STORED PRODUCT PESTS

Effect of temperature and diet on *Plodia interpunctella* (Lepidoptera: Pyralidae) development with special reference to Isomegalen diagram and accumulated degree days

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Abstract

Immature development times of the Indian meal moth, *Plodia interpunctella* were studied in the laboratory at four different constant temperatures (20, 23, 25, 27°C) reared on a standard diet (D1) and chocolate (D2). The minimal duration of development from oviposition to adult emergence was inversely related to temperature, ranging from 2.3±0.36 days to 50.5±0.5 days for D1 and from 36.7±0.53 days to 106.73±1.10 days for D2 for 27°C and 20°C, respectively. The minimum development threshold (tL), obtained from linear regression model of the development rates at the four studied constant temperature regimes, for total immature development is 15.3°C and 17.1°C for D1 and D2, respectively and the accumulated degree days (ADD) for *P. interpunctella* is 249.51°C for D1 and 358.4°C for D2 above the threshold.

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Introduction

Forensic entomology is the analysis of insect evidence for forensic and legal purposes. Forensic entomology is the branch of forensic science in which information about insects is used to draw conclusions when investigating legal cases relating to both humans and wildlife, although the term may be occasionally expanded to include other arthropods as well (Amendt *et al.*, 2007). In this context our article wants to deal with the stored-product forensic entomology.

The stored product aspect of forensic entomology involves the infestation of stored commodities by insects.

Infestations may include the harvesting and storage of crops and subsequent invasion by an insect pest and domestic invasion of kitchen products. This aspect also encompasses the infestation of food sold by retailers to the public, which Forensic entomology is the study of insects/arthropods in criminal and legal investigations. Although most recent attention has focused on the subspecialty known as "medico-criminal entomology", where insect development and succession on a corpse are used to estimate how long the decedent has been dead, there are additional areas where entomology is of legal interest (Amendt *et al*., 2007). Stored-product entomology includes the study of a variety of important insects with legal implications. The typical case involves infestations during the harvesting and storage of crops and subsequent invasion by an insect pest, the infestation of food sold by retailers to the public or the invasion of foodstuffs in the domestic kitchen by insect pests, which may result in prosecution and substantial fines. In such situations the size/developmental stage of insects, together with environmental conditions may be used to estimate the age of insects and therefore minimum time since infestation. Time frames may be used to determine whether infestations are the fault of the producer, the retailer, or are the result of long domestic storage or even deliberate contamination during the shelf life of the product (Hall, 2001).

The Indian meal moth, *Plodia interpunctella* (Hübner) is a

major economic insect pest of stored products and it is found on every continent except Antarctica. Among Lepidoptera, it is the most frequent pest infesting processed food (Süss *et al.,* 2013; Limonta *et al.,* 2016). Developmental rate, survival, and reproduction of Indian meal moth are affected by larval diet and environmental condition (Savov, 1973; Le Cato, 1976; Hoppe, 1981; Cline & Highland, 1985). Therefore, any model developed to predict phenology must take diet into account. Johnson *et al.* (1992) studied development of Indian meal moth at constant temperatures on wheat bran and ground almonds, walnuts, and pistachios to determine developmental thresholds, but only the temperatures of 22°C was studied (Johnson *et al.,* 1992).

Development data on different substrates are essential when it is necessary to estimate the beginning of the infestation in case of contamination of finished products. For this purpose, the degree-days from egg to adult of Indian meal moth on two larval diets (D1=standard diet, D2=chocolate) were evaluated. The developmental thresholds were estimated for *P. interpunctella* eggs and pupae, providing detailed information on the developmental rate, minimum development threshold and thermal constant of *P. interpunctella*. Data were collected following an approach usually used in forensic entomology, accordingly degree-day models are constructed, that can act as background for estimation time interval of food infestation The study reports a series of laboratory experiments that want to explore the effects of constant temperatures and larval substrate on the development of Indian meal moth with particular reference to ADD estimation and the Isomegalen diagram two approach usually used in medical forensic entomology

Materials and Methods

All test insects were from University of Milan laboratory, Department of Food, Environmental and Nutritional Sciences, maintained on a wheat bran diet (Locatelli & Limonta, 2004). Rearing conditions for non-diapausing larvae were 27°C, 60% RH, and a photoperiod of 16:8 (L:D) h.

