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Temperature effects on the embryonic development of *Aphthona abdominalis* (Coleoptera: Chrysomelidae), a natural enemy of *Euphorbia esula* (Euphorbiales: Euphorbiaceae)[1]

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Abstract:

The flea beetle *Aphthona abdominalis* Duftschmid was selected as a candidate for biological control o*f Euphorbia esula* L. (leafy spurge) in North America, where this introduced plant is a serious weed. The insect was collected and studied in Italy, where it occurs naturally. As part of the study of its life history, the effect of temperature on embryonic development was investigated at constant temperatures of 12, 15, 20, 25, 30, 35, 38, and 41°C, and variable temperatures were used as well. Survival and developmental rates were obtained. The median values used to calculate the time required for embryonic development through the thermal summation, a linear regression, and the logistic equation are discussed. The experimental data gave a developmental zero between 12 and 13°C. Embryos completed their development at constant temperatures from 15 to 38°C. Development required from 32.6 d at 15°C to only 4.5 d at 35°C constant temperatures. *A. abdominalis* was cleared and introduced into the United States during 1993.

Keywords:

Aphthona abdominalis, embryonic development, leafy spurge.

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Introduction

The flea beetle *Aphthona abdominalis* Duftschmid was released during 1993 in the United States (Montana and North Dakota) for the biological control of leafy spurge, *Euphorbia esula* L., a serious weed in North America (Dunn 1979). This poisonous plant, accidentally introduced into North America probably from Europe or Russia (Batho 1931, Dunn 1985) where it is not a problem, invaded rangelands and pastures of the northcentral United States and southern Canada, where it causes over 120 million dollars of losses annually (Watson 1985, Leitch *et al*. 1994). It is still spreading in North America, displacing natural vegetation and reducing the availability and value of land used for grazing (Watson 1985, Leitch *et al*. 1994). Because leafy spurge has a large pool of natural enemies in its area of origin, there is a good potential for a successful classical biological control program (Harris *et al*. 1985, Rees & Spencer 1992, Fornasari *et al*. 1993). *A. abdominalis* was collected and studied in Italy, where it occurs naturally.

The life history (Fornasari 1993) and host specificity (Fornasari & Pecora 1995) of *A. abdominalis* have been studied in the laboratory and in the field. The knowledge of the effect of temperature on the embryonic development of *A. abdominalis* is important for laboratory rearing and the use of this biological control agent in the field. The objective of the current study was to assess the effect of selected constant and variable temperatures on the embryonic development of *A. abdominalis.*

Materials and methods

Eggs of *A. abdominalis* were gathered from a colony of adults collected in the field in Italy (Tuscany) and reared in the laboratory. The adults were kept in plastic cages containing leafy spurge plants, on which the eggs were laid. The eggs for these studies were used within 4 hours of oviposition. They were collected using a fine camel's hairbrush and placed in 35-ml plastic cups provided with a layer of moistened plaster of Paris on the bottom. The effects of the following constant temperatures (± 0.5°C), under D:D photoperiod conditions, were evaluated: 12, 15, 20, 25, 30, 35, 38, and 41°C (Table 1). The relative humidity inside the cups was 70-80%. Groups of 20 eggs were placed in each cup and replicated 10-30 times for each temperature tested. A total of 2,685 eggs was studied at constant temperatures and 2,506 eggs at variable temperatures. The latter group of eggs was exposed at three environmental conditions having different daily mean temperatures: an outdoor cage, an unheated laboratory, and a heated laboratory. The cups containing the eggs for the studies at constant temperatures were placed into climatic cabinets. The number of hatched or collapsed eggs was recorded four times per day at 0800, 1200, 1600, and 2000 hours. The time required to control the eggs was 0.5-1 min per cup. The time required for embryonic development was reported as days and hundredths of a day and also expressed as 100/*y*, which represents the average percent development made by the embryos per day, at the given temperature. For each temperature, the mean number of days to hatch, their standard deviation, and the percentage of survival at each temperature were then calculated. The data obtained were also subjected to linear and nonlinear regression to show the pattern of the effect of temperature on the development of the eggs. For this purpose the thermal summation and

For this purpose the thermal summation and the logistic equation (Davidson 1944) were also used.

These experiments were conducted at the former location of the European Biological Control Laboratory (EBCL) in Rome, Italy.

Table 1. Duration, speed of development (100/*y*), and mortality of the eggs of *A. abdominalis* under constant temperatures.

