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Effect of temperature and prey in the biology of *Scymnus subvillosus* (Goeze)

(Coleoptera: Coccinellidae)

Departamento de Biologia

Ponta Delgada

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Dissertação para obtenção de Grau de Mestre em Biotecnologia em Controlo Biológico

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ABSTRACT

Scymnus subvillosus (Goeze) is an aphidophagous predator present in the Azores Archipelago (Portugal), but it occurs at low densities. Recently it has been observed exploiting *Melanaphis donacis* (Passerini) on *Arundo donax* L. The *Scymnus* spp., although less studied, have, in recent years, been the subject of research in the perspective of their use as biological control agents.

In this study we aimed to determine the concomitant effects of four constant temperatures (15°C, 20°C, 25°C and 30°C) and three aphid species on survival and development of immature stages of *S. subvillosus*, to access the ecophysiological suitability of *M. persicae, A. fabae* and *M. donacis* and finely the voracity and nutritional physiology of the 4th larval stage fed on *A. fabae*.

The development time from larvae to adult decreased with increasing temperature, ranging from 61.5 days at 15ºC to 10.4 days at 30ºC. To complete pre-imaginal development on *M. donacis*, LDT was estimated to be 11.7ºC and SET 196.3 ºD. At 15ºC, larvae failed to develop when fed on *A. fabae* and *M. persicae* but on *M. donacis* 22% of larvae have survived. . We also found that development time of immature stages were also prey-dependent, being *M. persicae* the least suitable.

Reproductive parameters were prey dependent, being roughly better with *A. fabae*.

Twelve hours 4th larval stage of *S. subvillosus* ingested 2.08 mg per day of biomass corresponding to an average of 10.5 aphids, allowing for a daily mean weight gain of 0.71 mg. The conversion efficiency and relative growth rate obtained was approximately 21% and 48% respectively.

The results obtained in the present study suggest that both, *A. fabae* and *M. donacis* are more suitable preys for development and reproduction of *S. subvillosus* than *M. persicae.*

Key-words: *S. subvillosus, A. fabae, M. donacis, M. persicae,* development parameters, reproductive parameters, nutritional physiology.

RESUMO

Scymnus subvillosus (Goeze) é um predador afidífago presente no Arquipélago dos Açores (Portugal), que ocorre em baixas densidades., recentemente, foi observado a explorar *Melanaphis donacis* (Passerini) em *Arundo donax* L. O Scymnus spp., embora menos estudadas, têm sido nos últimos anos, alvo de investigação na perspectiva da sua utilização como agentes de controlo biológico.

Este estudo teve como objetivo determinar os efeitos concomitantes de quatro temperaturas constantes (15° C, 20 °C, 25 C e 30°C) e de três espécies de afídeos na sobrevivência e desenvolvimento dos estados imaturos de *S. subvillosus* , para obter a adequação ecofisiológica de *M. persicae, A. fabae e M. donacis* e finalmente a voracidade e fisiologia nutricional da fase larval L4 alimentadas com *A. fabae* .

O tempo de desenvolvimento a partir de larvas a adulto diminuiu com o aumento da temperatura, variando de 61,5 dias a 15 º C para 10,4 dias a 30ºC. Para completar o desenvolvimento pré -imaginal com *M. donacis*, LDT foi calculado obtendo o valor de 11,7ºC e SET 196,3ºD. A 15ºC, as larvas não se desenvolveram quando alimentadas com *A. fabae* e *M. persicae,* mas com *M. donacis* 22 % das larvas sobreviveram. Assim sendo, o tempo de desenvolvimento dos imaturos também foi dependente da presa, sendo *M. persicae* a menos adequada.

Os parâmetros reprodutivos foram dependentes da presa, sendo melhor com *A. fabae.*

Larvas de *S. subvillosus* com doze horas no estado L_4, ingeriram 2,08 mg por dia de biomassa, correspondente a uma média de 10,5 afídeos, permitindo um ganho de peso médio diário de 0,71 mg. A eficiência de conversão e a taxa de crescimento relativa foi de aproximadamente 21 % e 48 %, respectivamente.

Os resultados obtidos no presente estudo sugerem que ambas, *A. fabae M. donacis* são presas mais adequadas para o desenvolvimento e reprodução de *S. subvillosus* do que *M. persicae*.

Palavras-chave: *S. subvillosus, A. fabae, M. donacis, M. persicae*, parâmetros de desenvolvimento, parâmetros reprodutivos e fisiologia nutricional.

