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Diet Effect on *Psacothea hilaris hilaris* (Coleoptera: Cerambycidae) Performance under Laboratory Conditions

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***Abstract* – As both climate and food can affect insect development, in order to deep the knowledge on *Psacothea hilaris hilaris* (Pascoe) (Coleoptera, Cerambycidae, Lamiinae) biology, four treatments were tested to evaluate the effects of diet composition and temperature on some biological traits of this longicorn beetle. *P. h. hilaris* survival, pre-imaginal developmental traits, adult size, sex-ratio, longevity, fecundity and duration of the oviposition period have been examined in order to evaluate the direct influence of the diet on larvae as well as the effect of pre-imaginal feeding on adult performances. Treatments investigating the maximum rearing temperature and the diet with the lowest content of mulberry showed the lowest rate of larval survival and adult longevity. Despite no variation in adult body size was estimated in relation to preimaginal rearing condition, significant differences were observed in adult performances: fecundity and oviposition behaviour were significantly higher at 25°C compared to 30°C, but no differences were recorded on the pre-oviposition period.**

***Keywords* – Artificial Media, Diet Quality, Exotic Species, Fecundity, Temperature Dependence.**

I. INTRODUCTION

The yellow spotted longicorn beetle *Psacothea hilaris hilaris* (Pascoe) (Coleoptera, Cerambycidae, Lamiinae), a species widely distributed in East Asia [1], has recently settled in Europe [2], [3]. It is an oligophagous pest that feeds on plants belonging to the Moraceae family, including mulberry trees (*Morus* spp.) and fig trees (*Ficus carica* L.) [4], [5], [6]. In Asia the insect is a serious pest damaging sericulture, as it seriously harms mulberry plants whose leaves are used to feed *Bombyx mori* L. [7], [8]. In Europe, where sericulture could augment due to the recent increase of the international price of silk, the insect can attack the still-existing mulberry fields or the new plantations, in addition to the those specimens used for ornamental purposes or scattered in the countryside. On the other hand, as the production of figs is important around the Mediterranean Basin, and many fig trees are grown in private gardens, the establishment of the pest in this area could become a threat for fig production. In Italy the insect, principally detected in association with fig trees cultivated in many private gardens and grown as ruderal species in undisturbed areas, is spreading in new areas as few natural enemies are found in association to this pest [3], [9].

Major damage is caused by larvae, which are xylophagous and bore tunnels in the trunks and branches of different diameters [10], [3]. After a first period of feeding under the bark, larvae tunnel into the xylem of the host tree. This results in considerable damage to the tree, which is progressively weakened, sometimes until death. The adults feed on the leaves and on the tender bark of the smallest branches and their feeding is usually of little economic importance [2].

P. h. hilaris larvae complete their development in the trunk where eggs were deposited. Therefore, larval development depends on the temperature, which varies according to the climate of the area considered, and on the nutritional value of the host, which depends on the species, the individual conditions and the season. Consequently, larvae must have adaptation mechanisms to the changes in temperature and food quality over the year. The quality of the host plant and the conditions of the host tissues can diversely affect insect biology and morphology, such as larval development in terms of length and number of molts, and diapause. Early nutrition has been shown to have important implications on adult phenotype and therefore on fitness in some species including *P. h. hilaris*[11], [10], [6], [12], [13], [14]. It is known that food quality is a determinant of the fecundity of herbivorous insects and that diverse components of host plant quality can directly affect egg production [15]. However, for many insects and also for Cerambycid it is difficult to separate the role of food from the surrounding environment as food is something they live in [16], [12],[17].

Rearing *P. h. hilaris* on an artificial diet under laboratory conditions allows standardization of nutrition and therefore comparison of environmental parameters possibly affecting its physiology. Artificial rearing of larvae will permit to overcome the nutritional variability of plant tissues according to the host and season [18]. However, as artificially reared insects need to be representative of natural populations [19], the choice of proper laboratory conditions in terms of alimentary quality and temperature is fundamental.

The aim of this work was to investigate *P. h. hilaris* preimaginal survival and development, and adult performances in relation to its larval nutritional needs under different laboratory conditions. Moreover, collecting data on the beetle survival and fecundity is essential to better understand the population dynamics as these parameters affect the rate of its increase [20], hence the study can contribute to deepen information useful for future researches in fields.

