

Effect of temperature on the biology of *Noctua atlantica* (Lepidoptera: Noctuidae), a species endemic to the Azores

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Abstract. The effect of constant temperatures (5, 10, 15, 20, 25 and 30°C) on several biological parameters of *Noctua atlantica* (Warren), a noctuid endemic to the Azores, was studied using an artificial diet as food. At 5 and 30°C no eggs hatched. Developmental time (egg, larval, pupal and egg-to-adult) differed significantly at each temperature. Pupal weight (males and females) was significantly higher at 10°C and lower at 25°C. Adult longevity and the pre-oviposition period were longer at 10 and 15°C and differed significantly from those observed at higher temperatures. The mean number of eggs laid per female (fecundity) was significantly higher at 15°C, and lower at 25°C. Pupal weight (males and females) was positively correlated with larval, pupal, egg-to-adult developmental times and with adult longevity. Female pupal weight was also positively correlated with the pre-oviposition period and with fecundity. Temperatures ranging from 10 to 20°C are the most suitable for the development of *N. atlantica*, suggesting that this species is well adapted to the climatic conditions found in their natural habitat. The number of day-degrees required for the development from egg to adult emergence was 1428.57 D°, and 117.79 D° for the pre-oviposition period. Thus, *N. atlantica* can have two generations per year at high altitudes (550 or 800 m) in the Azores.

INTRODUCTION

Noctua atlantica (Warren) (Lepidoptera: Noctuidae) is endemic to the Azores (Warren, 1905; Meyer, 1991, 1995; Hacker & Schmitz, 1996; Vieira, 1997, 1998) and is found on eight of the nine islands of the archipelago (Carvalho et al., 1999).

Its host plants are unknown, but *N. atlantica* is found in the Azorean ecosystem "Laurisilva". Adults are present throughout the year, except during winter at medium and high altitudes (Araújo, 1994; Vieira & Silva, 1994; Vieira & Tavares, 1994).

Lepidoptera native to the Azores were mostly surveyed for inventory purposes (Vieira, 1998). Recently, detailed studies of the community and biological characteristics of endemic noctuids, aimed at their conservation in the Azores, were undertaken.

To understand the annual life cycle and distribution of *N. atlantica* populations the influence of environmental factors, such as temperature, on development was studied. Temperature plays a critical role in determining the rate of development, survival and reproduction of insects (Olsen et al., 1998; Lysyk, 2000; Doerr et al., 2002). The rate of insect development is predictable using heat accumulation, based on day-degrees or physiological time (Wagner et al., 1984; Jones et al., 1997).

The objective of this study was to record the effect of temperature on mortality, developmental time, longevity and fecundity of *N. atlantica*. Additionally, a day-degree model was used to estimate the number of generations per year this species can achieve in its natural habitat.

MATERIAL AND METHODS

Development and survivorship

Adults of *N. atlantica* were collected overnight on Gramin-hais (S. Miguel-Azores, 800 m a.s.l.) using light traps (Pennsylvania type). Adult males and females were placed inside cages (50×50×50 cm) and supplied with an aqueous solution of sugar (10%). All experiments were conducted in climatic chambers

(Versatile Environmental Test Chamber, Sanyo, MLR-350) at constant temperatures of: 5 ± 1, 10 ± 1, 15 ± 1, 20 ± 1, 25 ± 1 and 30 ± 1°C. Photoperiod was maintained at 16L : 8D, while humidity was kept at 70 ± 5% at each temperature. Egg development time was determined using eggs obtained daily from adults of *N. atlantica*. Eggs were disinfected in 10% formaldehyde solution for 20 min and rinsed with distilled water. Three replicates, each of 100 eggs, were then placed in plastic containers, and allowed to hatch; they were checked every day. The period from neonate larva to pupation was the larval development time. Larvae were kept in groups until the 3rd instar, then, they were reared individually. Larvae were fed with small portions (1 cm³) of artificial diet (Poitout & Bues, 1970, modified by Oliveira, 1991), which were renewed every two days. Pupae were individually weighed and caged in small clear glass containers (4.5×3 cm). The mortality of larvae and pupae was recorded every day. Containers were checked twice a day (9:00 and 17:00 h) for emergence of adults. One male and one female were placed together in a clear glass container (8×5 cm) to allow mating and check the pre-oviposition period (number of days between female emergence day and the day when the first egg was laid). The fecundity (number of eggs laid by each female) and adult longevity (males and females) were also monitored daily. A cotton ball containing an aqueous solution of sugar (10%) was placed in each container, to nourish the adults.

