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

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Abstract

Among insects different defence mechanisms evolved against predation and parasitism. Defences can be chemical, behavioural and/or morphological. The larvae of the small aphidophagous coccinellid *Scymnus nubilus* are covered by a wax layer that might act as a defensive mechanism against ants and other predators. However, the degree of protection conferred by waxes in *Scymnus* larvae is not very well known.

The objective of the study was to evaluate some of the costs and benefits of wax production in *S. nubilus*. According to life history evolution theory, increased performances in one trait entail a decrease in another trait. If wax production is metabolically costly to larvae, we expect some costs on developmental time, growth or future reproductive potential. Because potential trade-offs are only evident when the organism are under stressful conditions, we evaluated the consequences of wax production to developmental time, alimentary physiology and potential reproductive effort in larvae, either wax and waxless (artificially removed).

From a physiological point of view we expect waxless larvae will display higher prey consumption in order to meet metabolic requirements for growth and wax production. To balance these costs and energetic allocation we propose that waxes might act as a defensive mechanism against an intraguild predator, the lacewing *Chrysoperla agilis*.

Our results showed that there were differences between the biological parameters of wax and waxless larvae, specifically, in the larval growth. Contrary to our prediction, prey consumption of wax and waxless did not vary significantly although relative weight gain was higher in wax larvae. This indicates that in order to produce waxes, resources need to be re-allocated and growth is reduced. Waxes reduced intraguild predation as predicted.

Key-words: *Scymnus nubilus*, morphological defence, cost-benefits of waxes, alimentary physiology, trade-offs, intraguild predation

Resumo

Diferentes mecanismos de defesa contra a predração e parasitismo evoluíram entre os insectos. Estes podem ser químicos, comportamentais ou estruturais. As larvas do pequeno coccinelídeo *Scymnus nubilus* estão cobertas por uma camada de ceras que podem actuar como um mecanismo defensivo contra formigas e outros predadores. No entanto, o grau de protecção conferido pelas ceras às larvas de *Scymnus* não é bem conhecido.

O objectivo deste estudo foi avaliar alguns dos custos e benefícios da produção de ceras nos *S. nubilus*. De acordo com a teoria da evolução das histórias de vida, um maior desempenho de um traço específico implica um decréscimo noutro traço. Se a produção das ceras é metabolicamente dispendiosa para as larvas, esperamos alguns custos no tempo de desenvolvimento, crescimento e futuro potencial reprodutor. Devido ao facto de potenciais *trade-offs* serem apenas evidentes quando um organismo está sobre condições de stress, avaliamos as consequências da produção de ceras no tempo de desenvolvimento, fisiologia alimentar e potencial reprodutor nas larvas com e sem ceras (removidas artificialmente). Do ponto de vista fisiológico, espera-se que a remoção das ceras das larvas induzirá um consumo maior de presas de modo a que se cumpram os requerimentos metabólicos necessários para o crescimento e produção de ceras. A equilibrar estes custos e a alocação energética, propomos que as ceras actuarão como um sistema defensivo contra a *C. agilis* um predador intraguilde.

Os resultados demonstram que existiram diferenças entre os parâmetros biológicos das larvas com e sem ceras, mais especificamente no crescimento larval. Ao contrário do previsto, o consumo de presas das larvas com e sem ceras não variou, embora o peso ganho tenha sido maior nas primeiras. Isto é uma indicação que para produzir as ceras há necessidade de distribuir os recursos e o crescimento é reduzido. As ceras reduziram a predação intraguilde, tal como esperado.

Palavras-chave: *Scymnus nubilus*, defesa morfológica, Custos-benefícios das ceras, fisiologia alimentar, trade-offs, predação intraguilde

Introduction

Among insects different defence mechanisms evolved against predation and parasitism. Defences can be chemical, behavioural and/or morphological. The majority of coccinelids are protected by chemical defences (Sloggett *et al*., 2011). Reflex bleeding is an example of a chemical defence in which coccinelid larvae and adults emit droplets of haemolymph that are distasteful and toxic (Hemptinne & Dixon, 2000). In ant-tending aphid colonies, a common behaviour in order to avoid ant attacks is to cover the larvae body with dead aphid such as in the case of *Aphidoletes aphidimyza* Rondani, or the green lacewing *Mallada desjardinsi* Navas (Lucas, 2005; Hayashi & Nomura, 2011). At the morphological level, the production of a wax cover, such as the ones found on larvae of *Scymnus* sp., act as a protection against predation and ant aggression (Völkl & Vohland, 1996).