II. MATERIALS AND METHODS

A. Stock population

A laboratory stock culture was founded by rearing the progeny of adult beetles collected on fig trees from May to September 2010 in Anzano del Parco (CO), Northern Italy (GPS coordinates 45°46'09.98" N, 9°11'52.86" E MAP datum WGS 84; altitude 324 m above sea level). In order to obtain numerous eggs, four to ten males and females (depending on the number of adults captured in the field) were placed together in plastic cages (30x20x18 cm) in a climatic chamber (temperature 25±0.5°C, photoperiod 16L:8D, R.H. 65±0.5%). Three times a week they were provided with fresh fig tree twigs (10-15 cm length, 1.0-2.5 cm diameter) as food and oviposition sites. In addition, water was always available.

B. Rearing condition

Two distinct environmental conditions were considered: 1) 25±0.5°C and 65±0.5% R.H., 2) 30±0.5°C and 65±0.5% R.H. The photoperiod was established in 16L:8D for all the experimental treatments. The temperature of 25°C was considered to compare the results obtained with the ones of [21], [22], and [23], while 30°C was chosen as this temperature is considered critical for *P. h. hilaris* development by Watari et al. [24]. New information on the biology at critical temperatures can elucidate the capability of the xylophagous beetle to adapt to Mediterranean regions characterized by high temperature in summer. To evaluate the effects of the quality of the larval feeding on larvae and adults, two different diets containing 25% or 40% of dried mulberry leaf powder (25DML and 40DML) were compared. The choice of an artificial diet containing leaves of one of the host plants seems opportune as similar diets were used in other experiments on *P. h. hilaris* [25], [21]. As *P. h. hilaris* larvae live inside trunks with up to 44.6% of cellulose, while the cellulose content in the two experimental diets varies from 6% to 7% in dry powder, preliminary tests were made to compare the longhorn beetle development on these diets with a low content of cellulose with the same added with cellulose powder up to 70%. As results did not show any significant difference in *P. h. hilaris* development (ANOVA F= 2.239;P= 0.141), the test were prosecuted on the two experimental diets without cellulose addition. The two artificial diet recipes were originally developed in Italy to rear *Bombyx mori* larvae, and they consist of mulberry leaf powder, soybean meal and fiber, wheat meal, corn starch, citric acid salt and vitamin mixtures, agar, β-sitosterol, and antifungal and antibacterial compounds[26]. The powder was hydrated and cooked in a microwave oven, cooled to room temperature and then maintained at 5°C until its utilization.

C. Preimaginal rearing

Preimaginal rearing started from eggs extracted three times per week from the small branches located in the adult boxes for stock culture. Each twig was examined under a stereomicroscope (Leica MZ 12.5, Leica Microsystems GmbH, Wetzlar, Germany; and Wild Heerbrugg M5A, Leica Geosystems GmbH, Heerbrugg, Switzerland) and when an oviposition site was detected, the bark around the egg was removed with a scalpel and the egg was extracted. Eggs were individually placed in 12-well tissue culture plates filled with artificial diet in which a small hole was excavated; to avoid dehydration and to allow observation, eggs were covered with a transparent cellophane foil.

As insect development is affected by both temperature and diet which interact [27], [28], 4 treatments were compared: Treatment 1, 25DML and 25±0.5°C; Treatment 2, 40DML and 25±0.5°C; Treatment 3, 25DML and 30±0.5°C; Treatment 4, 40DML and 30±0.5°C. The eggs obtained were equally divided in the two climatic chambers and checked daily until hatching. After egg hatching, a set of 50 newly-hatched larvae were prepared for each treatment. The larvae were singularly transferred into plastic boxes (5.5 cm diameter, 3 cm height), and fed *ad libitum* (about 30 g of artificial diet). Diet was changed once a week and the larvae were individually reared until adult emergence. Each larvae was numbered and weighed weekly, until pupation, on a digital precision balance (Sartorius TE64, Sartorius AG, Germany) and its head capsule measured under a stereomicroscope provided with an ocular micrometer scale to evaluate if a molt had occurred (Wild Heerbrugg M5A, Leica Geo systems GmbH, Heerbrugg, Switzerland). Pupae were then removed from their food and checked until emergence. Larval and pupation period was then calculated. Adults were sexed just after the emergence and females were then coupled with males coming from the same experimental treatment.

