

Effects of low temperatures on adult survival and reproduction of *Rhyzopertha dominica*

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Abstract

Laboratory trials were carried out on the influence of low temperatures on adult survival, reproduction and egg hatch of the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera Bostrichidae). Temperatures of 3, 7, 12, 17 and 27 °C were studied.

Results show the lethal time (LT) required for killing 5%, 50% and 95% of the tested insects was significantly affected by temperature but not adult age. Beetles died sooner at 27 °C than at the lower temperatures. In fact the LT₅ at 27 and 3 °C was 1.12 days and 2.27 respectively. On the contrary, the mortality rate of 95% was reached most quickly at 3 °C (12.57 days) and the time increased as temperature went up. The females of the lesser grain borer were able to oviposit at temperatures between 27 and 7 °C; but after 50 days of incubation at 27 °C only the eggs laid at 27 °C were able to hatch.

Key words: low temperatures, lesser grain borer, survival, reproduction.

Introduction

The lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera Bostrichidae) is an important stored grain pest.

Adults of the lesser grain borer are long lived; males and females reared on wheat at 32.3 °C and 70% RH live in average 19.67 and 17.21 weeks respectively (Birch, 1953). Females need to feed for about 15 days before starting to egg lay (Schwardt, 1933). Fecundity is influenced by temperature and moisture content of food, and it is largest at temperatures between 26 and 34 °C and a m.c. of 9-14% (Howe, 1950). The whole life cycle (from egg to adult) is completed in 25 days at the optimal conditions of temperature and m.c., that are 34 °C and 14% respectively (Birch, 1945, 1953).

In the future, grain protection and disinfestation from stored products insects will rely on alternative methods due to increasing legal restrictions and insecticide resistance. Modification of grain temperatures below the optimal values for insect reproduction and survival is a viable alternative for stored grain protection. Cooling of stored grain offers several advantages including decreased or cessation of feeding, reproduction stops, and mold production, even at moderately elevated humidity, is greatly reduced (Baldo *et al.*, 1987). Insects accustomed to warm environment die in a fairly short period. Death may be due to an accumulation of toxic products (Wigglesworth, 1965), or, also, the incapability to feed (Fields and White, 1997). Moreover grain chilling is a feasible technique to avoid reinfestation from insect pests, and it is also economically advantageous in comparison to traditional conditioning and insecticide treatments of stored-products (Rulon *et al.*, 1999).

Objectives of this study were to assess egg hatching, reproduction, and adult of the lesser grain borer under temperatures of 3, 7, 12, 17, and 27 °C.

Materials and methods

A culture of *R. dominica* was started from adults coming from the stock-colony, reared on soft wheat maintained at 27 °C, at the laboratory of the "Servizio Fitosanitario, Regione Emilia Romagna, sede di Ravenna".

The experimental feeding substrate for the insects consisted of soft wheat, *cv.* Genio. The wheat was treated at 80 °C for two hours into stove and then kept until use in 50-litre PVC tanks with a hermetic seal screw cap.

Before starting experiments a culture was set up in order to obtain adults of known age (i. e. the number of days from emergence from pupal cell). At this purpose the grains used for the mass rearing were sieved and adults collected were divided in seven groups of ten specimens of mixed age and sex; each group was put into a 11-cm Petri dish, and feed with 2 g/specimen of wheat grains. The presence of eggs was checked daily. Once eggs were found inside a Petri dish, adults were discharged and grains with eggs were put in a 2-liter glass jar together with 1000 g of feeding substrate. The jars were closed with a metal cap which had a 4.5-cm diameter hole closed with a fine-mesh brass net to prevent insects from escaping and to allow air circulation, and were maintained in an incubator at the temperature of 27 ± 1 °C.

The adults of *R. dominica* used in our experiments were obtained by sieving daily the jar content with 2 sieves of 1.7 and 0.4 mm. Groups of 6-18 unsexed adults were then transferred to 11-cm Petri dishes with 2 g grain/specimen (moisture content of wheat was 11%) and sealed with Parafilm®. Three age classes of insects were formed according to the time from their emergence: 1-3 d old; 4-5 d old and 6-7 d old. Each class of insects was tested at the following temperatures: 3, 7,

12, 17 and 27 °C (control). There were 3 replicates per experimental treatment. The dishes were checked daily to count and remove the dead insects. Following removal of dead insects, dishes were immediately placed back in the same incubator at the tested temperatures.