Scymnus nubilus Mulsant is a small aphidophagous ladybird frequent in the Mediterranean region and also present in the Azores islands (Soares *et al*., 2003; Soares *et al*., 2005; Soares, 2010). Compared to large-sized ladybirds *Scymnus* spp. may have weaker competitive abilities. Nevertheless, by surviving with low prey densities *Scymnus* are able to exploit aphid colonies at an earlier stage than larger ladybirds (Agarwala & Yasuda, 2001). Larvae of this species are covered by a thick wax layer produced by dorsal epidermal cells (Agarwala & Yasuda, 2001). This morphological defensive mechanism is found in several insect orders and has been associated with protection against the attack of predators or parasitoids (Eisner, 1994; Takabayashi & Takahashi, 1993; Völk & Vohland, 1996). This morphological defense mechanism balances their apparent absence of chemical defenses. Other functions have been attributed to the waxes such as protection against UV radiation and reduction of water transpiration (Agarwala & Yasuda, 2001). Their sticky nature not only makes the larvae difficult to bite into but also serves as an entangling agent. In the case of several *Scymnus* species these have been observed physically clogging the mouthparts of aggressive ants (Agarwala & Yasuda, 2001; Schwartzberg *et al*., 2010; Völk & Vohland, 1996). There are evidences that the larval wax also acts as mimetic mechanism. This has been found in some species that feed on mealy aphids attended by ants. In this case the larvae are not recognized as a threat (Majerus *et al*., 2007). Recently Schwartzberg *et al*. (2010) tested if the wax structures of *S. louisianae* J. Chapin acted as a defense mechanism against *Lasius neoniger* Emery. The results showed that the wax attenuated ant aggression towards the ladybeetle when compared to denuded larvae. Evolutionary, the success of Coccinellidae can be associated to the exploration of ant-tended insects as a food source which favored the development of defenses mechanisms such as larval waxes (Seago, *et al*., 2011).

S. nubilus does not occur isolated in their natural habitat. They belong to aphidophagous guilds and thus is subjected to an array of biotic interactions. Intraguild predation (IGP) is a type of interaction that occurs between species that share a common resource. It is an extreme form of competition that can have various ecological effects such as the alteration of the distribution, abundance and evolution of the species involved (Lucas *et al*., 1998; Félix & Soares, 2004). Besides the protection given by the waxes against ant attacks, any additional protection against IGpredators would be beneficial to the ladybeetle. Not much is known about the defensive potential of the *Scymnus* spp. waxes against several IGpredators. Völk & Vohland (1996) studied this subject regarding the carabid beetle *Platynus dorsalis* Pontoppidan and Agarwala & Yasuda (2001) with the syrphid larvae *Eupeodes freguens* Matsumura.

The objective of this study was to evaluate the costs and benefits of wax production *in S. nubilus*. It is expected that the production of waxes will be metabolically costly. According to life history evolution theory, increased performances in one trait entail a decrease in another trait. Considering wax production is, in metabolic terms, costly (Eisner, 1994) we expect some detrimental effects on developmental time, size or reproductive potential. Under a physiological point of view we expect that removing the waxes from larvae will induce higher prey consumption in order to meet metabolic requirements for growth and wax production. We also predict that wax cover confer protection against IGpredation.

2. Material & Methods

2.1. *Field work*

In a previous study the occurrence of *S. nubilus* waxless larvae was observed in the laboratory (Borges, pers. comm.). It was estimated that 10% of larvae did not developed waxes during the larval development. This is not reported in the literature.

In order to seek for the co-occurrence of wax and waxless *Scymnus* spp. larvae, adults and potential IGpredators, several aphid colonies were followed during a field work undertaken on the island of São Miguel, Azores (Portugal) in two different habitats; (i) corn fields and (ii) a coastal area (Santana). In 2011 corn fields were surveyed from June to November and the coastal area from June to August. In 2010 an exploratory field work was carried out to search for the co-occurrence of wax and waxless larvae. Considering the homogeneity of the corn field habitats, aphid colonies were randomly selected and checked once a week. For each plant there was a sampling time of three minutes. For the two years, the total corn plants surveyed was in a total of 44. In the coastal area (Santana) due to the heterogeneity of the habitat, the sampling method was adapted from Borges *et al*. (2011). Twenty sampling plots were disposed along two linear transects. Each sampling plot was a circular area of 5 m in diameter and separated from each other by 10 m. In each plot aphid colonies were selected and marked on different host plants. From these, a sample of aphids was collected and preserved on 70% ethanol. These samples were sent for identification to a specialist. Twice a week the number and size of aphid colonies as well as the presence of *Scymnus* spp. or potential IGpredators was registered.

2.2. *Insect rearing*

Larvae and adults of *S. nubilus* collected from corn fields were kept and reared in 2 L plastic cages at approximately 20±1ºC, 75±5% RH and a light regime of 16L:8D. A mixed diet of *Myzus persicae* (Sulzer) and *Aphis fabae* Scopoli reared on broad bean plants (*Vicia faba* L. Major) was provided *ad libitum*. Every two days the infested plants were replaced by new ones. Once a week, larvae and adults were transferred into new rearing cages. The eggs were separated into another 2 L plastic cage until larval eclosion.

Chrysoperla agilis Henry, Brooks, Duelli & Johnson (Neuroptera: Chrysopidae) is a common lacewing species found in the Azores (Borges *et al*., 2010) and a potential IGpredator for *S. nubilus*. They were reared in net cages at 20º±1ºC, 75±5% RH and a light regime of 16L:8D. Adults were fed with a mixture of honey, yeast and pollen grains dissolved in water. After the emergence of larvae, aphids were provided in broad bean plants until adult emergence. Once a week, old and new adults were transferred into a new cage.

2.3. *Development time, adult body weight and ovarioles number*

This experiment had two treatments: wax and waxless. During the first, larval wax was never removed whereas on the second one the waxes were removed artificially with a brush once a day until pupation.

