown that food availability (Kawauchi, 1981; Ferran et al., 1984; Stewart et al., 1991; Dixon & Guo, 1993; Dixon, 2000) and quality (Blackman, 1967; Nijima & Takahashi, 1980; Nijima et al., 1986; Michaud, 2005) affect the fecundity, fertility and percentage of hatching of coccinellids. The higher pre-oviposition time and lower fecundity observed when the prey was *A. fabae* compared to *M. persicae* could be result of an inferior nutritive quality of the first prey; however, the percentage of hatching was similar, signal of a possible strategy to maintain egg quality recurring to a longer vitellogenesis period and a decrease on the number of eggs laid. It is noteworthy that *A. fabae* significantly increased the pre-oviposition period and decreased adult longevity as well as fecundity and fertility of *C. undecimpunctata*. These results indicate that essential foods can show varying degrees of favourability, thus, enabling different development rates, fecundity and survival, as suggested by Hodek & Honěk (1996). On the other hand, our results indicate that a particular aphid may represent a highly suitable diet for larval development, but less suitable for adult reproduction, or vice-versa.

Comparing the biological and population parameters of *C. undecimpunctata* fed on single diets of *M. persicae*, *A. fabae* and *A. proletella*, we observed that, from the ecophysiological point of view, *M. persicae* and *A. fabae* are essential preys since full fell the presumptions previewed by Hodek & Honěk (1996), i.e., the prey supported the development, survival and reproduction of *C. undecimpunctata* and that *A. proletella* is an alternative prey. Generalist predators such as adult lady beetles have the tendency to consume alternative as well as essential preys,

probably enhancing considerably their ability to capitalize on short-lived and scattered opportunities as they seek out suitable sites in which to reproduce (Evans, 1999). According to Michaud (2005) in terms of biological control, alternative preys are especially important for sustaining coccinellid populations that survive largely on aphid species that exhibit “boom and bust” cycles of abundance, as they can function to reduce local extinction events when adequate prey are scarce.

In further works it will be gainful to evaluate how *C. undecimpunctata* could increase their performance by the enrichment of their diet using mix diets of essential preys or the different combinations of essential with alternative preys.

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