To study the time range of the egg period (*i.e.* time from oviposition to emergence of first instar larvae) under different constant temperatures, eggs were deposed on black 35 mm film. This provided a dark environment preferred for numbered the eggs. After a count, 200 eggs were placed in Petri dishes with 11 g of two different food source: D1 (Table 1) and D2 (chocolate "gianduiotto" with chopped hazelnuts: 59% fat, 37% carbohydrates, 4% protein chocolate), cut in approximately 0.5 cm thick slices.

Table 1. Composition of Diet 1 (D1) for rearing of *Plodia interpunctella* larvae.

Ingredient	Grams for 1000 g of diet
Wheat bran	400
Wheat flour	150
Corn flour	150
Wheat germ	125
Glycerol	75
Yeast	50
Honey	50

To evaluate the time required to hatching, eggs were collected with a fine brush within 1h of oviposition. After a count, 200 eggs were placed in Petri dishes with 11 g of two different food source: D1 (Table 1) and D2 (chocolate "gianduiotto" with chopped hazelnuts: 59% fat, 37% carbohydrates, 4% protein chocolate), cut in approximately 0.5 cm thick slices.

Eggs were put into a precision environmental chamber (PIARDI Mod. CC. 1500) at one of four desired temperature regimes, 20, 23, 25, and 27°C each ±1°C, respectively, with relative humidity set at 60±5% and photoperiod of 16:8 h (L:D). This procedure was repeated 3 times for each temperature regime.

Twice a day, the mean temperature at the center of actively feeding larvae was recorded, using a digital thermometer. Four of the longest larvae were removed from the substrate every 4 h from each of the 3 subsamples. The larvae were removed for measurement until 10% underwent pupation

According to forensic entomology approach, the longest individuals were measured to estimate the growths curve on different substrates Measurement was followed immediately under the binocular in 0.1 mm units using a Vernier caliper. This sampling method was justified because of the common practice of collecting a representative sample and selecting the largest maggots of the insect fauna at a death scene for measurement. In doing so, the investigating entomologist assumes that the largest larvae of a particular species are the oldest individuals and probably developed from the 1st oviposition (Byrd & Butler, 1998).

Length data analysis

Average lengths of larvae were plotted against time for the two diets and the four temperatures getting 8 developmental curves. Since development of larvae is a continuum process, time and temperature regulated, lengths measurement interval was arbitrary chosen, from less than 1 mm to stopped feeding larval stage. Moreover, since measurements could not be taken at the same time, but data analysis requires that every larval dimension be actually measured in each experiment, we extrapolate data missed from developmental curves length (Reiter, 1984).

Lower developmental threshold and accumulated degree days

For each of the tested diet Lower thresholds (tL) for development were estimated from the linear regression (STATA® software; TStat S.r.l., Sulmona, Italy) of the developmental rates (y =1/developmental time) on constant temperature (x).

The accumulated degree days (ADD) from egg to eclosion were calculated for each of the replicates (n=3), for each of the larval diet, and for each of the four constant temperature regimes, to obtain the overall ADD (mean ± S.D.), according to the following formula:

$$ADD = d\ (t - tL)$$

where d (days) is the developmental time, t is the rearing temperature (°C), and tL is the lower developmental threshold temperature (°C). It was considered not relevant to perform a further statistical analysis because the ADD value for each of the diet was estimated thrice (Campbell *et al.*, 1974; Tun-Lin *et al.*, 2000).

Data analysis

A one-way analysis of variance was used to compare data using SPSS® Statistic (Version 24 for Windows, SPSS Inc. Chicago, IL, USA). Where significant differences occurred, Tukey's Honestly Significant Difference test was applied for mean separation ($P<0.05$).

Results

Growth curves

The mean minimum duration of development (±S.D.) from oviposition to egg-hatching at each of the four studied temperature regimes is given in Table 2. Significant differences were observed in relation to temperature and diet.