To determine the existence of potential trade-offs in wax and waxless individuals the development time, adult body weight and ovarioles number of *S. nubilus* was determined. Twenty-five couples were separated into 5 cm diameter x 2 cm height plastic boxes (5 couples per box). They were allowed to reproduce for 24 hours in order to obtain fresh eggs. The adults were then removed and the eggs were observed twice a day until eclosion of larvae. New eclosed larvae were weighed and individually kept in 3 cm diameter x 1 cm height plastic boxes. Larval stage was checked twice a day at 9am and 4pm for the presence of exuviae. The development time from egg to adult was determined.

The adults were weighed 12h after emergence and sex was determined according to the coloration of the head: yellowish for males and predominantly dark brown for females (Raimundo & Alves, 1986). After 5 days, *S. nubilus* females were dissected in order to determine the number of ovarioles. All weighings were made in a Sartorius ultramicrobalance SE 2.

Individual fitness (r) was adapted from Sadeghi & Gilbert (1999, 2000):

$$R=[\ln(m.V)]/D$$

where ln = natural logarithm, m = survival (1 or 0), V = potential fecundity and D = development time. Fitness was only calculated for surviving females where D is the value of their development time. Since this is the case, the value given for the variable m was always 1. The value of potential fecundity was given by the ovarioles number of each female.

2.4. *Larval alimentary physiology*

Larval alimentary physiology tests were performed with 12 hours old 4th instar larvae obtained as described previously. Thirty larvae (15 wax and 15 waxless) were isolated and subjected to 12 hours of starvation before conducting the tests. For the wax treatment, the waxes of the larvae were not removed during their development. For the waxless treatment the waxes were removed daily since larval eclosion. Larvae were provided with 40 *M. persicae* adults for 24 hours. Both, larvae and aphid biomass were determined, before and after the test. In order to determine the weight loss due to dehydration, 40 *M. persicae* were confined in 3 cm plastic boxes 3cm diameter x 1 cm during 24h. They were weighed before and after the referred period.

Biomass consumption (BC) and weight gain (WG), expressed in absolute and relative terms, were calculated. Conversion efficiency (CE%) was also calculated (adapted from Borges, 2008):

$$BC = PWi - PWf - PWd$$

$$BClw = \frac{PWi - PWf - PWd}{LWi} \times 100$$

$$WG = LWf - LWi$$

$$WGlw = \frac{LWf - LWi}{LWi} \times 100$$

$$CE\ \% = \frac{LWf - LWi}{BC} \times 100$$

where PWi is the prey initial weight, PWf the final prey weight and PWd the aphid weight loss due to dehydration. LWi and LWf are the larval initial and final weights respectively.

2.5. *Intraguild Predation*

To test if the presence of waxes provided a defensive morphological structure against IGpredation two treatments were made: wax and waxless. Waxes were not removed on the wax treatment and for waxless these were only removed before conducting the test.

The developmental stages of the predators were selected according to weight so this would not be a factor conditioning the intraguild predation direction. For *S. nubilus* (0.91±0.09 mg) and *C. agilis* (0.93±0.07 mg) (t-test: t = 0.186, p>0.05). The selected instar for *S. nubilus* was the fourth and the second for *C. agilis*.

In order to obtain the larval stages, eggs of *S. nubilus* and *C. agilis* and were separated daily from rearing cages and transferred to 3 cm x 1 cm plastic boxes. Their development was checked twice a day. After molting into the selected instars, larvae were fed for 12h followed by a period of 24h of starvation to increase their hunger.

Before the experiment, one *S. nubilus* and one *C. agilis* larvae were weighed and placed in a petri dish for 24h. After this period the predators were examined under a stereoscope to determine survival of the predators (n=15).

For each treatment, a test control to estimated survival rate was performed. Fifteen larvae of each species were isolated in a petri dish for 24h.

IGP levels were estimated from the rates of predation for *S. nubilus* (RPsn) and *C. agilis* (RPca), which were calculated as follows (Félix & Soares, 2004):

$$RPsn = (P(ca,sn)\ SRca / N)\ 100$$

$$RPca = (P(sn,ca)\ SRsn / N)\ 100$$

where “P(ca,sn)” = number of individuals of *C. agilis* killed, “P(sn,ca)” number of individuals of *S. nubilus* killed, “SRca” survival rate of *C. agilis* in control, “SRsn” survival rate of *S. nubilus* in control and “N” number of replicates. Survival rates were calculated from the control tests.

The symmetry index of Lucas et al. (1998) was used. This index expresses the number of replicates in which a given predator was eaten over the total number of replicates where IGP ocurred.

2.6. *Statistical analysis*

For the trade-offs experiment, the t-test was used to compare the time of development, adult weight, number of ovarioles and fitness of wax and waxless larvae. The t-test was also used to compare wax and waxless prey consumption (either in absolute and relative terms), weight gain (either in absolute and relative terms) and conversion efficiency. Data normality and variance homogeneity were assessed by the Kolmogorov-Smirnov and Levene´s tests, respectively. All data was analysed with SPSS (2010) statistical package version 19.