D. Adult rearing

Each couple was placed in a plastic cage in the same climatic conditions as preimaginal stages, according to the respective treatment and their emergence date. Males that died before the females were substituted with others coming from the same treatment. Adult lifespan was calculated on all the specimens by checking rearing cages daily, from emergence until death. To standardize post-emergence conditions all the adults were fed only with fig twigs (10-15 cm length, 1.0-2.5 cm diameter) provided in abundance to guarantee both nutrition and oviposition sites.

Twigs were removed daily and the bark surrounding all the pits detached in order to evaluate: the pre-oviposition period of each female (calculated for each female from the day of emergence until the first egg detection in the twigs); lifetime fecundity (calculated for each female from the first day of oviposition until death, by counting all the eggs in the twigs); age specific fecundity (the average number of eggs laid by females of the same age were calculated in order to obtain the oviposition curve). Adult body size was calculated with a caliper as length of the body from the head to the apex of the elytra. Specimens were classified into small, medium and large classes according to Fukaya[23].

E. Data analysis

Larval and pupal survival rate and developmental time, number of larval instars, head capsule width measure frequency in different larval instars, larval weight, adult emergence, sex ratio, body length, adult lifespan and survival, pre-oviposition period, mean number of eggs per female, lifetime fecundity, age specific fecundity, and maximum number of eggs laid per day were analyzed using SPSS® Statistic (Version 19 for Windows, SPSS Inc. Chicago, IL, USA). Normally distributed data were described by the mean and standard error and treatments were compared with a univariate analysis of variance (ANOVA) through the general linear model procedure. Results from treatments showing significant overall changes were subjected to post hoc Tukey's test with a Type I error rate <0.05.Factorial MANOVA was applied to evaluate the effect of sex on the survival in the different treatments. The correlation between the oviposition period and the total number of eggs laid per female was analyzed with the Spearman rank order correlation coefficient.

Kaplan-Meier survival curve analysis [29] was used to compare the death rate among different experimental groups in both larvae and adults. Log rank, Breslow and Tarone-Ware tests were then applied to compare survival curves [30].

III. RESULTS

A. Larval and pupal survival rate and developmental time

The larval survival rate varied according to the diet and the environmental condition. Major mortality was observed in both diets at 30°C with values from 64.52% in 40DML to 85.96% in 25DML. At 25°C the mortality was 53.85% with 25DML and 44.00% with 40DML. Considering each larval instar, the most remarkable larval death occurred always in the first and second instars. Death rarely occurred in mature larvae and in pupal stage (Table 1). Figure 1 shows Kaplan-Meier survival curves of *P. h. hilaris* larvae applied to the four treatments. The rate was the highest when *P. h. hilaris* was reared at 25°C and on the mulberry high-content diet (40DML). At 30°C half of the population died within 20 days after hatching with 40DML diet, and within 28 days with 25DML diet. At 25°C only the population with 25DML showed a death rate major that 50% in the larval period after 105 days. The log rank, Breslow, and Tarone-Ware statistics evidenced significant differences among the curves of the larvae reared in each of the four treatments (P< 0.001).

Table 1: Percentage of mortality in the different larval instars and in pupae in the different treatments.[*T*= 1: 25DML 25°C; 2: 40DML 25°C; 3: 25DML 30°C; 4: 40DML 30°C]

T	Larval instar							Pupae
	1	2	3	4	5	6	7	
1	38.46	7.69	3.85	-	1.92	1.92	-	7.69
2	30	12	-	-	-	2	-	-
3	56.14	10.53	7.02	5.26	1.75	1.75	3.51	-
4	43.55	11.29	4.84	3.23	1.61	-	-	4.84

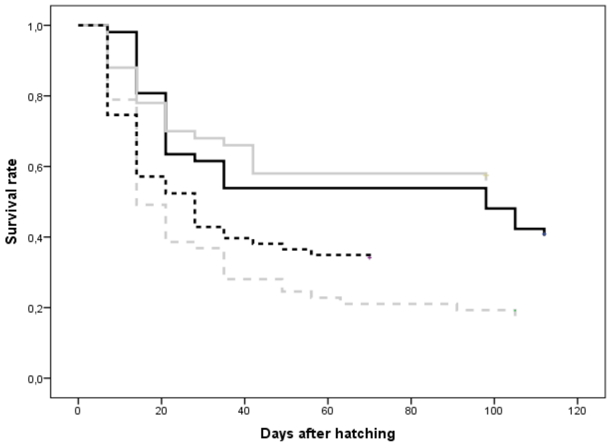

Fig.1. Kaplan-Meier survival curves of *P. h. hilaris* larvae on different treatments (black and solid line: 25DML 25°C; grey and solid line: 40DML 25°C; black and broken line: 25DML 30°C; grey and broken line: 40DML 30°C).