Temp, °C	No. eggs	Mean ± SD, d	100/*y*	Mortality, %
12	208	–	–	100.0
15	608	32.65 ± 6.34[a]	3.06[a]	42.6
		39.36[b]	3.08[b]	
20	324	17.92 ± 1.18[a]	5.58[a]	28.4
		14.23[b]	7.39[b]	
25	572	8.40 ± 1.49[a]	11.91[a]	31.3
		8.68[b]	11.70[b]	
30	219	5.22 ± 0.57[a]	19.15[a]	32.0
		6.25[b]	16.02[b]	
35	307	4.53 ± 0.81[a]	22.07[a]	36.5
		4.88[b]	20.33[b]	
38	220	5.08 + 1.19[a]	19.67[a]	48.6
		4.31[b]	22.92[b]	
41	227	–	–	100.0

-, Not applicable.
[a] Observed.
[b] Calculated.

Results and discussion

Constant Temperatures. The time required for development under constant temperatures ranged between 4.5 ± 0.81 and 32.6 ± 6.34 d, at 35 and 15°C, respectively, and the calculated values range between 4.3 and 39.4 d (Table 1). The data are presented graphically in Fig. 1 and show a very good correlation between the observed and calculated values. The relationship between duration of egg development and temperature is represented by a hyperbola (Fig. 1), which has its asymptote at 12.06 ± 0.92°C and a thermal constant (Thc) of 111.40 ± 18.29 DD (Table 2). This calculated developmental zero agrees with the experimental data, because no development was observed at 12°C and 57.4% of the eggs hatched at 15°C.

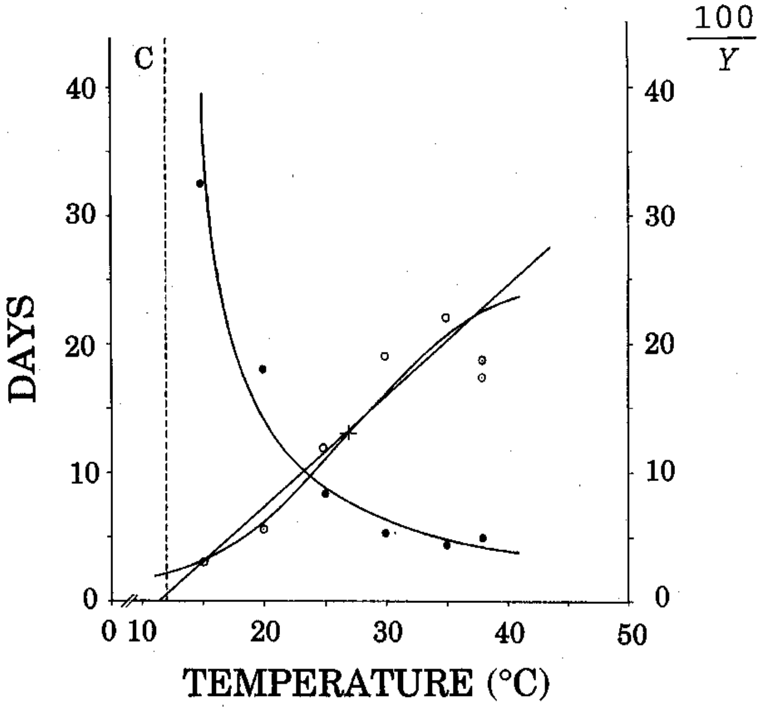

Fig. 1. Duration (days) and rate (100/*y*) of development of the eggs of *A. abdominalis* under constant temperatures. Developmental zero (c, dotted line) for the thermal summation and the linear regression is reported. The flex point of the logistic curve is also shown (x = 27.045, *y* = 13.169*).* Observed points are reported as closed circles (days) and open circles (100/*y*).

The rate of development is expressed by a linear regression and a sigmoid logistic curve in Fig. 1, where the reciprocals for developmental time are plotted against temperature. The high correlation coefficient for the linear regression (0.96, Table 2) shows the good correlation between temperature and rate of development (Fig. 1). The Thc obtained was 115.9 DD for linear regression and 91.1 DD for the sigmoid curve (Table 2). The sigmoid curve better expresses the course of the rate of development, especially at lower temperatures. The developmental zero obtained using the three methods (Table 2) was similar for the thermal summation (12.1 ± 0.9°C) and the linear regression (11.4°C), but lower for the logistic curve (6.2°C). This underestimate of the lower threshold for development by the logistic curve has also been observed for other insects (Crovetti *et al.* 1981, 1982; Belcari & Loi 1983; Loi & Fornasari 1985).