Day-degrees

The recorded developmental times were: egg development time (oviposition to hatching), larval development time (hatching to pupation), and pupal development time (pupa to adult emergence) and pre-oviposition period. The threshold temperature for development was estimated by the x-intercept method, i.e., through least squares regression of the mean development rate over temperature, and extrapolating the regression line back to the x-axis. The day-degrees (D°) required for development of each life stage were estimated using the method reported by Arnold (1959).

Weather variables

The meteorological data, from two agro-climatic stations at 550 and 800 m, were provided by the Institute of Meteorology/Azores. Calendar monthly averages of temperature recorded daily at 12:00 GMT for 30 years (1951–1980) were used.

Statistical analysis

All data were transformed by $\sqrt{x + 0.5}$ and compared using analysis of variance (ANOVA), followed by Scheffé tests with $P < 0.05$, except the differences between the pupal weights of the sexes at each temperature, which were analysed using t -tests. Percentage of eggs that hatched was analysed using a Kruskal-Wallis test (Zar, 1996).

Pearson correlation analyses were performed between pupa weight and other variables. Mortality data was analysed using the Multiple Comparison test for Proportions (Zar, 1996). All analyses were performed using SPSS 10.0 Windows (SPSS Inc., 1999).

RESULTS

Development and survivorship

At 5 and 30°C no eggs of *N. atlantica* hatched and the highest percentage hatched at 15°C (Table 1). At 10°C percentage of egg hatch was not determined since cannibalism occurred. Percentage of egg hatch decreased with increase in temperature (Table 1), but not significantly ($F = 1.330$; $df = 2, 8$; $P = 0.317$).

TABLE 1. Percentage (mean \pm SD) of *N. atlantica* eggs that hatched at 5, 10, 15, 20, 25 and 30°C.

Temperature (°C)	n	Hatched eggs (%)
5	406	0.00 \pm 0.00
10	418	—
15	237	91.23 \pm 8.07
20	353	82.37 \pm 12.11
25	446	78.26 \pm 9.73
30	382	0.00 \pm 0.00

N. atlantica larval and pupal mortality were significantly influenced by temperature ($F = 23.477$; $df = 3, 513$; $P < 0.001$). Larval mortality was significantly higher at 25°C and pupal mortality at the lowest temperature (10°C) (Table 2).

The number of days required for egg, larval, pupal and egg-to-adult (total) development decreased significantly with increase in temperature ($F = 8674.352$; $df = 3, 342$; $P < 0.001$), ($F = 1938.727$; $df = 3, 342$; $P < 0.001$), ($F = 4049.504$; $df = 3, 342$; $P < 0.001$) ($F = 7606.543$; $df = 3, 342$; $P < 0.001$) (Table 3).

Pupae were significantly heavier at 10°C, and lightest at 25°C ($F = 75.167$; $df = 3, 342$; $P < 0.001$) (Table 4). At each temperature, the pupae of females were significantly heavier than those of males ($t = -3.192$; $df = 86$; $P = 0.02$ t -test for 25°C), ($t =$

TABLE 2. Percentage of mortality of larvae and pupae of *N. atlantica* at 10, 15, 20 and 25°C.

Temperature (°C)	n	Mortality (%)		
		Larval	Pupal	Total
10	91	15.38b	33.77a	43.96a
15	116	6.90b	6.48b	12.93b
20	130	13.08b	6.19b	18.46b
25	180	47.78a	6.38b	51.11a

Means in each column that are followed by a different letter are significantly different ($P < 0.05$).

–3.715; $df = 104$; $P < 0.001$ t -test for 20°C), ($t = -7.482$; $df = 99$; $P < 0.001$ t -test for 15°C) and ($t = -3.195$; $df = 49$; $P = 0.02$ t -test for 10°C).

Longevity of the adults and pre-oviposition period were significantly affected by temperature ($F = 166.158$; $df = 3, 342$; $P < 0.001$ and $F = 52.772$; $df = 3, 109$; $P < 0.001$, respectively). These two parameters were significantly longer at 10 and 15°C than at the higher temperatures (Table 4). The mean number of eggs laid per female was significantly higher at 15°C ($F = 14.495$; $df = 3, 137$; $P < 0.001$) (Table 4).

TABLE 3. Development times (mean \pm SD) of egg, larval, pupal and egg-to-adult (total) stages of *N. atlantica* at 10, 15, 20 and 25°C.

