



Cold hardiness of immature and adult stages of the Mediterranean flour moth, *Ephestia kuehniella*

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ABSTRACT

The cold hardiness profile of immature and adult stages of the Mediterranean flour moth, *Ephestia kuehniella* was investigated in the laboratory. Supercooling point (SCP) of early instars, late instars, pupae and adults of *E. kuehniella* was determined using a circulating bath with a cooling rate of 1 °C/min. Mean SCP of pupae was significantly lower (−23.3 °C) than that for early and late instars (−16.1 and −19.5 °C, respectively), but did not differ significantly from that for adults (−21.6 °C). Moreover, mortality at sub-zero temperature was estimated by cooling eggs, early instars, late instars, pupae and adults to −5, −7.5, −10 and −12.5 °C for 30, 60, 90 and 120 min. Main effects of temperature, exposure time and developmental stage on mortality proved to be significant. Two-way interactions as well as the three-way interaction between all tested factors also proved to be significant in most cases. Generally, pupae and adults were the most cold-tolerant, followed in decreasing order by late instars, early instars and eggs. However, when exposure temperature declined to −12.5 °C, no significant differences were observed between the developmental stages in any exposure, suggesting that temperatures as low as −12.5 °C are equally detrimental to all developmental stages. Complete mortality was observed only when early instars, late instars and adults were exposed to −12.5 °C for 120 min. In all tested temperature regimes mean lethal time (LTime₅₀) of pupae was higher compared to the other developmental stages. Similarly, in all exposure times mean lethal temperature (LTemp₅₀) of pupae was lower in relation to the other stages. Non-freezing injury above the SCP was well documented for all stages of *E. kuehniella* indicating a pre-freeze mortality. The potential of using low temperatures to control *E. kuehniella* is discussed.

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1. Introduction

The Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), is one of the most common and important pests in storage facilities, attacking especially flours, grains and dry plant materials. Control of *E. kuehniella* is a necessity worldwide. Conventional chemicals, grain protectants and fumigants, are extensively used around the world to achieve preventive and therapeutic control of *E. kuehniella* in stored commodities. However, the efficacy of chemical insecticides is inconsistent since its use can lead to insecticide resistant populations (Fields, 1992; Mason and Strait, 1998; Loganathan et al., 2011). Moreover, the use of insecticides results in insecticide residues in the treated product and other adverse environmental consequences. Thus alternative methods need to be explored further to optimize monitoring tools and develop other ways of controlling *E. kuehniella*.

Physical control methods, such as use of extreme temperatures, particularly low temperatures, have been extensively used to control stored-product insects as they provide several advantages over chemical control, such as: i) no residues left on the product after treatment, ii) effective against insecticide resistant strains and iii) low risks for the applicators (Fields, 1991, 2001; Arthur, 1996; Fields and White, 2002). Temperature, duration of exposure, species, developmental stage, acclimation, relative humidity, age, cooling rate, accumulation of cryoprotectants and gender determine the survival of insects at low temperature (Lee, 1991; Mason and Strait, 1998; Fields, 1992, 2001; Andreadis et al., 2005, 2008, 2011; Eliopoulos et al., 2011). Data regarding *E. kuehniella* cold hardiness have been very limited so far and with quite controversial and non-comparable results (Mathlein, 1961; Sømme, 1966, 1968; Cox, 1987; Locatelli et al., 1991; Dohino et al., 1999).

The objective of this study was to investigate extensively the cold hardiness profile of *E. kuehniella* at different stages of development (egg, early and late instar larvae, pupa and adult) in respect to IPM programs against stored-product pests. For this purpose various parameters, such as capacity of supercooling, mortality at

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low temperatures as well as lethal temperatures and times after prolonged exposure to sub-zero temperatures above the supercooling point (SCP) were determined. Factors such as temperature level, duration of exposure and age were taken into consideration.

2. Materials and methods

2.1. Insect rearing

The colony of *E. kuehniella* originated from a flour mill in Athens, Greece. Rearing took place in incubators at 25 ± 1 °C, $65 \pm 5\%$ r.h., and total darkness. Insects were maintained in clear plastic boxes ($17 \times 11 \times 5$ cm) each one containing 150–200 moth eggs and 200–250 g of semolina, which provided the larvae with excess food throughout their larval development. Emerging adults remained in the same boxes. All experimental insects were non-acclimated.

2.2. Determination of supercooling points

Early and late instars of *E. kuehniella* prior to determination of SCP were put individually into plastic boxes and left for 3–4 h without food in order to evacuate their gut of any ice nucleating bacteria. Each insect stage sample was placed individually into a transparent plastic capsule (16×7 mm) and immobilized with a cotton plug. A copper-constantan thermocouple (Digitron 2000T, Kalestead Ltd, U.K.) was attached inside the capsule in contact with the surface of each sample to monitor body temperature. Each capsule bearing a sample with a sensor was placed separately in a test tube (diameter 1.7 cm; height 17.5 cm), which was then immersed in a circulating bath (Model 9505, PolyScience, Illinois, U.S.A.) with a solution of ethylene glycol and water (1:1). Cooling rate was set at 1 °C min^{-1} from 20 °C. The supercooling point (SCP) of the individual was taken as the lowest temperature reached before an exothermic event that occurred due to release of latent heat. Overall 10 replicates were prepared for each experimental group.