Table 2. Equations and constants characteristic for the eggs of *A. abdominalis* (under constant temperatures).

Thermal summation curve	$y(x - 12.17) = 111.40$ DD
	$c = 12.06 \pm 0.92$
	Thc = 111.40 ± 18.29 DD
Linear regression curve	$y = 0.863x - 9.865$
	$r = 0.957$
	$c = 11.43$
	Thc = 115.91 DD
Logistic curve	$\frac{100}{y} = \frac{26.337}{1 + e^{4.37924465-0.16192618x}}$
	Flex; $x = 27.0$, $y = 13.2$
	$c = 6.17$
	Thc = 91.12 DD
Mortality	100% = 10.7°C cold, 42.1°C warm
	50% = 16.7°C cold, 36.1°C warm
	25% = 22.1°C cold, 30.7°C warm
	Min. (18.8%) = 26.4°C

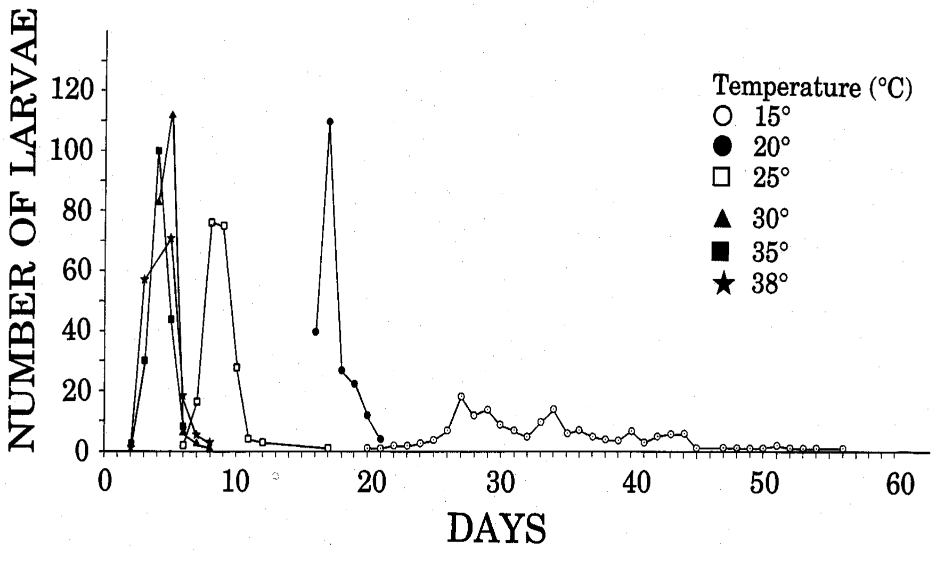

Fig. 2. Temporal distribution of hatching for eggs of *A. abdominalis* kept at constant 15, 20, 25, 30, 35, and 38°C.

Figure 2 shows the distribution in time of hatching at the various constant temperatures tested (n = 232 at each temperature). Eggs held at temperatures of 20, 25, 30, 35, and 38°C had a small and rather uniform variation in the time needed to develop. On the contrary, hatches at 15°C were spread over a long period (Fig. 2). No eggs hatched at 12 and 41°C.

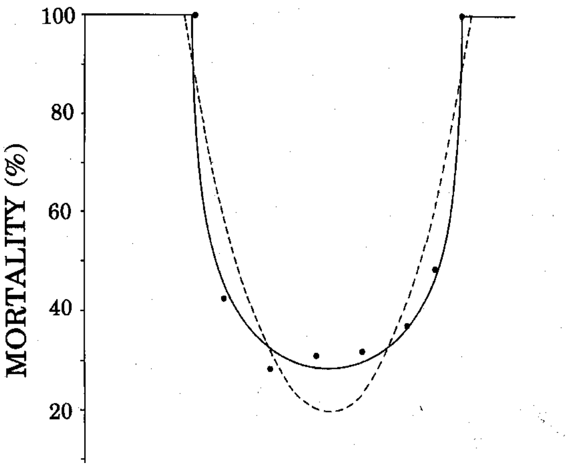

Fig. 3. Mortality curve for the eggs of *A. abdominalis* under constant temperatures, obtained from experimental data interpolated (solid line) curve and calculated (dotted line) curve.