Statistical analysis did not show any significant difference in the length of larval development among the treatments (F= 2.31; df= 3; P>0.05). Larval development took from a minimum of 49 days in 25DML 30°C, to a maximum of 266 days in 40DML 30°C. The medium number of days of larval period was 112.67±44.35 in 25DML and 94.14±3.67 in 40DML 25°C, 84.00±14.43 in 25DML and 97.63±10.44 in 40DML at 30°C. Pupal development was influenced by the diet and the temperature (F=2.96; df=3; P<0.05). Minimum developmental time resulted for treatment 25DML 30°C. On the contrary maximum developmental time was observed at 25°C with the diet with highest content of mulberry powder (Table 2).

B. Number of larval instar

Different larvae in the same treatment showed a slight variability in the number of molts before pupation. Larvae pupated from the fourth to the eighth instar in both treatments at 30°C and from the fifth to the seventh in both treatments at 25°C. The majority of larvae pupated in the fourth and fifth instar at 40DML 25°C, at 25DML 30°C, and at 40DML 30°C (respectively 53%; 50%; and 55.6%) and in the sixth instar at 25DML 25°C (60%).

C. Larval head capsule width

Table 3 refers also to the range of variability of head capsule width in different treatments and the days spent in each larval instar. To notice that a superimposition between different head capsule measures is present in different instars in the same treatment; overlapping values increase as the larvae grows up from the first to larval maturity. Despite a great variability from larvae to larvae, younger larvae (first and second instar) usually molted in about one week, while they took a longer period as they grew up. The major mean period of permanence in an instar was 66.50 ±3.5 days in the VII instar at 40DML at 25°C.

Table 2: Larval and pupal period length in the different treatments. Different letters are representative of significantly different values at P < 0.05, on the basis of one-way ANOVA and Tukey's MRT.

	Larval period			Pupal period		
	Minimum	Maximum	Mean ± SE	Minimum	Maximum	Mean ± SE
DML25 25°C	84	154	112.67±4.345a	6	17	11.6±0.7ab
DML40 25°C	70	133	94.14±3.67a	6	18	12±0.55b
DML25 30°C	49	161	84.00±14.43a	5	14	9.13±0.99a
DML40 30°C	56	266	97.63±10.44a	7	17	10.22±0.60ab

Table 3: Larval head capsule width, mean period in each larval instar and range of larval instar in the different treatments.

Larval instar	Head capsule width range (mm)				Days spent in each larval instar (mean ± SE)			
	25DML 25°C	40DML 25°C	25DML 30°C	40DML 30°C	25DML 25°C	40DML 25°C	25DML 30°C	40DML 30°C
I	0.49 - 0.90	0.57 - 0.82	0.49 - 1.22	0.24 - 0.98	8.36 ± 0.47 (n = 36)	7.6 ± 0.58 (n = 41)	7.98 ± 0.93 (n = 43)	7.48 ± 0.38 (n = 58)
II	0.82 - 1.96	0.73 - 1.55	0.82 - 2.37	0.82 - 2.37	8.09 ± 0.53 (n = 32)	7.91 ± 0.90 (n = 35)	7.13 ± 0.52 (n = 23)	7.30 ± 0.84 (n = 35)
III	1.22 - 2.94	1.63 - 2.86	1.39 - 3.84	1.47 - 3.27	10.11 ±1.91 (n = 27)	8.22 ±0.58 (n = 29)	8.88 ±1.23 (n = 18)	10.13 ±1.98 (n = 28)
IV	1.96 - 3.67	2.61 - 3.92	2.12 - 4.08	1.96 - 3.92	12.88 ±1.44 (n = 25)	12.79 ±1.10 (n = 29)	21.00 ±3.54 (n = 13)	11.76±1.26 (n = 25)
V	2.53 - 4.16	3.27 - 4.49	2.69 - 4.33	2.37 - 4.16	25.38 ±3.67 (n = 24)	37.41 ±3.79 (n = 29)	26.44 ±4.92 (n = 9)	45.15±11.04 (n = 20)
VI	3.35 - 4.57	3.43 - 4.90	2.86 - 4.73	2.86 - 4.24	60.42±14.13 (n = 19)	37.69 ±3.93 (n = 13)	60.20±28.40 (n = 5)	44.00 ± 7.75 (n = 7)
VII	3.67 - 4.73	4.16 - 4.49	2.94 - 4.41	2.94 - 2.94	42.00 ± 4.95 (n = 5)	66.50 ±3.50 (n = 2)	63.00±35.00 (n = 2)	24.50±10.50 (n = 7)
VIII	-	-	3.10 - 4.73	3.10 - 3.10	-	-	14 (n = 1)	0 (n = 1)

