Forensic Science International 252 (2015) 29–32

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Technical Note

New developmental data for *Cynomya mortuorum* (L., 1761) in Belgium (Diptera: Calliphoridae)

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ARTICLE INFO

Article history:
Received 22 July 2014
Received in revised form 10 April 2015
Accepted 15 April 2015
Available online 22 April 2015

Keywords:
Forensic science
Forensic entomology
ADD
Lower threshold
Cynomya mortuorum

ABSTRACT

The calliphorid *Cynomya mortuorum* (L., 1761) is a forensically important species mainly found in the Palearctic region. Knowledge about its biology and ecology is scarce. Thermal constants as well as developmental time were studied at constant and variable regimes of 5 average temperatures: 14, 16, 18, 20 and 22 °C, respectively. Total developmental time varied between 15.82 ± 0.40 days at 22 °C and 28.67 ± 2.38 days at 14 °C, for the constant regime, and between 16.05 ± 0.67 days at 22 °C and 32.79 ± 1.77 days at 14 °C, for the variable regime. No significant differences were observed between ADD, and threshold at the constant (ADD: 277.39 ± 14.78 DD; lower threshold: 4.72 °C) and variable regimes (275.99 ± 14.16 DD; lower threshold: 5.05 °C).

1. Introduction

For over a century, flesh flies and blow flies (Diptera: Sarcophagidae and Calliphoridae) have been important auxiliaries of forensic entomology, as they help solve cases [1–6]. The accumulated degree-days method (ADD) is one tool used to evaluate minimum Post-mortem Interval (PMI) using these insects. Although this method has been used in various contexts [7–11], Marchenko [12] was the first to estimate the developmental rates of 10 necrophagous fly species using a temperature sum, following the accumulated degree-day model. Each ectothermic insect species requires a particular number of degree-days above a theoretical threshold. These two constants (total ADD and threshold) are species-dependant.

Since Marchenko's work, the thermal constants (ADD and temperature threshold) were determined for several other Diptera species of forensic interest such as: *Chrysomya megacephala* (Fabricius, 1794), *Sarcophaga (L.) tibialis* Macquart, 1851, *Muscina stabulans* (Fallén, 1817), and *Synthesiomyia nudiseta* (van der Wulp, 1883) [13–16]. Despite an the increasing number of species with published data, much remains undocumented regarding these data as well as information about species biology, ethology, and development. Among the dipteran family Calliphoridae, the genus *Cynomya* Robineau-Desvoidy, 1830 is composed of two vicarious species, *Cynomya mortuorum* (L., 1761) and *C. cadaverina* Robineau-Desvoidy, 1830. The geographic distribution of *C. mortuorum* extents across the entire Palearctic region and partly overlaps that of *C. cadaverina* in Alaska and the Far East of Russia [17–19].

C. mortuorum appears to be relatively common in Fenno-Scandinavia, Denmark [17] and the Czech Republic (H. Šuláková, personal communication), but it is rarely observed in Switzerland [5]. The few observations in Switzerland have indicated *C. mortuorum* presences mainly in meadows at altitudes ranging between 400 m and 3000 m [5]. In Belgium, over a period of 14 years, Frederickx et al. [20] reported 100 *C. mortuorum* in the yearly collections made by students of Gembloux University. This should be compared to the 1486 specimens of the very common species *Calliphora vicina* Robineau-Desvoidy 1830 reported over the same period of time. *C. mortuorum* has been reported several times as an agent of myiasis [21,22]. Rognes [17] reared *C. mortuorum* from samples taken on small mammals carcasses (Soricidae, Muridae). To date, no synanthropic preference has ever been demonstrated [23]. The average length of its development cycle in natural conditions ranges from 25.4 to 31.5 days in Southern Finland, and between 46.7 and 72.3 days at the polar circle (''subarctic Finland'') (Nuorteva [24,25] *in* Rognes [17]). Its phenology extends from April to October in Finland [17]. Immature stages have been described by Erzinçlioglu [26]. Megnin [1] considers *C. mortuorum* as belonging to what he named the ''second squad''.

Because *C. mortuorum* is missing in the forensic entomology toolbox, we decided to obtain data on the thermal constants (ADD and lower threshold) of this species.

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http://dx.doi.org/10.1016/j.forsciint.2015.04.018
0379-0738/

2. Material and methods

2.1. Origin and colonies rearing

The laboratory colonies of *C. mortuorum* used in this study were originally collected as first and second instar larvae. They were harvested on a human corpse discovered in Outrelouxhe, Belgium (alt = 250 m), on May 1st, 2012. In the rearing room, the adult fly colonies were maintained in mesh rearing cages (40 cm × 40 cm × 40 cm) at room temperature (15° and 21 °C during night and day, respectively). The room was programmed with a (16:8 L:D) circadian rhythm. The difference between night and day temperatures were chosen based on temperatures commonly found in Belgium. Each cage contained 150–200 flies. They were provided *ad libitum* with water, sugar and powdered milk. After emergence and over a period of three days, adult flies were provided with a piece of beef heart used as a protein source to promote the ovarian development of females [27]. The room temperature was monitored using a data logger (Testo 175-T1).