The mean minimum duration of development (±S.D.) from oviposition to eclosion (total immature development) at each diet and of the four studied temperature regimes is given in Table 3. As the temperature increased, the minimal developmental time was significantly lower. At the same temperature the minimal development time on diet 2 was always higher than D1.

The lengths (mm) and time (days) measured during larval artificial development experiments on D1 and D2 were reported in Table 4 and represented in Figures 1 and 2.

Developmental threshold and thermal constant

In our experiments, development of *P. interpunctella* was linearly related to temperature (R^2=0.98, P<0.01 and R^2=0.89, P<0.01, for D1 and D2 respectively) between 20°C and 27°C. The minimum development threshold (tL) for total immature development was extrapolated by linear interpolation of the two models developed for D1 and D2 (Figure 3).

It was 15.3 and 17.1°C for D1 and D2 respectively. This information has allowed the calculation of the ADD (oviposition-emersion) for both tested larval diet. Different degree-days for each diet were observed (average 249.51°C for D1 and 358.4°C for D2) (Table 5).

Isomegalen diagrams

As expected the development time (age) of *P. interpunctella* is related to temperature and time relationship, but our results show that also the substrate used by larvae during development play a fundamental role.

For entomologic forensic study is fundamental to provide a relatively simple diagram for the estimation of the minimum interval of infestation knowing the length of larvae observed in the food infested and the storing temperature. Following the original approach proposed by Reiter (1984) two isomegalen (equal length) diagrams were drown (Figures 4 and 5) for D1 and D2.

Table 2. Minimal developmental times (mean days ± S.D.) of *Plodia interpunctella* from oviposition to egg hatching, at four constant temperatures, on two different larval diets (D1 and D2).

Temperature °C	D1	D2
20	1.26±0.12^{d}	1.49±0.01^{e}
23	0.96±0.05^{c}	1.16±0.09^{d}
25	0.46±0.05^{ab}	0.56±0.05^{b}
27	0.39±0.03^{a}	0.48±0.03^{ab}

The means followed by different letters are significantly different (HSD Tukey, P<0.05).

Table 3. Minimal developmental times (mean days ± S.D.) of *Plodia interpunctella* from oviposition to adult eclosion, at four constant temperatures, on two different larval diets (D1 and D2).

Temperature °C	D1	D2
20	50.50±0.50^{e}	106.73±1.10^{g}
23	34.97±0.72^{c}	76.90±0.78^{f}
25	24.97±0.29^{b}	38.87±0.47^{d}
27	21.30±0.36^{a}	36.70±0.53^{c}

The means followed by different letters are significantly different (HSD Tukey, P<0.05).

Table 4 . Larval development of *Plodia interpunctella* on D1 and D2 at 20 °C, 23°C, 25°C and 27°C.

Length(mm)	Diet 1 (days)				Diet 2 (days)			
	20°C	23°C	25°C	27°C	20°C	23°C	25°C	27°C
0.2	0	0	0	0	0	0	0	0
0.5	9	0	0	0	10	7.5	0	0
1	10	5	5.5	3	11.5	9	6	5.5
1.5	11	6	6.5	3.5	13	14	7	6.5
2	12.5	7	7.5	5.5	14.5	16	11	10.5
3	18	9	8	8.5	24	21	13.5	13
4	20.5	11.5	11	10	27	23	16.5	15.5
5	21.5	14	12	11	30	28	20	18.5
6	24	15	12.5	12	32	34.5	21	19.5
7	26	16	13	12.5	36.5	37	22.5	20.5
8	27	18	13.5	12.75	42	39	26	22.5
9	27.5	19	14	13	48.5	43	27	26
10	30	20	15	13.5	54	44	28	27
11	31	21	16	14	57	45	29.5	28
12	32	22	17	14.5	58	47	31	s.f.m.
13	s.f.m.	23	17.5	15	s.f.m.	50	s.f.m.	s.f.m.
14	s.f.m.	24	18	16	ND	ND	ND	ND

s.f.m. = stopped feeding maggots stage; ND = length not achieved.