Table 4: Trend of the larval weight in the different treatment.

Treatment	Sex	Maximum weight (g)	Larval weight before pupation (g)	Meanweightloose (%)
25DML 25°C	F	1.155±0.058	0.920±0.040	20.361
	M	1.506±0.011	0.978±0.015	35.086
40DML 25°C	F	1.170±0.462	0.975±0.375	16.839
	M	1.031±0.046	0.879±0.046	14.684
25DML 30°C	F	1.240±0.096	0.943±0.063	23.930
	M	1.146±0.122	0.869±0.064	24.152
40DML 30°C	F	0.993±0.792	0.805±0.071	18.955
	M	0.983±0.060	0.835±0.048	15.110

D. Larval weight

Larval weight rapidly increased during the first 9 weeks to a maximum of 1.5 g in 25DML 25°C and then decreased in the following weeks until pupation. Larval weight was not significantly influenced by the treatment (maximum weight F = 2.365, df=3, P>0.05; larval maturity: F=1.163, df=3, P>0.05).Weight loose before pupation varies greatly from larvae to larvae. The ANOVA analysis on arcsin (inverse-sine, square root) transformation of weight loose percentage before pupation showed significant differences (F= 5.93; df =3; P<005) between specimens reared on the poorest diets (25DML at both temperature) and the ones reared on the richest (40DML at both temperatures). 25DML diet caused a major weight loose (Table 4) .

E. Adults emergence

In Treatment 40DML 25°C and 40DML 30°C the adult emergence was 56% and 29.03% respectively. When the larvae fed on the diet with the lowest mulberry content (25DML), the survival rate decreased and the adult emergence was 38.46% in Treatment 25DML 25°C and only 15.78% in 25DML 30°C.

F. Adult sex-ratio, size, survival rate and lifespan

The sex ratio revealed the predominance of females over males. The percentage of the females emerged from the diet 25DML (respectively 74% at 25°C and 75% at 30°C) was the highest in comparison to the diet 40DML at both temperatures (respectively 41% at 25°C and 53% at 30°C).

All adults were allocated in the medium and large class of dimension [23]. Dimensions did not differ significantly between sex (F=2.516; df=1; P=0.119) or among treatments (F=2.65; df=3 P=0.061). Specimens ranged from 2.2 to 3.2 cm with a mean values of 2.59±0.03 cm (n= 74).

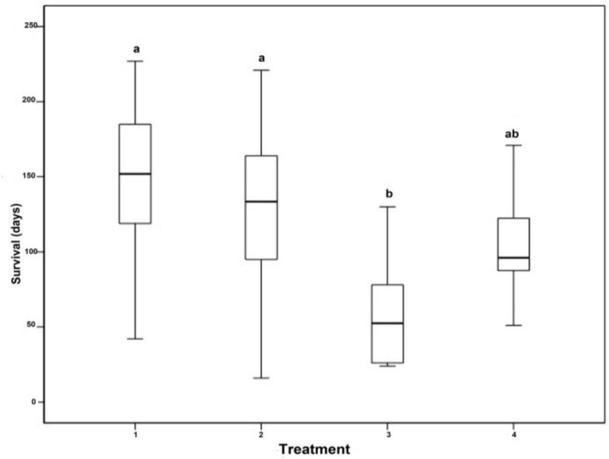

Fig.2. Box plot comparing the adult survival period (in days) on the two diets at the two temperatures [1: 25DML 25°C; 2: 40DML 25°C; 3: 25DML 30°C; 4: 40DML 30°C]. Different letters are representative of significantly different values at $P < 0.05$, on the basis of one-way ANOVA and Tukey's MRT

Beetle longevity ranged from 1 to 227 days. Adult lifespan differed according to the treatment (F=4.96; df=3; P=0.005) and the sex (F=6.34; df=1; P=0.015). MANOVA analysis showed no interactions among the treatment and the sex (F=0.11; dF=3; P=0.953). The mean longevity was significantly the lowest in 25DML 30°C (Fig. 2). In general, males lived longer than females. The mean longevity of males was 128.81±15.39 days (n=21) ranging from 5 to 227 days, while females lived on average 90.73±8.35 days (n=45), ranging from 1 to 203 days.