The mortality of the eggs kept under constant temperatures (Table 1) is graphically interpolated as a parabola (Fig. 3). The apparently high mortality recorded, also at optimal temperatures under constant temperature regimes, is the result of the fact that eggs laid throughout the oviposition period during different generations were used in these studies; the average high mortality reflects the variations in fertility at different times of the year (Fornasari 1993). The eggs laid at different times of the year were homogeneously distributed in the groups tested at each temperature. A mortality of 100% is reached at the extremes of 10.7 and 42.1°C (Table 2), whereas 50% mortality is reached at 16.7 and 36.1°C and 25% at 22.1 and 30.7°C. The minimum mortality (18.8%) is estimated to be at 26.4°C.

Variable Temperatures. The time required for development under variable temperatures is reported in Table 3. This information allows a better understanding of the biology of this flea beetle in the field. During these studies, 35 groups of eggs were kept under different mean temperatures, ranging from 16.2 to 26.3°C*,* with extremes from 11 to 34°C (Table 3). The effect of variable temperatures is evident in speeding up development (Table 3), if compared with constant temperatures (Table 1). In fact, it is known (see also Wigglesworth 1972) that usually variable temperatures lead to a faster development, provided the high and low values fall within the extreme thermal limits for that species. For instance, at constant 15 and 20°C*,* embryonic development took ≈33 and 18 days, respectively (Table 1), whereas with a mean temperature of ≈16 (range, 13-20°C) and 20°C (range, 12-31°C*)* it took about half as long (≈15 and 11 days, respectively) (Table 3). The temperature variation during the period of the experiment ranged between 4 and 18°C (Table 3). The mortality ranged from 1.9 to 36.5%, except for a group of 124 eggs laid on 17 October, which had a mortality of 73.4%. Nevertheless, the high mortality in this group of eggs was very likely caused by their lower fertility at that time of the year.

Table 3. Observed values of the duration, speed of development (100/*y*), and mortality of the eggs of *A. abdominalis* under variable temperatures.

Temp. °C							
Mean	Max	Min.	Range	Mean development time (days ± SD)	100/*y*	No. eggs	Mortality, %
16.16	19	13	6	17.12 ± 0.762	5.842	60	28.4
16.27	20	14	6	13.41 ± 1.244	7.459	120	20.0
16.42	20	14	6	14.02 ± 1.781	7.133	60	11.7
17.34	20	14	6	13.90 ± 1.374	7.193	60	31.7
17.90	20	15	5	12.44 ± 0.831	8.039	60	31.7
18.40	20	16	4	12.48 ± 0.782	8.014	100	10.0
18.80	24	11	13	13.80 ± 0.523	7.246	27	25.9
18.90	20	16	4	11.59 ± 1.435	8.628	140	12.9
18.94	24	11	13	14.39 ± 1.153	6.949	54	33.4
19.16	25	12	13	11.46 ± 0.505	8.727	119	10.1
19.69	25	12	13	13.12 ± 0.823	7.619	42	4.8
20.06	25	12	13	13.19 ± 1.220	7.581	115	13.1
20.25	27	14	13	11.38 ± 0.932	8.783	75	13.3
20.29	31	14	17	7.84 ± 0.986	12.758	37	0
20.36	26	14	12	11.83 ± 1.097	8.454	40	12.5
20.80	27	12	15	9.52 ± 0.601	10.500	25	16.0
21.28	30	13	17	6.61 ± 0.650	15.120	98	10.3
21.40	27	15	12	5.64 ± 0.773	17.716	49	8.2
21.60	24	19	5	7.03 ± 1.131	14.224	124	73.4
21.68	31	13	18	5.59 ± 1.202	17.891	59	5.1
22.10	31	13	18	5.41 ± 1.009	18.467	97	3.1
22.26	30	17	13	5.67 ± 0.766	17.647	25	30.0
22.80	25	20	5	9.06 ± 2.462	11.034	20	20.0
23.00	24	19	5	6.80 ± 1.086	14.697	128	24.2
23.10	24	20	4	8.02 ± 2.647	12.462	260	36.5
23.74	30	17	13	3.92 ± 1.277	25.517	46	19.57
23.80	27	20	7	9.00 ± 2.405	11.111	37	21.60
23.90	25	20	5	5.39 ± 1.377	18.556	23	21.70
24.24	30	17	13	6.92 ± 1.212	14.457	25	40
24.55	29	17	12	6.67 ± 0.985	21.483	105	27.6
24.76	27	20	7	7.36 ± 1.761	13.580	90	14.5
25.10	27	18	9	6.72 ± 1.337	14.876	75	28.0
25.60	27	23	4	6.68 ± 0.556	14.970	27	7.4
25.72	27	23	4	9.81 ± 2.449	10.196	32	18.75
26.30	34	21	13	4.67 ± 0.683	21.428	52	1.90

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