Discussion and Conclusions

Development of *P. interpunctella* was linearly related to temperature between 20 and 27°C, and expressed by the Accumulated Degree Days of 249.51°C for D1 and 358.4°C for D2, whit threshold of 15.3°C and 17.1°C for D1 and D2 respectively. These values were slightly different from what reported by Johnson *et al.* (1992) that observed a threshold temperature (tL) of 14°C. This difference could be attributed to variation in experimental method (extrinsic factors), but also intrinsic factors (such as geographic adaptation (Honek, 1996) could explain the difference in the temperature-dependent development observed. Assuming an average constant temperature, as is the case with stored products the isomorphen-

Table 5. Accumulated degree days (ADD) of *Plodia interpunctella* at four constant temperatures, on two different larval diets (D1 and D2). The means followed by different letters are significantly different (HSD Tukey, P < 0.05).

Temperature °C	ADD_D1	ADD_D2
20	237.4±2.4[a]	309.5±3.2[d]
23	269.24±5.6[c]	453.7±4.6[f]
25	242.2±2.8[ab]	307.0±3.7[d]
27	249.2±4.2[b]	363.3±5.2*e*

The means followed by different letters are significantly different (HSD Tukey, P<0.05).

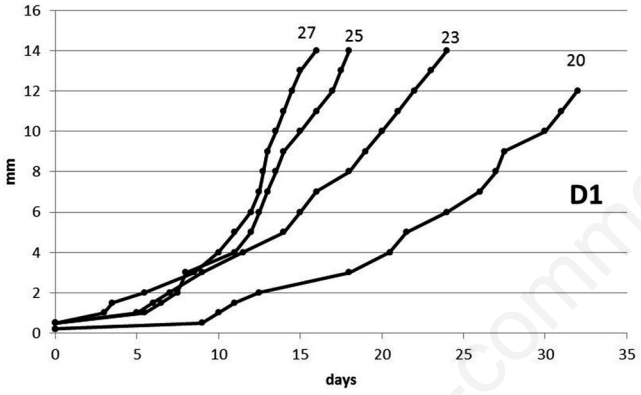

Figure 1. Larval length (mm) of *P. interpunctella* on D1 from hatching to pupation at 4 different temperature regimes.

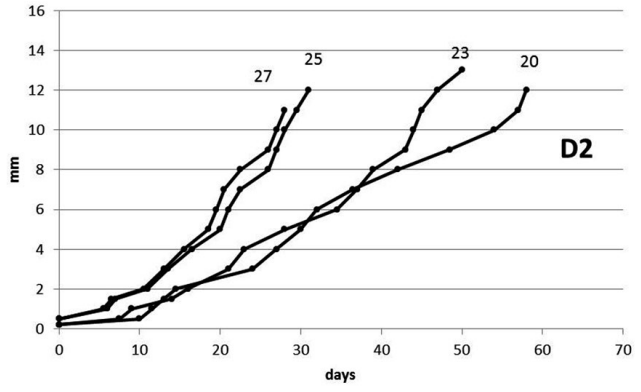

Figure 2. Larval length (mm) of *P. interpunctella* on Diet 2 from hatching to pupation at 4 different temperature regimes.

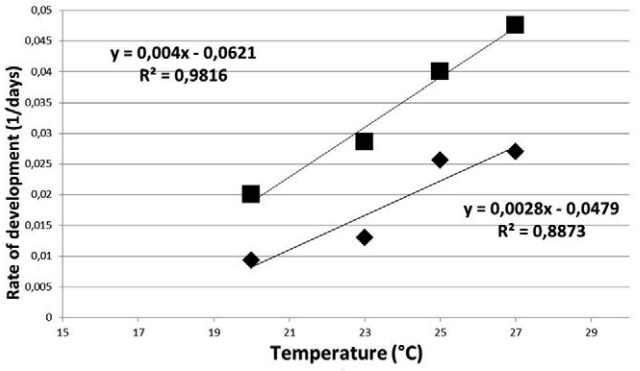

Figure 3. Linear regression of rearing temperature and rate of development (for ■ Diet 1and for ♦ Diet 2).