2.3. Determination of lethal time and lethal temperature

The time and temperature, at which 50% of the treated insects died, was determined by cooling groups of 30 individuals (three replicates of 10 individuals for each treatment) to sub-zero temperatures (–5, –7.5, –10 and –12.5 °C) for 30, 60, 90 and 120 min. Groups of ten individuals of each developmental stage were placed in the bottom of thin-walled test tubes (diameter 1.2 cm; height 10 cm) plugged with foam rubber and then they were immersed into the circulating bath as for the SCP experiment, but directly from room temperature to the desired temperature. Early and late instars were treated in the same way as mentioned above to evacuate the gut. The bath temperature was checked during each experiment using the same thermocouple used for the SCP experiment. After exposure, individuals were transferred to 25 °C under a 16:8 h (L:D) photoperiod for subsequent mortality assessment. Eggs and pupae were left undisturbed at the room temperature for 10 days and considered to have died if no hatch or emergence was noted by that time. Early and late instar larvae as well as adults were assumed to be dead if they did not respond to a gentle prodding after 24 h recovery at 25 °C.

2.4. Statistical analysis

Differences between treatment means of SCP and mortality at each developmental stage were compared by one-way analysis of variance (ANOVA), followed by Tukey's-b test for multiple comparisons. Treatment differences were considered significant at

$P < 0.05$. Lethal time and temperature for 50% mortality (LTime₅₀ and LTemp₅₀, respectively) of all treatments were calculated by probit analysis after correction for control mortality using Abbott's formula (Finney, 1952). Differences of LTime₅₀ and LTemp₅₀ values were based on non-overlapping confidence intervals. Statistical analyses were performed using Minitab 15 Statistical Software (Minitab Inc., 2007).

3. Results

3.1. Supercooling point

In general, all developmental stages of *E. kuehniella* (except eggs which could not be assessed due to their small size) showed an enhanced ability to supercool. However, SCP differed significantly between the developmental stages ($F_{3,36} = 14.729$; $P = 0.000002$). More specifically, mean SCP of pupae was significantly lower (–23.3 °C) in relation to early (L₁–L₃) and late (L₄–L₅) instars (–16.1 and –19.5 °C, respectively), but did not differ from that of adults (–21.6 °C) (Fig. 1).

Similarly, the age of larvae affected significantly their ability to supercool, since SCP of late instars (–19.5 °C) was significantly lower compared to that of early instars (–16.1 °C) (Fig. 1).

3.2. Mortality at sub-zero temperatures

Mortality data of each developmental stage exposed to sub-zero temperatures are shown in Table 1. Complete mortality was achieved only for larvae and adults exposed to –12.5 °C for 120 min. Generally, pupae and adults were more cold-tolerant followed in decreasing order by late instars, early instars and eggs.

Differences among various stages were significant in almost all exposure times at –5 °C (30 min: $F_{4,14} = 7.6$; $P = 0.004$; 60 min: $F_{4,14} = 5.5$; $P = 0.013$; 90 min: $F_{4,14} = 6.0$; $P = 0.01$; 120 min: $F_{4,14} = 17.1$; $P < 0.001$), –7.5 °C (30 min: $F_{4,14} = 6.1$; $P = 0.009$; 60 min: $F_{4,14} = 12.1$; $P < 0.001$; 90 min: $F_{4,14} = 11.1$; $P < 0.001$; 120 min: $F_{4,14} = 9.4$; $P = 0.002$) and –10 °C (30 min: $F_{4,14} = 5.3$; $P = 0.015$; 60 min: $F_{4,14} = 3.9$; $P = 0.04$; 90 min: $F_{4,14} = 3.8$; $P = 0.04$; 120 min: $F_{4,14} = 2.7$; $P = 0.09$). The only exception was that of the individuals exposed to –12.5 °C where mortality levels did not differ significantly with developmental stage at any exposure time (30 min: $F_{4,14} = 1.3$; $P = 0.327$; 60 min: $F_{4,14} = 2.2$; $P = 0.142$; 90 min: $F_{4,14} = 3.1$; $P = 0.067$; 120 min: $F_{4,14} = 2.3$; $P = 0.132$). This may be attributed to the increased mortality observed at this temperature (70–100%) (Table 1). At each temperature differences

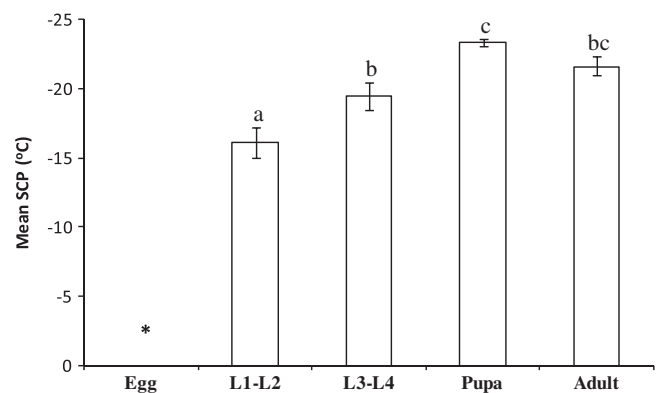


Fig. 1. Mean supercooling point (°C) of adult and immature stages of *E. kuehniella*. Each point represents mean \pm SE ($n = 10$) (*SCP of egg could not be calculated due to its small size, mean SCP values labeled with the same letter are not significantly different, Tukey's-b test, $P < 0.05$).

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