1. INTRODUCION

Ladybird predators (Coleoptera: Coccinellidae) are known as important biological control agents, especially against aphid populations (Hodek, van Emden and Honěk, 2012). Several species are used in biological control programs under its various strategies: classical, augmentative, inundative, in different crops and cultivation systems, either outdoors and greenhouses (Hodek and Michaud, 2008; Cabral et al., 2009). These predators have been extensively studied due to their biological, ecological and behavioral characteristics such as polyphagy, high voracity and rapid numerical and aggregative response (Hodek and Honěk, 1996; Obrycki and Kring, 1998; Dixon, 2000). Indeed the first case of successful biological control program was achieved with a ladybird species, *Rodolia cardinalis* (Mulsant) against the cottony cushion scale *Icerya purchasi* Maskell on citrus plantation in California (USA) (Dixon, 2000).

Among ladybirds the small *Scymnus sp.*, although less studied, have, in recent years, been the subject of research in the perspective of their use as biological control agents (Uygun and Atlihan, 2000; Wanntorp, 2004; Pluke *et al*., 2005; Woin et al., 2006). Comparing with larger species, the *Scymnus* spp., due to its small size and lower voracity, will be apparently less competitive and efficient as biological control agents. However, persisting at low densities of prey and presenting high longevities (Tawfik et al., 1973, Borges et al., 2011, 2013), these small ladybirds may be able to explore aphid colonies on earlier and /or later stages (Agarwala and Yasuda, 2001) and for a longer period of time, which would give them the possibility to exploit different spatial and temporal niches other than the larger ladybirds.

Scymnus subvillosus (Goeze) is an aphidophagous predator recorded for the Azores Archipelago (Portugal) (Soares et al. 2003; 2006), but occurring at low densities. It has been observed exploting *Melanaphis donacis* (Passerini) on *Arundo* *donax* L. This coccinellid is a cosmopolitan species, found in France and Mediterranean regions, Madeira archipelago (Portugal), Caucasus, Siberia, Minor Asia, North Africa and Germany (Gourreau, 1974; Raimundo and Alves, 1986). In the Portuguese mainland, it is common in arboreal agro-ecosystems, including apple orchards, orange, peach, plum, walnut and oak, where it exploits *Hyalopterus pruni* (Geoffroy) (Homoptera: Aphididae) (Raimundo and Alves, 1986). In Turkey, *S. subvillosus* is widespread (Atlihan and Güldal, 2009), and it is an important natural enemy against 15 aphid species (Aslan and Uygun, 2005). There it is common in agro-ecosystems and natural habitats as well, including stone fruits especially in *Prunus* spp. orchards, *Mallus* spp., and *Poplus* spp., on apple, plum, cherry, pine and alder (Aslan and Uygun 2005).

The temperature and the nutritional quality of prey are decisive factors in the biological performance of insect predators, altering for instance the development time of pre-imaginal stages, longevity and reproductive parameters of adults (e.g., fecundity, fertility), and by this way contributing to differential population growth (McCaffrey and Horsburgh 1986; Niijima et al., 1986; Gibson et al., 1992; Hodek, 1993; Mohaghegh et al., 2001; Skirvin and Fenlon, 2003; Kontodimas and Stathas, 2005; Michaud, 2005; Cabral et al., 2006; Jalali et al., 2010). The estimation of the biological characteristics of natural enemies, such as time of development and the thermal constant may contribute to select the most appropriate biocontrol agent to be used under certain environmental conditions (Perdikis and Lykouressis, 2002) as well as the best conditions either thermal or nutritional for mass production.

In this study we aimed i) to determine the concomitant effects of four constant temperatures (15°C, 20°C, 25°C and 30°C) and three aphid species on survival and development of immature stages of *S. subvillosus*, ii) to access the ecophysiological suitability of *M. persicae*, *A. fabae* and *M. donacis* as prey to *S. subvillosus* and iii) the voracity and nutritional physiology of the 4th larval stage fed on *A. fabae*.