Figure 3 shows Kaplan-Meier survival curves of *P. h. hilaris* adults in the four treatment. The log rank, Breslow, and Tarone-Ware statistics evidenced significant differences among the curves of the adults reared in each of the four treatments (P< 0.001). While at 25±0.5°C half of the observed population survived up to 135 days in 25DML 25°C and 151 days in 40DML 25°C, the survival period was reduced at the highest temperature; at 30±0.5°C, a half of the adults survived for43 days from the emergence in 25DML and for 91 days in 40DML.

G. Pre-oviposition period

Results showed that the age at which females of *P. h. hilaris* started oviposition was not significantly different in the various treatments (F=2.231; df=3; P=0.108) (Table 5) and for this reason data of females were pooled together.

In Figure 4 the frequency of the ages (expressed as days from emergence) when oviposition was first observed is illustrated. The histogram shows that beetles were well-synchronized, with the majority of females starting oviposition within 15 to 25 days after their emergence.

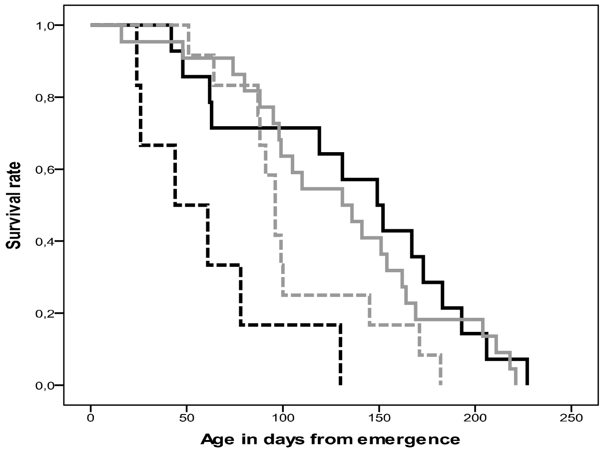

Fig.3. Kaplan-Meier survival curves of *P. h. hilaris* adults emerging from different treatments (black and solid line: 25DML 25°C; grey and solid line: 40DML 25°C; black and broken line: 25DML 30°C; grey and broken line: 40DML 30°C).

H. Lifetime fecundity

The number of eggs laid per day per female ranged from 2 to 22, and the total number of eggs laid during the entire life ranged from 32 to 393 with an average of 150.56±20.74. ANOVA applied to lifetime fecundity (expressed as number of eggs/female) showed significant differences according to the treatment (F= 3.415; df= 3; P= 0.032): specimens belonging to 25DML 30°C oviposited only few eggs during their lifetime (Table 5).

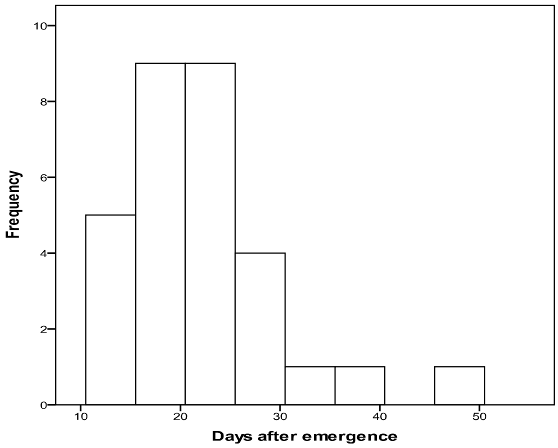

Fig.4. Pre-oviposition period of *P. h. hilaris* under laboratory condition.

The oviposition period ranged from 4 to 160 days with an average of 77.45±8.02 days. Significant differences were found in the duration of the oviposition period in dependence on the treatment (F=4.127; df=3; P=0.017). The shortest period was found in 25DML 30°C, while the longest in 25DML 25°C.