Concerning the IGP test, the symmetry indices for each combination were compared with the theoretical index of 50% corresponding to a symmetrical interaction, using a Chi-square test (SPSS, 2010). The strength of the IGP between the two species for a given combination was assessed using the χ^2 and was considered (i) symmetrical, when the χ^2 value was not significant, which indicated that the rate of predation of the two species was similar, (ii) asymmetrical, when the χ^2 value was significant and (iii) not significant asymmetrical, when the χ^2 value was not significant but the rate of predation of the two species differed.

3. Results

3.1. *Field work*

In 2010, in corn field, 29 *Scymnus* spp. were observed from a total of 24 prey colonies. From these, 75.9% were larvae and 24.1% adults. The only IGpredator observed was *Aphidoletes* sp (9 larvae). In 2011 the number of ladybirds was higher with 47 *Scymnus*, from 21 aphid colonies (59.6% larvae and 40.4% adults). One hundred and twelve *Aphidoletes* sp. were observed throughout the colonies and one lacewing larva on an aphid colony. Mummies of aphids were also observed.

The vegetation cover of the coastal area is presented in Table 1. On the coastal area, a total of 53 *Scymnus* spp. were observed from 97 prey colonies. Only 11.3% of these were adults. The highest number of *Scymnus* larvae was found on *Arundo donax* L. and *Tropaeolum majus* L.. Four plant species did not have any ladybird larvae or co-occurring predators (Table 1). *Aphidoletes* sp.

was again the most abundant IGpredator with a total of 116 individuals versus two syrphids and one coccinelid larvae. These last IGpredators were observed individually on a colony of *Aphis oenotherae* and *Aphis fabae,* respectively (Table 1).

Naturally occurring waxless larvae were not observed during the field work.

Table 1- List of the plant species found in Santana and their corresponding aphids, number of *S. nubilus* larvae observed and co-occuring predators.

Host Plant	Aphid species	*Scymnus* sp.		Main co-occurring predators and ants			
		Larvae	Adults	Ants	*Aphidoletes* sp.	Syrphid	Coccinelid
Arundo donax L.	*Melanaphis donacis* Passerini	13	0	+	+	-	-
Chrysanthemum segetum L.	*Aphis* sp.	1	1	+	-	-	-
Daucus carota L.	*Aphis fabae* Scopoli	5	1	+	+	-	-
Galactites tomentosa Moench	*Brachycaudus cardui* L.	0	0	+	-	-	-
Malva pseudolavatera Webb & Berthel	*Aphis fabae* Scopoli	3	2	+	+	-	-
Mentha suaveolens Ehrh.	*Aphis ruborum* Börner	0	0	+	+	-	-
Oenothera biennis L.	*Aphis oenotherae* Oestlund	2	1	+	+	+	+
Opuntia stricta (Haw.) Haw.	*Aphis fabae* Scopoli	0	0	+	-	-	-
Parietaria judaica L.	*Aphis fabae* Scopoli	7	0	+	+	-	-
Rubus ulmifolius Schott	*Aphis ruborum* Börner	1	0	+	+	-	-
Rumex aquaticus L.	*Aphis fabae* Scopoli	0	0	+	-	+	-
Solanum mauritianum Scop.	*Aphis fabae* Scopoli	0	1	+	-	-	-
Sonchus asper (L.) Hill	*Uroleucon sonchi* L.	3	0	+	-	-	-
Tropaeolum majus L.	*Aphis fabae* Scopoli	12	0	+	+	-	-

3.2. *Development time, adult body weight and ovarioles number*

Male wax larvae developed significantly faster than the waxless larvae (t-test: t = -3.195, p<0.005) but for females no significant difference was found (t-test: t = -0.407, p>0.05) (Figure 1).

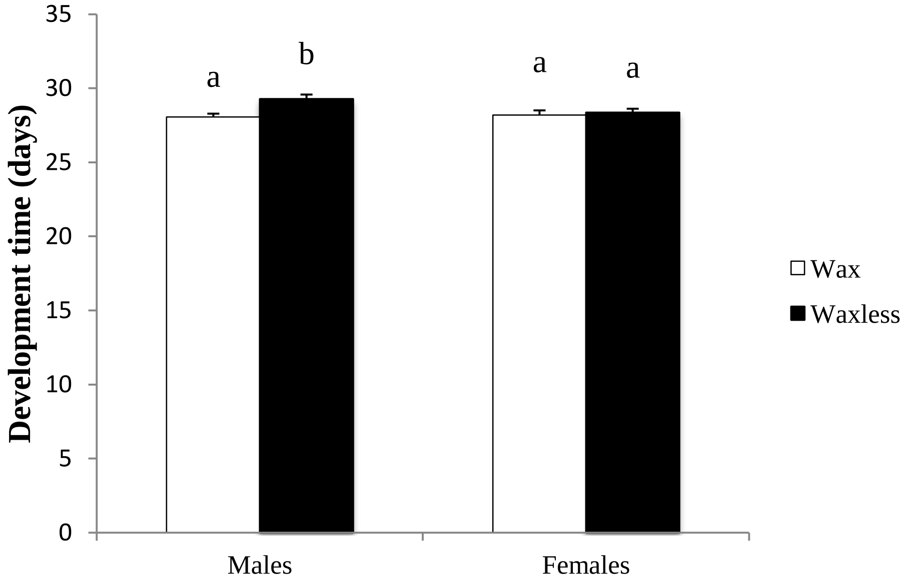

Figure 1- Development time (days±SE) from egg to adult for males and females of wax and waxless *S. nubilus* (for each sex different letters mean significant differences).