2. MATERIAL AND METHODS

2.1 The insects

Scymnus subvillosus adults were collected in the field, on *Arundo donax* L., in S. Miguel Island, Azores (Portugal). The predator was found foraging and reproducing on *Melanaphis donacis* (Passerini). Ladybird adults were collected and reared in the laboratory at 25 ± 1°C, 75 ± 5% RH and a light regime 16L:8D under fluorescent lamps. The predator was fed on a mixed diet of *Myzus persicae* (Sulzer) and *Aphis fabae* (Scopoli) (Homoptera: Aphididae), provided *ad libitum*, and complemented with pollen and honey. The mixed diet was provided to avoid food adaptation (Rana et al., 2002) and also to supply a wider group of nutrients. The prey species were reared on *Vicia faba* L. major at 15 ± 1°C, 75 ± 5% RH and a light regime 16L: 8D under fluorescent lamps.

2.2 Temperature and prey-dependence for development of the immature stages of *S. subvillosus*

To determine the effect of temperature and prey on the development of the immature stages of *S. subvillosus*, the development time from egg to adult emergence was followed at 12 experimental treatments, corresponding each one to the following temperature, 15°C, 20°C, 25°C and 30°C and prey regimes of *A. fabae*, *M. donacis* or

M. persicae (N = 30). Each larvae was kept individually in a plastic box (diameter: 5cm, height: 3 cm) and was fed once a day *ad libitum,* with single diets of *A. fabae, M. donacis* or *M. persicae*. All assays were performed at 75 ± 5% RH, with a photoperiod of 16L: 8D. Immature stages were observed twice a day (10:00 am and 17:00 pm) to record moulting and adult emergence.

The larval development time and survival were determined for each diet at each tested temperature. With the data on development at the different temperatures (15°C, 20°C, 25°C and 30°C), the lower developmental threshold (LDT) was determined. The (LDT) is the temperature below which a particular stage of an animal cannot develop. The relationship between the inverse of the development time and the temperature is nearly linear (Honěk and Kocourek, 1990). According to this, the following linear relationship was considered (Mota et al., 2008):

$$1/DT = aT \ + b$$

Where DT corresponds to the development time of the pre-imaginal, which is inversely proportional to the temperature (T), and *a* and *b* are the regression parameters. The LDT corresponds to T, when no development occurs, that is, when 1/DT = 0 (Honěk and Kocourek, 1988).

Sum of effective temperatures (SET), is the thermal time T which is the number of day degrees (°D) above lower developmental threshold for completion of a developmental stage (Hodek, van Emden and Honěk, 2012).

The sum of effective temperatures (SET) was calculated according to the model (Mota et al., 2008):

$$SET = \frac{[DT_{15}(15 - LDT_{15})] + [DT_{20}(20 - LDT_{20})] + [DT_{25}(25 - LDT_{25})] + [DT_{30}(30 - LDT_{30})]}{4}$$

DT_x- development time to temperature x

LDT_x- lower developmental threshold to temperature x

2.3 Prey suitability of *A. fabae*, *M. donacis* and *M. persicae* for *S. subvillosus*

The suitability of *A. fabae, M. donacis* and *M. persicae* as prey for *S. subvillosus* was evaluated by determining development data such as development time of immature stages (N=30), survival rate, weight of females soon after emergence and reproductive parameters, e.g., pre-oviposition time, fecundity and fertility. To evaluate reproductive performance of adults, 10 couples were formed and followed for 20 days on each prey diet. Each couple was kept inside a plastic box (diameter: 5cm height: 3cm) and fed *ad libitum* with single diets of *A. fabae*, *M. donacis* or *M. persicae*. All females were weighed before to the tests for reproductive parameters. Couples were observed once a day to obtain data on pre-oviposition time, fecundity and fertility.

All assays were performed at 25± 1°C, 75±5% RH and a light regime 16L: 8D under fluorescent lamps.

2.4 Voracity and nutritional physiology of the 4th larval stage of *S. subvillosus* fed on *A. fabae*

The larvae used on this experiment were previously fed *ad libitum* with *A. fabae* and reared under the same abiotic conditions of the previous experiments until they reached the 4th larval stage. The experiments were performed on *A. fabae* because it was the prey on which the predator presented faster development, high fecundity and fertility. To determine aphid natural mortality and weight loss due to dehydration, a control test was performed. Ten aphids were placed inside a plastic box (N=15). At the beginning of the experiments as well as at the end of the experiment (8:00 am/8:00 pm) the weight of aphids was recorded to measure the weight loss due to dehydration (PW_d) and the survival rate (S_r) of the aphids.

The test of consumption was performed with 12 hours old 4th instar larvae. Then larvae were subjected to starvation for a period of 12 hours, followed by a 24 hours test where they were allowed to consume prey.