The Spearman rank order correlation coefficient applied to the oviposition period and the total number of eggs laid/female did not evidenced a statistically significant linear relationship between the two parameters (P=0.680).

Table 5. Oviposition behaviour recorded under different conditions and ANOVA results. (Means followed by different letters in the same column are significantly different at P<0.05 by one-way ANOVA and Tukey's post-hoc test).[Treatment 1: 25DML 25°C; 2: 40DML 25°C; 3: 25DML 30°C; 4: 40DML 30°C].

Treatment	Females tested	Pre-oviposition period (days)	Number of eggs/female (mean ± std err.)	Maximum number of eggs/female	Maximum number of eggs/female/day	Oviposition period (days)
1	8	24.88±3.76 a	128.88±29.92 b	227	22	100.88±16.01 c
2	14	13.07±3.44 a	172.35±31.56 b	393	9	86.54±10.23 bc
3	5	17.67±4.18 a	9.50±4.17 a	32	2	33.67±14.84 a
4	5	23.20±3.54 a	82.60±36.57 ab	220	12	42.60±12.05 ab

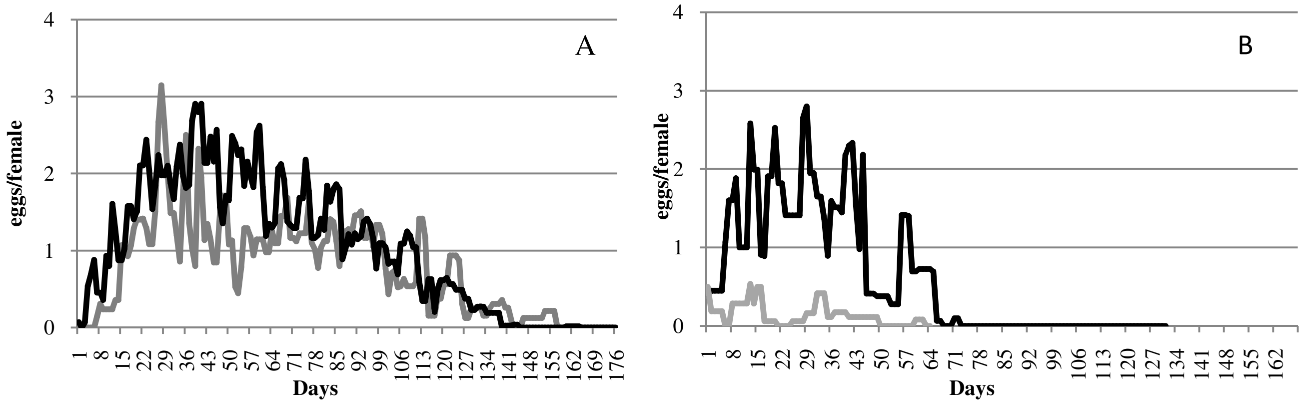

Fig.5. Fecundity curves of *P. h. hilaris* in the different treatments. To better visualize the curves were plotted separatedly in relation to the environmental conditions [(A =25.0±0.5°C where grey line 25DML and black line 40DML);(B=30.0±0.5°C where grey line 25DML and black line 40DML)].

I. Age specific fecundity

Figure 5 shows the curves resulting from the mean daily oviposition of females in dependence on their age. The daily rate of oviposition increased rapidly after emergence and reached a maximum depending from rearing conditions, then it declined and leveled off. Oviposition generally occurred until female death. Considering the specimens reared at 25DML 25°C the oviposition peaked in the period between the days24 - 44 from the beginning of oviposition and the females laid half of the eggs during the first 63 days; in 40DML 25°C a long peak period was observed between days 11 - 85 from the beginning of oviposition with the half of the eggs laid within 53 days; in 25DML 30°C only small picks were observed and the females laid half of the eggs within 16 days; in 40DML 30°C a long peak period was observed between days 7 - 45 and the half of the eggs laid in 42 days. Significant differences were found in the duration of the oviposition periods in dependence on rearing conditions (ANOVA F=4.127; df=3; P=0.017). However, the oviposition period was more affected by environmental condition than by the diet; major differences were observed between females reared at 25±0.5°C and 30±0.5°C T with the 25DML diet (Table 5).