There was a significant difference between both treatments for the adult weight at emergence (Figure 2). The male (t-test: t=8.036, p<0.001) and female (t-test: t=4.766, p<0.001) adult weights were significantly higher when no waxes were removed during the development of the larvae.

There was no significant difference between treatments for the ovarioles number (t-test: t=-1.326, p>0.05) and fitness (t-test: t = -0.877, p>0.05) (Figure 3).

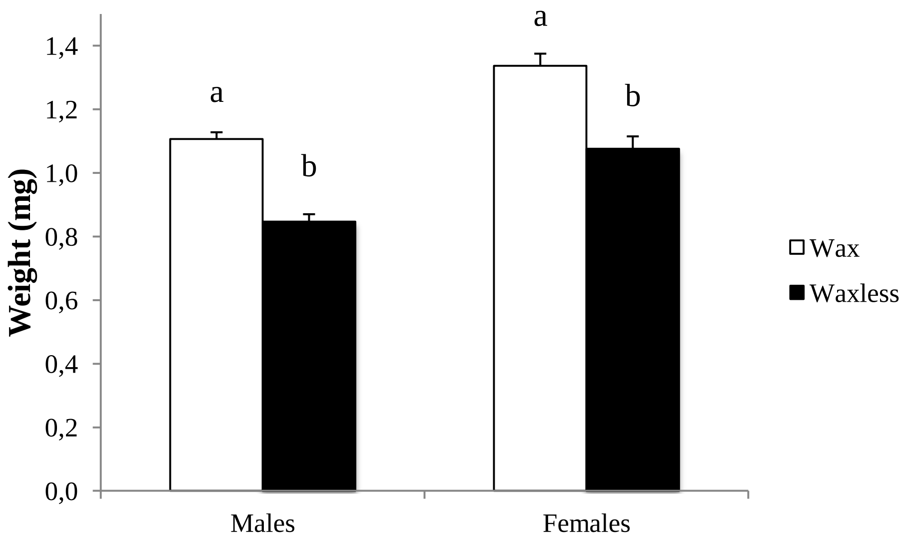

Figure 2- Mean weight (mean±SE) of *S. nubilus* adults at emergence.

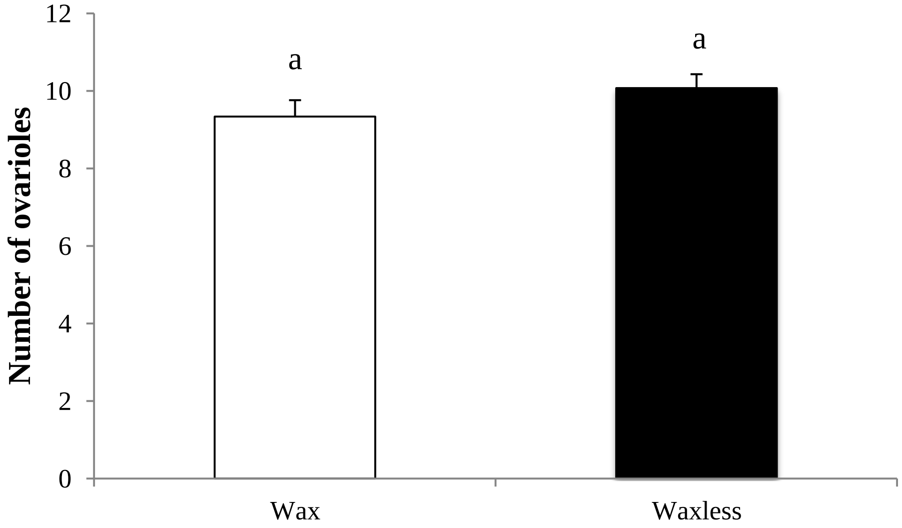

Figure 3- Mean number (mean±SE) of ovarioles for the wax and waxless *S. nubilus* female adults.

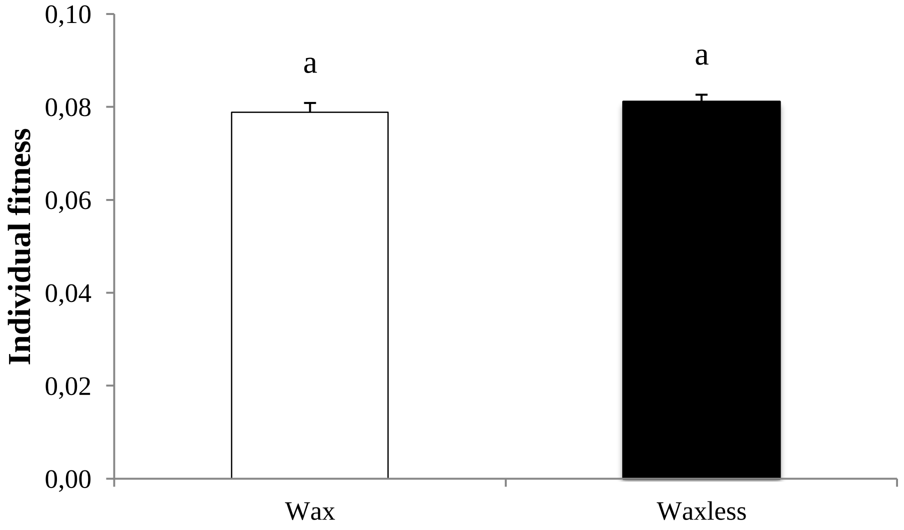

Figure 4- Individual fitness of wax and waxless *S. nubilus* larvae.