T(°C)	n	Development time (days)			
		Egg	Larva	Pupa	Total
10	51	26.14 \pm 1.69a	112.14 \pm 7.24a	79.45 \pm 3.33a	217.73 \pm 9.37a
15	101	14.57 \pm 0.54b	61.49 \pm 3.85b	35.62 \pm 4.55b	111.60 \pm 3.77b
20	106	7.41 \pm 0.57c	43.66 \pm 5.35c	25.98 \pm 1.67c	77.05 \pm 5.36c
25	88	6.39 \pm 0.49d	41.15 \pm 4.91d	17.16 \pm 1.53d	64.60 \pm 4.72d

Means in each column that are followed by a different letter are significantly different ($P < 0.05$).

The weights of both male and female pupae were positively correlated with larval ($R^2 = 0.393$; $df = 345$; $P < 0.001$), pupal ($R^2 = 0.496$; $df = 345$; $P < 0.001$) and egg-to-adult development times ($R^2 = 0.447$; $df = 345$; $P < 0.001$), and adult longevity ($R^2 = 0.317$; $df = 345$; $P < 0.001$). Female pupal weight was also significantly correlated with pre-oviposition period ($R^2 = 0.224$; $df = 112$; $P = 0.017$), and the mean number of eggs laid per female ($R^2 = 0.272$; $df = 140$; $P = 0.001$).

Day-degrees

For the range of temperatures used, the lower temperature threshold (t_b) and the day-degrees required for the development of *N. atlantica* were effectively determined by the linear model, as shown by the high coefficient of determination obtained for all the developmental stages (all $R^2 > 0.93$) (Table 5).

The lower developmental thresholds (t_b) for eggs, larvae and pupae were 5.69, 0.36 and 5.97°C, respectively. The number of

TABLE 4. Pupal weight (males and females), adult longevity (males and females), pre-oviposition period and number of eggs laid per female (means \pm SD) of *N. atlantica* at 10, 15, 20 and 25°C.

Temperature (°C)	n	Pupal weight	Longevity of adults	n	Pre-oviposition period	n	Number of eggs/female
10	51	578.84 \pm 68.07a	29.20 \pm 9.78a	15	19.80 \pm 3.65a	21	207.05 \pm 269.49ac
15	101	513.58 \pm 58.45b	33.51 \pm 13.93a	44	17.73 \pm 7.64a	47	473.62 \pm 300.87b
20	106	517.15 \pm 77.36b	14.64 \pm 8.20b	33	7.82 \pm 2.20b	41	252.32 \pm 231.76a
25	88	409.25 \pm 76.30c	7.69 \pm 3.14c	21	6.10 \pm 1.58b	32	97.84 \pm 119.58c

Means in each column that are followed by a different letter are significantly different ($P < 0.05$).

TABLE 5. Temperature threshold (t_b) and the day-degrees required for the development of the different stages of *N. atlantica*, with the respective coefficient of determination (R^2).

Stage	Intercept	Slope	R^2	t_b	Day-degrees (D°)
Egg	-0.0478	0.0084	0.96	5.69	119.05
Larva	-0.0004	0.0011	0.93	0.36	909.09
Pupa	-0.0173	0.0029	0.99	5.97	344.83
Egg-Adult	-0.0023	0.0007	0.99	3.29	1428.57
Pre-oviposition	-0.0445	0.0085	0.92	5.24	117.79

day-degrees required for the development from egg to adult emergence was 1428.57 D° and 117.79 D° to start of oviposition. For a complete generation a total of 1546.36 D° is necessary (Table 5).

For the calculation of the day-degrees required for the development of *N. atlantica* in the field, $t_b=3.29^\circ\text{C}$ (obtained from the linear model for the total development time) and 1st January as the initial date, because it is commonly used for agronomic purposes (López et al., 2001), were used. This and the meteorological data for two localities at 550 and 800 m, indicate that

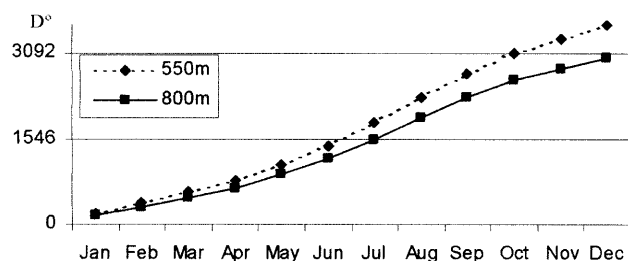


Fig. 1. The accumulation of day-degrees during the course of a year for *N. atlantica*, assuming a $t_b = 3.29^\circ\text{C}$, at localities at two altitudes (550 and 800 m) on S. Miguel Island, Azores.

this species can have 2 generations per year in its natural habitat (Fig. 1).