Because the aphid prey dehydrated considerably on a 24 hours period, the predator was provided with 10 aphids in two feeding periods (8:00 am and 8:00 pm): in a total of 20 aphids per day/larvae. In order to determine the biomass consumption, the aphids were weighed before and after the 24hour feeding period. The number of prey consumed and naturally dead was recorded. In the beginning and at the end of the test the prey biomass (PW) and larvae weight (LW) were also recorded.

Voracity was estimated as the number of preys entirely or partially eaten (P). The voracity (V), biomass ingested (BI), relative growth rate (RGR %) and conversion efficiency (CE %) was also calculated (Borges, 2008) :

$$V_{S.subvillosus} = P \times S_r$$

$$BI_{\ S.subvillosus} = \frac{PW_f - PW_i - PW_d}{PW_i}$$

$$RGR\ \% = \frac{LW_f - LW_i}{LW_i} \times 100$$

$$CE\ \% = \frac{LW_f - LW_i}{BC} \times 100$$

Where PW_i and PW_f are prey initial and final weights respectively, LW_i and LW_f are the larval initial and final weights respectively.

2.5 Statistical analysis

Data normality and variance homogeneity were evaluated by the Kolmogorov-Smirnov and Levene's tests respectively. When normality and homogeneity of variance were confirmed, the parametric test of ANOVA was used and for multiple comparisons the Tukey test was performed. When data was not normal, non-parametric Kruskal-Wallis test was used, with multiple comparisons made by the Mann-Whitney test with the correction of Bonferroni.

The Bonferroni correction is a multiple-comparison correction used when several dependent or independent statistical tests are being performed simultaneously. To perform a Bonferroni correction, the critical *P v*alue (α) is divided by the number of comparisons being made. In the present study, 3 diets tested, therefore the critical *P* value is 0.017 (α/3).

SPSS v. 15.0 was used to perform statistical analysis.

3. RESULTS

3.1 Temperature and prey-dependence for development of the immature stages of *S. subvillosus*

Our results showed that the immature stages development time of *S. subvillosus* decreased with increasing temperature, as expected within the range of temperatures tested (Tables I, II and III).

At 15ºC, only a few couples laid eggs and most of them were infertile. Due to this fact, statistical analyses on developmental data did not include embryonic development. To overcome the reduced number of eggs obtained at 15ºC, couples were maintained at 25°C to allow oviposition. The eggs were then collected and transferred to 15°C in order to obtain new born larvae to follow its development.

Lower development threshold LDT (ºC) and sum effective temperatures SET (̊D) for immature development of *S. subvillosus* fed on *M. donacis* range from 9.4ºC for L1 to 12ºC for L4 and SET from 16.7°D for L2 to 70.6°D for pupa (Table IV). To complete pre-imaginal development, LDT was estimated to be 11.7ºC and SET 196.3ºD (Table IV). The thermal parameters of larvae fed on *A. fabae*, *M. persicae* was not calculated because data from 3 temperatures (20°C, 25°C and 30°C) leads to an inaccurate and unrealistic estimates of the LDT and SET values.

Table I: Development time of immature stages (days ± SE) of *S.subvillosus* under constant temperatures (15°C, 20°C, 25°C and 30°C). L_1, L_2, L_3, L_4 and P: first, second, third and fourth larval stages and pupa, respectively fed with *M. persicae*.

Developmental stages	15°C	20°C	25°C	30°C	ANOVA (F) or Kruskal-Wallis ($\chi 2$)
L_1	___	2.34 ± 0.15a*	2.03 ± 0.07ab	1.84 ± 0.08b	F = 3.84, df = 2, p = 0.029
L_2	___	1.67 ± 0.15a	1.57 ± 0.21a	0.97 ± 0.60b	$\chi 2$= 11.06, df = 2, p= 0.004
L_3	___	1.85 ± 0.24a	1.69 ± 0.43ab	1.42 ± 0.53b	F = 3.36, df = 2, p = 0.046
L_4	___	6.04 ± 0.14a	4.21 ± 0.65b	4.17 ± 0.44b	F = 47.17, df = 2, p ≤ 0.0001
P	___	7.77 ± 0.26a	4.14 ± 0.19b	3.86 ± 0.10b	F = 272.28, df = 2, p ≤ 0.0001
Total	___	19.5 ± 0.23a	13.8 ± 0.30b	12.3 ± 0.17c	F = 258.32, df = 2, p ≤ 0.0001

* Means in each row followed by different letters significantly different at p < 0.05 (ANOVA or Kruskal-Wallis).