3.3. *Larval alimentary physiology*

There was no significant difference on aphid consumption during 24h between the wax and waxless treatments in absolute terms (t-test: t=0.968, p>0.05) and relative terms (t-test: t=1.328, p>0.05) (Figure 5 and 6).

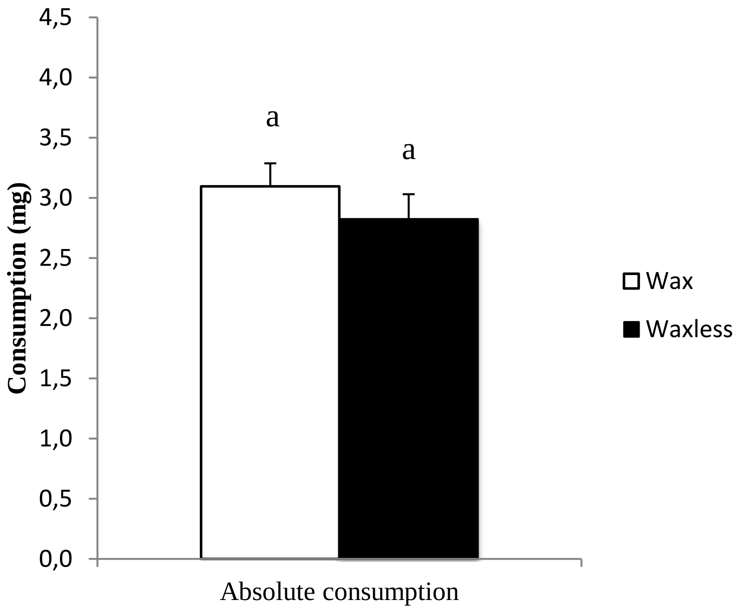

Figure 5- *S. nubilus* larvae absolute consumption (mean±SE) of aphids during 24h.

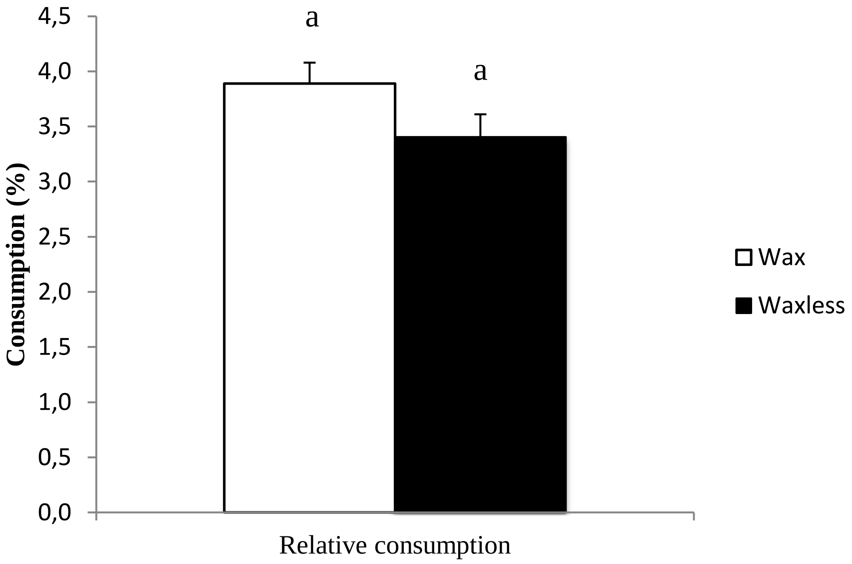

Figure 6- *S. nubilus* larvae relative consumption (mean±SE) of aphids during 24h.

The absolute weight gain (t-test: t=2.025, p=0.052) (Figure 7) and the conversion efficiency (t-test: t=1.114, p>0.05) (Figure 9) did not differ for waxy and waxless larvae. However if weight gain was expressed relatively to body weight (Figure 8) a significant difference was detected (t-test: t=2.488; p<0.05).