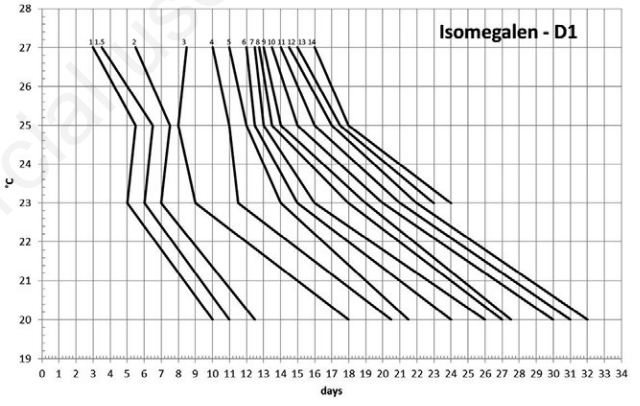

Figure 4. Isomegalen diagram of larvae developed on D1, each line corresponds to the time that larvae need to achieved the same larvae length at temperatures between 20°C and 27°C.

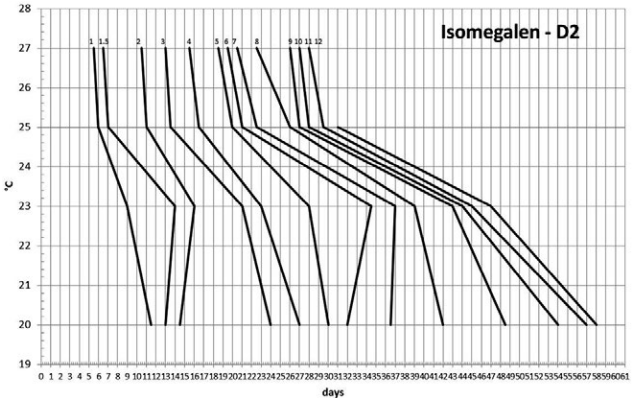

Figure 5. Isomegalen diagram of larvae developed on D2, each line corresponds to the time that larvae need to achieved the same larvae length at temperatures between 20°C and 27°C.

diagram could provide a quick and precise minimal estimate of the time of infestation since it being able to determine the age of the larva retrospectively. As already known, the comparison with different diets indicated confirmed that not only the temperature but also the larval diet influenced the developmental time (Johnson *et al.,* 1992; Subramanyam & Hagstrum, 1993). Larval growth of *P. interpunctella* on chocolate (D2) and standard (D12) diets was very different, as would be expected with such large differences in mean developmental rate for each diet. The high ability of *P. interpunctella* to reproduce in nut-containing chocolate is in accordance with earlier report (Hoppe, 1981) and the attraction to nut-containing chocolate is adaptive for the gravid females, since it provides a suitable substrate for feeding larvae. As reported in Olson *et al.* (2005), wheat-based rearing diet shows the shortest developmental time of larvae, but nut-containing chocolate is the second best one (Olsson *et al.,* 2005). Nevertheless, our data raise an important observation: if the minimum infestation interval in larvae developed on the chocolate diet was estimated using models developed on wheat (or other substrates), a strongly erroneous estimate would be obtained. The results of our study highlighted the difficulty in developing predictive models for insects with a wide range of feeding substrates. A different model will likely be required for each product attacked. Also, not all products within a single packaging plant are stored under the same temperature and humidity conditions. All of these factors add to the complexity of any proposed model.

Studies with insect nutrition have indicated that variations in the quantity and quality of a suitable food can have important effects on insect development (GENÇ, 2006). In fact, if the minimum infestation interval in larvae developed on the chocolate diet was estimated using models developed on wheat (or other substrates), we would be obtained a strongly erroneous estimate. The results of our study highlighted the difficulty in developing predictive models for insects, as *P. interpunctella*, with a wide range of feeding substrates, but also the importance to know these data. In case of customer complaints, predictive models of larval growth on different substrates under different temperature conditions are useful to establish the beginning of infestation to better determine the responsibilities and weak points of the food chain. Current challenges would be to define a different model for each product attacked. Predictive models could be developed using the average temperature and humidity found in the food industries, as well as in the warehouses of finished products and distribution environments.

Hopefully this research will inspire similar study variations involving different factors (temperature, humidity, larval substrate) and stored product pests.

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