Table II: Development time of immature stages (days ± SE) of *S.subvillosus* under constant temperatures (15°C, 20°C, 25°C and 30°C). L_1, L_2, L_3, L_4 and P: first, second, third and fourth larval stages and pupa, respectively fed with *A. fabae*.

Developmental stages	15°C	20°C	25°C	30°C	ANOVA (F) or Kruskal-Wallis (χ2)
L_1	___	2.15 ± 0.07a*	1.80 ± 0.06b	2.07 ± 0.04a	χ2 = 11.07, df = 2, p ≤ 0.0001
L_2	___	2.08 ± 0.04a	1.03 ± 0.09b	1.20 ± 0.08b	χ2 = 39.4,df = 2, p ≤ 0.0001
L_3	___	1.63 ± 0.10a	1.38 ± 0.12ab	1.08 ± 0.08b	F = 7.5, df = 2, p = 0.001
L_4	___	5.11 ± 0.14a	4.22 ± 0.09b	3.75 ± 0.13c	F = 32.45, df = 2, p ≤ 0.0001
P	___	7.14 ± 0.08a	4.75 ± 0.07b	4.17 ± 0.08c	F = 411.89, df = 2, p ≤ 0.0001
Total	___	17.34 ± 0.14a	13.17 ± 0.12b	12.08 ± 0.20c	χ2 =11.07, df = 2, p ≤ 0.0001

*Means in each row followed by different letters significantly different at p < 0.05 (ANOVA or Kruskal-Wallis).

Table III: Development time of immature stages (days ± SE) of *S. subvillosus* under constant temperatures (15°C, 20°C, 25°C and 30°C). L_1, L_2, L_3, L_4 and P: first, second, third and fourth larval stages and pupa, respectively fed with *M. donacis*.

Developmental stages	**15°C**	**20°C**	**25°C**	**30°C**	**Kruskal-Wallis ($\chi 2$)**
L_1	7.11 ± 0.37a*	2.95 ± 0.06b	1.97 ± 0.05c	1.77 ± 0.07c	$\chi 2$ = 76.66, df = 3, p ≤ 0.0001
L_2	6.01 ± 0.28a	1.81 ± 0.14b	1.22 ± 0.08c	0.82 ± 0.08d	$\chi 2$ = 35.54, df = 3, p ≤ 0.0001
L_3	6.61 ± 0.37a	2.17 ± 0.12b	1.67 ± 0.12c	0.89 ± 0.07d	$\chi 2$ = 31.61, df = 3, p ≤ 0.0001
L_4	20.63 ± 0.45a	7.06 ± 0.15b	4.5 ± 0.13c	3.25 ± 0.19d	$\chi 2$ = 28.96, df = 3, p ≤ 0.0001
P	23.31 ± 0.83a	8.0 ± 0.09b	5.63 ± 0.08c	3.75 ± 0.09d	$\chi 2$ = 29.60, df = 3, p ≤ 0.0001
Total	61.50 ± 1.96a	22.00 ±0.22b	14.75 ± 0.11c	10.50 ± 0.13d	$\chi 2$ = 21.86, df = 3, p ≤ 0.0001

* Means in each row followed by different letters significantly different at p < 0.05 (Kruskal-Wallis).

Table IV: Lower development threshold (LDT) and sum of effective temperatures (SET ± SE) required for the development of the immature stages of *S. subvillosus* fed on *M. donacis*, with the respective coefficient of determination (R^2), slope (a) and intercept (b). L1, L2, L3, L4 and P: first, second, third and fourth larval stages and pupa, respectively.

Stages	a	b	R^2	LDT (°C)	SET (°D)
L1	0.0301	-0.2825	0.9687	9.4	34.2 ± 2.13
L2	0.0629	-0.7302	0.9766	11.6	16.7 ± 1.40
L3	0.0512	-0.5962	0.9901	11.6	20.1 ± 0.97
L4	0.0171	-0.2046	0.9983	12.0	59.1 ± 1.19
P	0.0143	-0.1693	0.993	11.8	70.6 ± 2.18
Total	0.0052	0.0606	0.9949	11.7	196.3 ± 5.93

3.2 Prey suitability of *M. persicae*, *A. fabae* and *M. donacis* for *S. subvillosus*

Table V: Development time of immature stages (Days ± SE) of *S. subvillosus fed* with 3 different preys **(**different letters mean significant differences followed in each row).