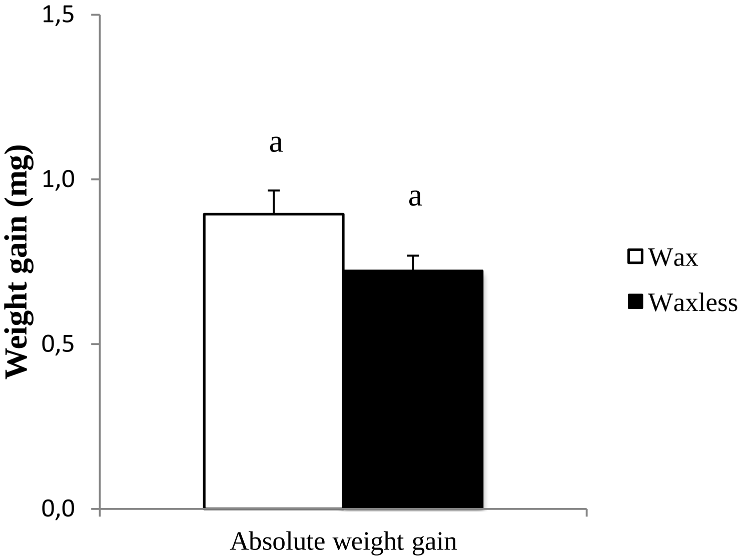

Figure 7- Absolute weight gain (mean±SE) (mg) for each of the treatments during 24 hours.

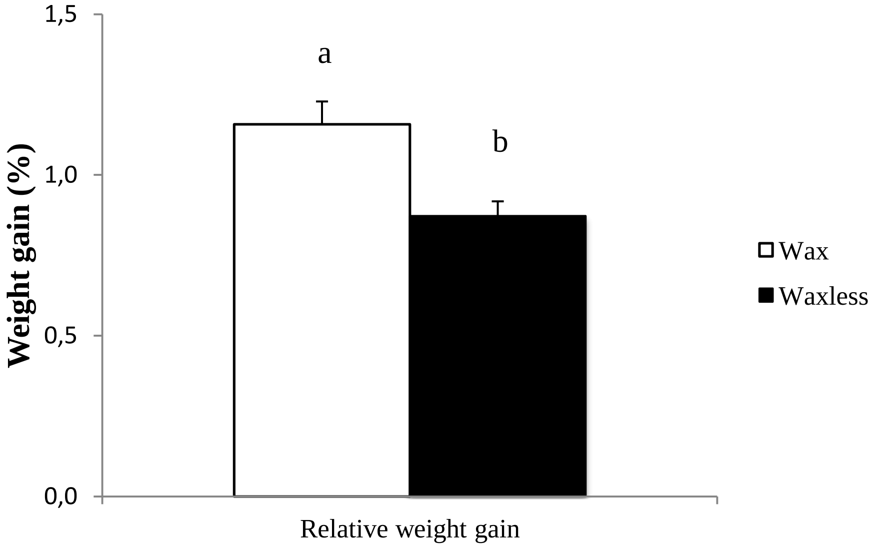

Figure 8- Relative weight gain (mean±SE) (mg) for each of the treatments during 24 hours.

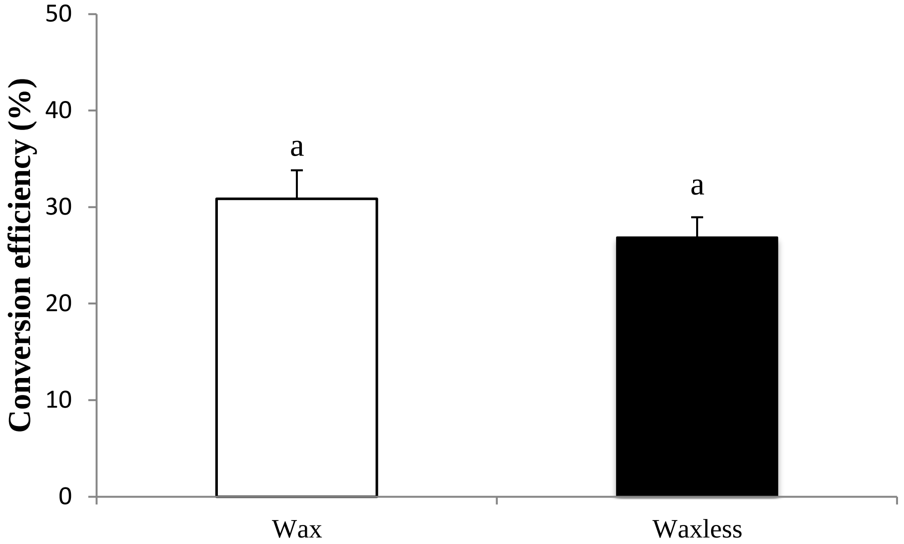

Figure 9- Feeding efficiency (mean±SE) of the larval treatments.

3.4. *Intraguild predation*

The weight of *S. nubilus* and *C. agilis* did not show statistical differences for the wax (t-test: t=0.186, p>0.05) and waxless treatment (t-test: t=-1.267, p>0.05).

All waxless larvae were preyed by *C. agilis* whereas the rate of predation decreased to 79% in the combination with the wax *S. nubilus* larvae. In one replicate of the wax treatment both larvae survived. There was a significant asymmetrical IGP in the combination *C. agilis* versus waxless *S. nubilus* (χ^2=0.001, df=1, p<0.05). No significant asymmetry was observed for the other treatment (χ^2=0.037, df=1, p>0.05).