2.2. Growth and development under different temperature regimes

When eggs were required, a dish containing one piece of beef heart (around 10 g) was placed into one or several mesh cages and removed 4 h later. Each piece of meat with the newly laid eggs was transferred (for a maximum of 250–300 eggs) on approximately 250 g of fresh mixed beef heart. Each set of 250 g of fresh mixed beef heart was finally placed into home-made plastic rearing boxes (23 cm × 17.5 cm × 10 cm). A 1 cm-thick layer of dry sand covered the bottom of the boxes to provide a pupation medium. The rearing boxes were then placed into an environmental chamber (SANYO Incubator MIR 553, Sanyo Electric Biomedical, Japan). To ensure a suitable relative humidity; a container filled with water was also placed inside the environmental chamber. From June 2012 until November 2013, this procedure was repeated a minimum of eight times for each temperature regime. Five different daily mean temperatures (14, 16, 18, 20 and 22 °C, respectively) were chosen following the recommendations of Bergant and Trdan [28]. The conditions corresponded to temperatures commonly found in Belgium.

Two temperature regimes were selected for each of the five desired temperatures. During the constant regime (Tcst), the temperature was kept constant day and night, for each of the five desired temperature levels. During the variable regime (Tvar), the environmental chambers were programmed to obtain the corresponding daily average temperature while observing a lower temperature during the dark period, in order to mimic natural nocturnal conditions (10–16 °C, 12–18 °C, 14–20 °C, 16–22 °C and 18–24 °C, respectively). All treatments were maintained with a photoperiod of 16:8 (L:D) (hours) and the temperature gradually changed (over a 15 min period) between the dark and light cycles. A data logger (Testo 175-T1) was inserted into each incubator to monitor hourly actual temperatures.

Emerging adults were recorded daily and stunned with a short puff of CO_2 before being removed from the box. To avoid temperature bias in each incubator, rearing boxes were randomly replaced inside each incubator.

2.3. Developmental threshold and thermal constant

In order to collect data to assess the thermal constants and lower threshold of *C. mortuortum* under variable and constant regimes, a total of 63 and 72 rearing boxes were used. Shortest developmental time (first day when a total of 10 specimens had emerged) was used for calculations and the corresponding sum of degree accumulated to complete the cycle recorded. Lower thresholds (w) for development were estimated from the linear regression of the developmental rates (y = 1/developmental time) on average temperature, for variable and constant regimes. The thermal constant (K) was calculated from the equation $K = d(T - w)$, where d is the developmental time (days), T is the average rearing temperature (°C), and w is the lower developmental threshold temperature (°C). Following Nabity et al. [29], a thermal constant was calculated for each box at each of the five average temperatures (from egg to adult) to obtain the overall K (mean ± standard deviation (SD)). Values of K are the number of degree-days (DD) above the threshold (w) needed for total development.

To avoid any perturbation to the larvae, they were left untouched until adulthood. The ADD corresponding to the different larval stages were therefore not assessed.

2.4. Data analysis

The statistical analysis was performed using Microsoft® Excel 2010/XLSTAT©-Pro (Version 2013.5.08, Addinsoft, Inc., Brooklyn, NY, USA). The significance level was set at $p \leq 0.05$. Equality of variances between each set was confirmed with an F-test.

3. Results

3.1. Developmental threshold and thermal constant

The observed growth rates were greater with higher temperatures. Under variable growth conditions, total duration ranged from 32 ± 1.77 days at 13.99 °C to 17.05 ± 0.67 days at 22.25 °C (Table 1). Under constant temperature conditions, duration ranged from 29.67 ± 2.38 days at 14.45 °C to 16.82 ± 0.40 days at 22.11 °C (Table 2). Differences between total development times under the 2 different regimes studied were found to have no statistical significance with the exception of the 14 °C (t test, <0.0001) and the 18 °C (t test, =0.013) respectively.

The development data under variable temperatures (Table 1), resulted in a lower temperature threshold of 5.05 °C and an accumulated degree-days (ADD) of 275.99 ± 14.16 DD. Using the developmental data obtained under constant temperatures (Table 2), the lower temperature threshold reached 4.72 °C with an ADD of 277.39 ± 14.78 DD to complete total development (Table 3). The difference between observed ADD for the two regimes of temperatures was not significant (t test, p = 0.066).

4. Discussion

C. mortuorum (Diptera, Calliphoridae) is a characteristic, large species that is present in Belgium, although collected with low frequency. Its relative absence in Flanders (Northern Belgian territory) is probably related to a lack of observation [20]. Indeed, it

Table 1
Average and standard deviation (SD) (in brackets) of developmental times, sum of degree accumulation to complete development from egg to adult under a variable temperature regime, at each temperature (see Section 2 for the range of each temperature).

Temperature (°C)		N	TVar	
Set	Average		Egg to adult (d)	Sum of degree (°C)
22	22.25 (0.01)	19	16.05 (0.67)	351.52 (15.00)
20	20.17 (0.03)	11	18.09 (0.94)	359.77 (19.00)
18	18.15 (0.04)	12	21.25 (1.10)	381.09 (20.47)
16	16.50 (0.01)	13	24.08 (1.58)	393.05 (25.96)
14	13.99 (0.09)	8	32.79 (1.77)	433.59 (23.17)

N = number of rearing boxes.