Temperature	*M. persicae*	*A. fabae*	*M. donacis*	Kruskal-Wallis (χ2)
15°C	-	-	61.5 ± 1.96	-
20°C	20.3 ± 0.14a	17.5 ± 0.31b	21.6 ± 0.15c	$\chi2 = 48.43$, df = 2, $p \leq 0.0001$
25°C	13.8 ± 0.26a	13.2 ± 0.12a	14.9 ± 0.04b	$\chi2 = 26.54$, df = 2, $p \leq 0.0001$
30°C	12.3 ± 0.17a	12.3 ± 0.25a	10.4 ± 0.10b	$\chi2 = 23.01$, df = 2, $p \leq 0.0001$

At 20°C, larvae of *S. subvillosus* developed significantly faster fed on *A. fabae* and slower on *M. donacis*. At 25°C it developed significantly slower on *M. donacis* and significantly faster on that prey at 30°C. (Table V).

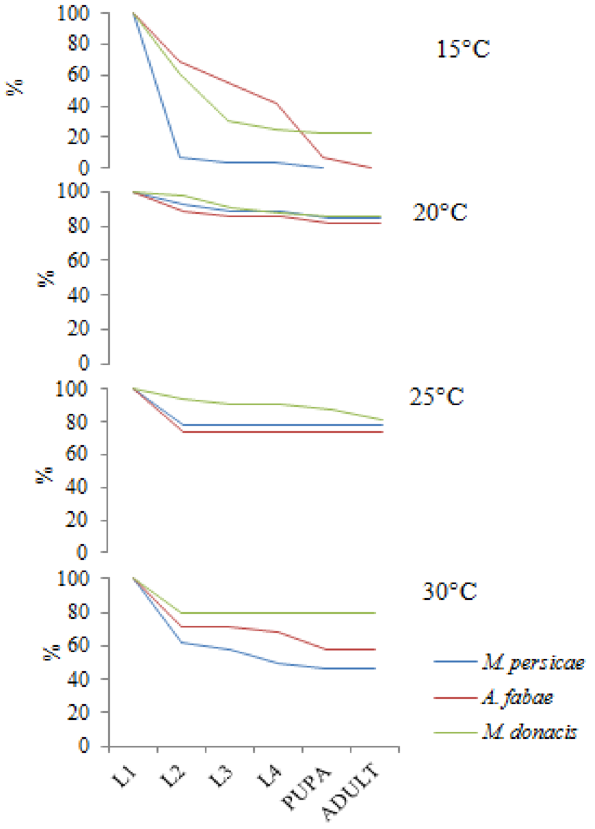

Figure 1: Survival rate (mean ± SE) of immature stages of *S. subvillosus* fed with different preys.

Concerning survival rates, we found that at 15 °C larvae failed to develop into adult when fed on *A. fabae* and *M. persicae* but on *M. donacis* 22% of larvae have survived. At 20 and 25°C larval survival was high, ranging from 74.1% to 85.7% (Figure 1).

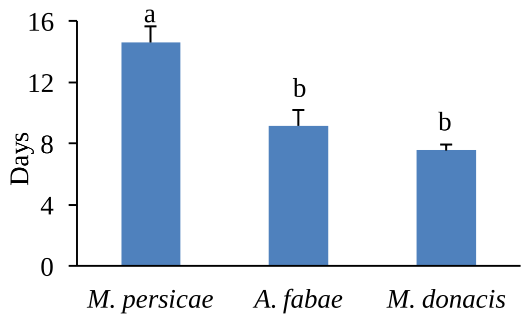

Figure 2: Pre-oviposition time (Days ± SE) of *S. subvillosus* females at 25°C fed with 3 different preys (different letters mean significant differences).

Pre-oviposition time was significantly longer when females fed on *M. persicae* and no significant differences were found for *A. fabae* and *M. donacis* (ANOVA, F = 17.95, df= 2, $p \leq 0.0001$) (Figure 2).