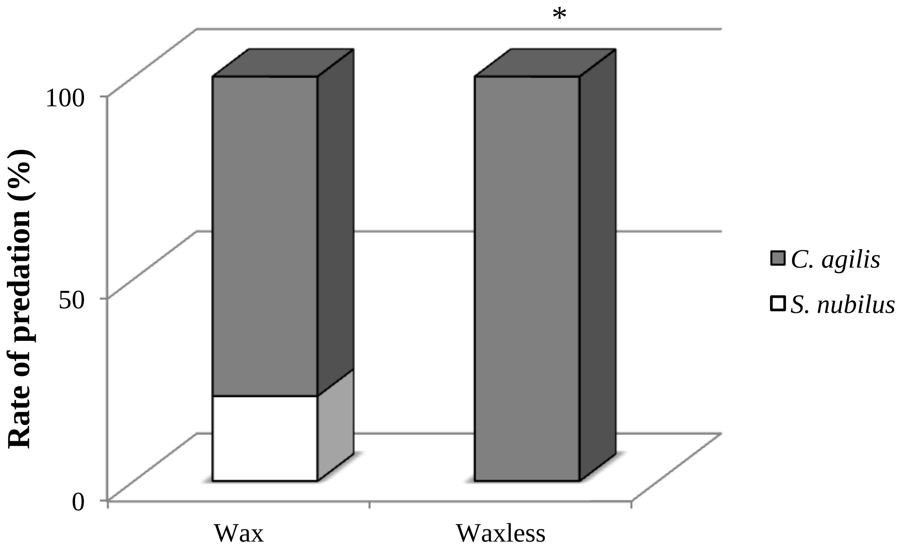

Figure 6- Rate of predation (RP) of the fourth instar of *S. nubilus* and second instar of *C. agilis* for the two ladybird wax and waxless treatments. * indicates significant asymmetrical IGP.

Discussion

Contrarily to our expectations, no natural occurring waxless larvae were found in the field work. These expectations were due to the fact that previous observations made in the laboratory conditions revealed that the some larvae of *S. nubilus* did not produce wax during their development (Borges, pers. comm.). The absence of waxless larvae could be associated either to an expected susceptibility to intraguild predation, as our results seem to indicate, or a low probability to find them due to low frequency.

As hypothesized, the stress of removing the wax cover in larvae resulted in some differences in the biological parameters and emphasized a trade-off between the investment on larvae wax production and adult biomass, Indeed some trade-offs are only evident when the organism is under stressful conditions (Stearns, 2004). The daily artificial removal of waxes in the larvae resulted in smaller adults. Nevertheless females of the wax and waxless treatments showed the same rate of development and reproductive investment in gonads. There was, therefore, a reduction of investment in soma in order to maintain fitness. Sato *et al*. (2009) showed that *Harmonia axyridis* Pallas larvae forced to reflex bleed originated lighter adults than adults developed from larvae where no stimulation was given.

Contrary to what we predicted, waxless larvae did not increase prey consumption, in both absolute and relative terms. Absolute weight gain was also not statistically different between treatments. However the significance of the test is close to the 0.05 limit of significance. This result is not completely unexpected because wax larvae were heavier than waxless ones. Once the initial larval weight was factored out, statistical differences arose. Conversion efficiency was similar for both treatments. This seems to indicate that there is a displacement of resource allocation caused by the wax removal. While the consumption and conversion efficiency into biomass did not differ, there is indication that there was an investment made in the wax production over growth, from the waxless larvae. The organisms must allocate the food resources between metabolism, growth and reproduction. If the energy acquired only covers metabolic costs then the larvae will disinvest in body growth (Hodek & Honěk, 1996). The results suggest that removing the waxes will entail a large resource allocation to regeneration of the cottony cover, leaving less matter and energy to devote to body growth. Fourth instar larvae have greater metabolic costs due to the process of preparation for pupation (Hodek & Honěk, 1996). Although the larval alimentary physiology was not tested for all instars, it may be that the fourth instar is more vulnerable to the induced stress of removing the waxes. Indeed, the initial body weight of the fourth instar larva from wax and waxless treatments was similar (t-test: t=-0.537, p>0.05).

Concerning to IGP, as we expected, the larval wax cover seems to act as an intraguild defense mechanism against *C. agilis* although to a small extent since the rate of predation of *C. agilis* was 79%. Nevertheless this is an indication of their defensive potential against an IGpredator such as the lacewing that has greater aggressiveness (Lucas *et al*., 1998). Even though wax-producing coccinellid predators seem to have evolved to exploit ant-tended soft body sternorrhynchan insects as a food source (Seago *et al*., 2011), they may have a secondary function as a defensive mechanism against other intraguild predators. Völkl & Vholand (1996) demonstrated that they effectively enhanced *Scymnus interruptus* Goeze survival against the carabid beetle *Platynus dorsalis* Pontopiddan. Agarwala & Yasuda (2001) found that *Scymnus posticalis* Sicard waxes were also effective against the attack of syrphids although the protection was not absolute. Besides the small size of *S. nubilus* that allows them to forage in areas of low prey density and the ability to prey on ant-tending aphid colonies (Agarwala &Yasuda, 2001) at a time when bigger predators have not yet been established (Schwartzberg *et al*., 2011), the waxes also confer some degree of protection against IGpredators that may explore the same resources spatially and temporally.

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