IV. DISCUSSION

The use of an artificial diet, developed for *Bombyx mori,* allowed to assess that the content of mulberry leaf is relevant for an oligophagous insect as *P. h. hilaris* in the same way as it is in the silkworm, which is monophagous. In our experiments the different content of mulberry leaf in the diets affected the survival and the behaviour of *P. h. hilaris*. In *B. mori*, different substances play an important role in attraction to the food, biting and swallowing behavior, as in its growth [31]. The same hypothesis might be put forward in the case of *P. h. hilaris*: some secondary metabolites of the mulberry trees can play a role in promoting the diet consumption, while others are probably involved in its physiology and reproduction.

Besides many features of the environment, such as resource availability, temperature and diet quality, can impinge directly on the developing larvae and indirectly on adults [12]. The cue is to which extent food quality and temperature can influence insect performance without visible variation of the phenotype and physiology.

The research has provided evidence that all the treatments tested allowed to complete the development obtaining adults able to mate and reproduce. To notice that the climatic condition used, photoperiod included, allowed *P. h. hilaris* to complete the cycle without the need of vernalization in line with the information provided by [21]. However food quality and temperature influenced larval survival and adult performances. Major mortality was observed in younger larvae as it normally happens in natural conditions. The highest temperature and the poorest diet resulted in a reduced survival of the xylophagous insect. When the larvae survived to the first two instars, the development (in term of length, molt and weight) was similar in all the rearing conditions. On the contrary, pupal period was influenced not only by temperature but also by the concentration of mulberry leaves on which larvae were reared: 30°C, as expected, anticipated the emergence of few days, but at the same temperature the richest diet delayed adult emergence.

The information acquired on larval development allowed to find results in line with the ones provided by [22] and [24]. The major part of larvae pupated in the fourth and fifth instar like in Japanese population. Only preimaginal development took a longer period in the 1-3 instars. Food quality did not exert a profound effect on adult body size. In fact all specimens were allocated in the larger class of dimension [23]. However in the present research we observed that, even if different diets did not result in significant differences in body size, the performances of the adults can be influenced by the quality of food. This is also emphasized at higher temperatures. *P. h. hilaris* subjected to treatments at 30±0.5°C showed the lowest performances in terms of percentage of adult lifespan and fecundity. Moreover individuals reared in 25DML 30°C, on the diet with the lowest mulberry leaf content and the highest temperature, were significantly the worst in nearly all the examined parameters and were able to oviposit few eggs during their lifetime. Due to the ectothermic metabolism of insects, lower performances of the artificially-fed specimens at the highest temperature could have also been affected by an increased metabolic activity, which should have been sustained by an apt diet composition. Lower performances at 30°C confirm the study by Watari *et al.* [24], where this temperature is considered beyond the range of temperatures optimal for *P. h. hilaris* development. This result raises doubts about the invasiveness of *P. h. hilaris* in some Mediterranean areas characterized by summer with temperatures that can peak above 30°C.

The described research allowed to add information also on *P. h. hilaris* life cycle. *P. h. hilaris* like other species belonging to the tribe of Lamiinae[32], [33], [34], [35] and [36], demonstrated to be characterized by a longer lifespan than other Cerambycid species with a maximum survival of 227 days in males and 203 days in females. The preoviposition period is slightly longer than that observed by Iba[37] for *P. h. hilaris* females reared on mulberry. However, as adults in the present research were reared on *Ficus* twigs, future research should investigate if the host plant species on which the adult feeds, can have a fundamental role in oogenesis and may affect the time of the first oviposition. In fact in most Cerambycid species adult feeding is an essential prerequisite for egg maturation and oviposition [32], hence the food quality might have an important role in determining oviposition starting date. The species also exhibited a great oviposition potential with a large individual variability and a maximum of 393 eggs per female in the treatment characterized by the lowest temperature and 40DML diet. As age specific fecundity strictly influence the intrinsic rate of increase of a population, the information acquired in the present study emphasizes the potentiality of this exotic long horned beetle in establishing in a new area and spreading[38], [39] and [3]. In *P. h. hilaris* the oviposition period showed different peaks and a final slow decline, while in other Cerambycid species like *Anoplophora glabripennis* (Motschulsky) and *Corymbia rubra* (L) a linear decrease was recorded [35], [40].

As the food quality in larval feeding results to be one of the factors affecting adult performances, further research is needed to obtain data on the feeding behaviour on fig extract as this is the principal host of *P. h. hilaris* in Italy, while in the native countries mulberry is the preferred plant.

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