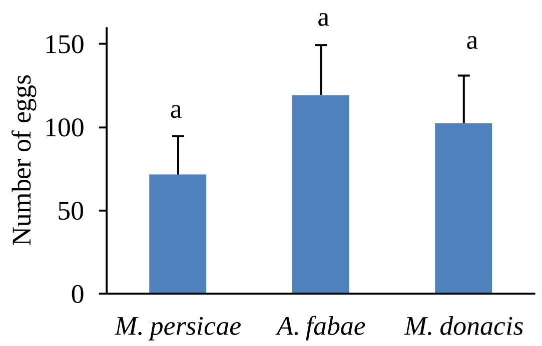

Figure 3: Fecundity (mean ± SE) of *S.subvillosus* at 25°C fed with *M. persicae, A. fabae* and *M. donacis.*

Fecundity did no significantly differ with prey species (ANOVA, F= 1.59, df = 2, p = 0.224). Females of *S. subvillosus* laid on average a minimum of 71 eggs and a maximum of 119 eggs when fed on *M. persicae* and *A. fabae*, respectively. Despite the absence of significant differences in the mean number of eggs laid, we found that the proportion of ovipositing females was not the same *e.g*. 90%, 70% and 50% for *M. donacis A. fabae* and *M. persicae*, respectively.

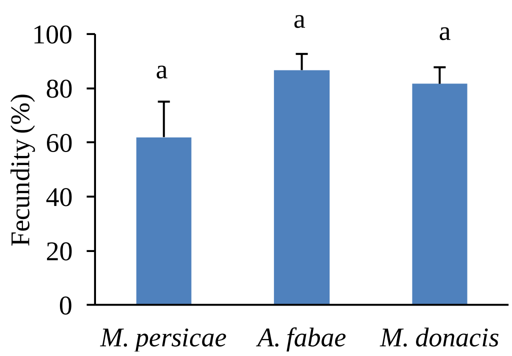

Figure 4: Percentage of fertility (mean ± SE) *S. subvillosus* at 25°C.

The fertility of *S. subvillosus* eggs was high with prey *A. fabae* (86.7%) and lower on *M. persicae* (62%) but no significant differences were observed. (Kruskal-Wallis $\chi 2$ = 5.03, df = 2, p= 0.081) (Figure 4).

3.3 Voracity and nutritional physiology of the 4th larval stage of *S. subvillosus* fed on *A. fabae*

Table VI: Feeding parameters of the 4^{th} larval stage of *S. subvillosus.*

Feeding parameters	**Mean ± SE**
Larval weight initial (mg ± SE)	1.69 ± 0.08
Larval weight final (mg ± SE)	2.40 ± 0.06
Mean weight gain (mg ± SE)	0.71 ± 0.11
Voracity (number of aphids eaten ± SE)	10.5 ± 0.78
Voracity (mg of biomass ingested ± SE)	2.08 ± 0.29
Relative growth rate (%)	47.7 ± 1.93
Conversion efficiency (%)	21.2 ± 9.42

The mean weight gain by a 12 hours 4^{th} larval instar was 0.71 mg, corresponding roughly to 50% of their initial body weight (1.69 mg) (Table VI). The 2.08 mg of aphid biomass ingested, correspond to approximately 10.5 aphids eaten, is converted to larvae biomass on a rate of 21% (Table VI).

4. DISCUSSION

Environmental temperature is one of the most important factors determining physiological process on poikilothermic species with direct implications on development rate and survival (Honěk and Kocourek, 1990; Chapman, 1998). Several authors have shown that food quality also affects the development rate and survival (Blackman, 1967; Obrycki and Orr, 1990; Kalushkov, 1998; Kalushkov and Hodek, 2001; Işikber and Copland, 2002), as well reproductive performance, such as fecundity and fertility (Blackman, 1967; Niijima et al., 1986; Hodek, 1993; Michaud, 2005).

As we expected, developmental time of the immature stages decreased with increasing temperature. Our results also indicated that developmental rate and survival were concomitantly temperature and prey-dependent, which is consistent with previous studies (Atlihan et al., 1999; Satar and Uygun 2012). Survival rate was higher when larvae fed on *M. donacis*. Developmental rate decrease at 20°C and 30ºC feeding on *A. fabae* and *M. donacis*, respectively. The most paradigmatic example was the incapacity of *S. subvillosus* to complete the development at 15ºC fed with *M. persicae* and *A. fabae*. The importance of prey quality on developmental rate could be revealed by differences by the sum of effective temperatures (SET). In our experiment, *S. subvillosus* required 196.3°D, but the same species requires 329°D and 286°D to develop from egg to adult when fed *H. pruni* and *A. gossypii*, respectively (Atlihan and Chi 2008; Satar and Uygun, 2012).