DISCUSSION AND CONCLUSIONS

Although insects are not subject to constant temperatures in nature, a study under controlled conditions can provide a valuable insight into the population dynamics of a particular species (Summers et al., 1984). Our results demonstrate the effects of temperature on larval and pupal mortality, preimaginal development times, adult longevity, pre-oviposition period and fecundity of *N. atlantica*.

Low ($<10^\circ\text{C}$) or high ($>20^\circ\text{C}$) temperatures had a detrimental effect on *N. atlantica* fecundity, as is recorded by Mays & Kok (1997) for *Evergestis rimosalis* (Lepidoptera: Pyralidae). At each temperature, female pupae were significantly heavier than male pupae. In contrast, the pupal weights of female and male *Pseudaletia sequax* (Lepidoptera: Noctuidae) do not differ (Foerster, 1996).

Development time was inversely related to temperature, when temperature is favourable, as observed in other Lepidoptera (Mcavoy & Kok, 1992; Jones et al., 1997; Howell & Neven, 2000; Tobin et al., 2001; Honek et al., 2002). Besides the inverse linear relationship between total development time and temperature, larval and pupal mortality was also significantly higher at the highest and lowest temperatures used. However, the mortality results show that *N. atlantica* larvae were less tol-

erant of the highest temperature (i.e., 25°C), and the pupae of the lowest temperature (i.e., 10°C) used.

According to Honek et al. (2002) the thermal constants of insect species and even those of populations within a species vary greatly. Since these differences may be caused by bias in the experimental data, as well as biological variation, the relevance of these differences may be tested using the "rate isomorphy" hypothesis (Honek et al., 2002). According to this hypothesis the same proportion of the total development time is spent in each developmental stage independent of temperature. Consequently, the lower development threshold should be identical for all developmental stages. According to Honek et al. (2002), there are three reasons why extreme temperatures may violate rate isomorphy: i) mortality may differ at low temperatures; ii) the developmental time may be imprecisely measured at high temperatures; and, iii) a small bias in the developmental rates measured at the highest or lowest temperatures will cause a large shift in the lower development threshold. The discrepancy between the values of the lower developmental threshold of larvae and those of eggs and pupae, could be due to the high larval mortality observed at 25°C . According to Wagner et al. (1984), mortality increases with temperature beyond the optimum, making the study of development difficult at high temperatures. Furthermore, the number of day-degrees required for *N. atlantica* larval development was high, which is to be expected when temperature is at or above the optimum (Wagner et al., 1984).

On the other hand, *N. atlantica* pupal weights were significantly higher at 10°C , and lower at 25°C . Honek et al. (2002) also record a decrease in *Autographa gamma* (Lepidoptera: Noctuidae) pupal weight with increase in temperature. Our results and those of these authors, show the temperature at which insects grow to the largest body size is always below the optimum for development time and fecundity. In *N. atlantica* the highest fecundity was achieved at 15°C .

Our results indicate that *N. atlantica* is adapted to the temperatures (8 to 20°C) recorded in their natural habitat. Larvae are found in the field usually during late Winter and Spring, when ambient temperatures are low (ranging from $9-14^\circ\text{C}$) and pupae and adults during Summer, when temperatures are high (around 18°C). As predicted by the lower developmental threshold for the eggs ($T_b = 5.69^\circ\text{C}$), no eggs of *N. atlantica* hatched at 5°C .

As suggested by Wagner et al. (1984) and Briere & Pracros (1998) a linear model is useful because it is easy to use and requires minimal data. This model can be used for field predictions when extreme field temperatures rarely occur (Worner, 1992). In the Azores, at middle and high altitudes (where *N. atlantica* occurs), the average monthly temperatures vary between 8 and 20°C . Therefore, according to these authors and our results, the linear model should be suitable for predicting population fluctuations of *N. atlantica*. According to this model, *N. atlantica* can have up to two generations per year on S. Miguel, with overlapping generations due to the high developmental heterogeneity of the population.

In future, the effect of other factors that significantly influence development rates and other biological parameters, such as photoperiod, diet and food availability, will be studied in order to better understand and protect the endemic Lepidoptera in our Region.

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