Growth and development of insect species occur only across specific range of temperature (Nedvěd and Honěk, 2012) and thus the thermal parameters as lower developmental threshold and thermal constant are useful indicators to predict its potential distribution and abundance (Messenger, 1970). The LDT of *S. subvillosus* was estimated as 11.7°C for total immature stages development fed on *M. donacis*. Satar and Uygun (2012) determined 10.26°C from egg to adult of *S. subvillosus* fed on *A. gossypii*. These values are quite similar to other *Scymnus* species (*S. hofmanii*: 10.1°C, *Scymnus frontalis*: 11.7°C fed on *Diuraphis noxia* (Mordvilko); *S. syriacus*: 11.3°C; *S. levaillanti*: 11.7°C fed *A. gossypii*; (Kawauchi, 1985; Naranjo et al., 1990; Emami et al. 1998; Uygun and Atlihan, 2000). Atlihan and Chi (2008) estimated a lower LDT for *S. subvillosus* fed on *H. pruni*; 7.1°C. Some of the differences can be partially explained by the differences on regional acclimatization. Satar and Uygun (2012) collected their *S. subvillosus* in the Mediterranean region where mild winters occur whereas Atlihan and Chi (2008) collected individuals in Turkey (Van region) where there is a harsh and long winter condition. The combination of a high LDT and a low SET guarantees a fast development at high temperatures, in contrast to cold adapted species whose LDT is low and SET high (Trudgill, 1995).

Egg production requires nutritional intake beyond a maintenance level, and thus high quality food is important for supporting reproductive capacity (Seagraves, 2009). Our results showed that *M. donacis* and *A. fabae* are equally suitable preys and on the other hand *M. persicae* leads to worse biological performance. In the Scymninae the pre-oviposition period varies with temperature and prey suitability even among closely related species (Naranjo et al., 1990; Uygun and Atlihan, 2000; Nedvěd and Honěk, 2012).

From an ecophysiological point of view, the three prey species tested on our study can be considered as essential foods. Indeed, under the light of Hodek, van Emden and Honěk (2012) criteria, the consumption of those prey species provided growth and development of larvae and reproduction by adults. Other essential prey for *S. subvillosus* includes *Aphis sambuci*, *Hyalopterus pruni*, and *Aphis gossypi* are reported as essential foods for *S. subvillosus* (Klausnitzer, 1992; Atlihan and Chi, 2008; Atlihan and Guldal, 2009; Satar and Uygun, 2012). However, essential foods, show varying degrees of favorability, enabling different developmental rates, fecundity, and survival (Hodek, 1993; Hodek and Honek, 1996; Kalushkov, 1998; Kalushkov and Hodek, 2004; Soares et al., 2005; Cabral et al., 2006). Given that, we consider that *A. fabae* and *M. donacis* are more suitable food resources than *M. persicae*. Those results could reflect the co-ocurrence between *S. subvillosus* and the *A. fabae* and *M. donacis* typically found in Azorean costal habitats, contrarily to *M. persicae*. Indeed according to Rana, Dixon and Jarosik 2002, the prey specialization increase fitness on ladybirds.

Body-weight increase following predation is a good indicator of energy intake and associated costs (Frazer, 1988). The mean daily weight gain by the 4^{th} larval stage of *S. subvillosus* during 24h was 0.71 mg after a daily consumption of 10.5 aphids, which correspond to a 2.08 mg of biomass ingested. The ladybird handled around 6.3 mg o biomass, but has ingested only 2.08 mg, which means that after spending energy on basal metabolism, excretion and others activities, she assimilates only about 33% of weight of their preys. The larvae increased for doubled their size, 21% of the biomass ingested (average of 0.44 mg) is converted to larvae biomass so close 79% was spent on basal metabolism and metabolic activities of the larvae. The relative growth rate was approximately of 48%. Borges (2008), obtained similar value for conversion efficiency for *S. nubilus*, was 26%. Our results show that the high mortality of the prey may be because the ladybird satiates quickly and eats little of each aphid and spends much energy looking for a better or new prey.

5. GENERAL CONCLUSIONS

Our results clearly show the concomitant effect of temperature and prey on developmental time and survival rate of immature stage of *S. subvillosus*. Least evident were the differences concerning fecundity and fertility.

This study indicates that *M. persicae, A. fabae* and *M. donacis* are essential prey species for *S. subvillosus*. However diets differ in suitability, with *M. donacis* and *A. fabae* being better than *M. persicae* but equally suitable. The higher survival rate was observed with *M. donacis* at 20 ºC and 25ºC.

According to our results and published data, optimal temperature for population growth of *S. subvillosus* and for its mass production could be somewhere between 20 to 25ºC.

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