After the death of the last specimen in each group the dish was checked to detect eggs: every grain was observed under a binocular microscope and in case of finding eggs, usually deposited singly or in groups along the ventral furrow, the grain with eggs was isolated with a paper cylinder. Dishes were then placed in an environmental chamber maintained at 27 ± 1 °C in order to allow the egg hatching and insect development. After 50 days, i.e. approximately the time necessary to complete the development from egg to adult (Hagstrum and Flinn, 1994; Hagstrum, 1996), the dishes were checked again for offspring.

The survival of *R. dominica* adults at the tested temperatures was calculated as daily percent of survivors transformed to Probits and regression of Probits on

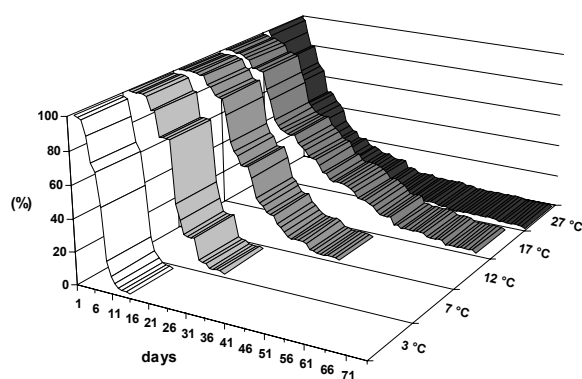


Figure 1. The survivorship of *R. dominica* adults exposed to different temperatures.

natural logarithm of time were calculated. Then the lethal time (LT) for 5%, 50% and 95% for each temperature and class of age were estimated using the linear calibration technique (Snedecor and Cochran, 1967) and compared by two way ANOVA with means separation procedure following the method of Fisher's least significant difference (LSD).

Results and discussion

The times required to kill the 5%, 50% and 95% of the tested insects varied significantly among temperatures but not age of beetles (table 1).

About the differences in mortality level obtained at different temperatures it is possible to note that the insects started to die faster at the control temperature than at the lower temperatures tested (figure 1). In fact at the temperature of 27 °C the 5% of the insects died just after 1.12 day whereas the same level of mortality was reached after 2.27 days at 3 °C. For this level of mortality no differences occurred among temperatures of 7, 12 and 17 °C. The time required to kill the fifty percent of beetles (LT_{50}) was also affected by the temperature. In this case the level 50% of mortality was reached faster by the insects exposed to 3 °C, followed by those exposed at the temperatures of 27 °C and 7 °C. No difference in LT_{50} was observed between the specimens maintained at 12 and 17 °C. Finally, it was demonstrated that the 95% of insects were killed faster at the lowest temperature (12.5 days). At the temperature of 7 °C the same level of mortality was reached in about 21 days. No differences were observed between the control temperature (27 °C) and those of 17 or 12 °C (table 2).

About the ability of the beetles to reproduce under low temperatures, it was noted that within the range of temperatures between 27 and 7 °C the insects were able to oviposit. Only in a Petri dish maintained at 17 °C the eggs were absent. In any case none of them, with the

Table 1. Two way ANOVA for regression curves of Probit on Natural logarithm (Ln) of time.

	LT_5				LT_{50}				LT_{95}			
	df	Mean square	F	p	df	Mean square	F	p	df	Mean square	F	p
Age	2	0.24	0.78	0.47	2	0.02	0.22	0.81	2	0.04	0.17	0.85
Temperatures	4	6.77	21.82	<0.0001	4	2.77	26.24	<0.0001	4	3.85	16.38	<0.0001
Interaction	8	0.13	0.43	0.89	8	0.12	1.21	0.33	8	0.19	0.82	0.59
Residual	30	0.31			30	0.11			30	0.24		

Table 2. Comparison of LT at different temperatures by 95% LSD.

Temperatures (°C)	n	LT_5 (days)	95% confidence interval	LT_{50} (days)	95% confidence interval	LT_{95} (days)	95% confidence interval
3	9	2.27	b (1.55-3.31)	5.34	d (4.28-6.66)	12.57	c (9.03-17.49)
7	9	5.80	a (3.97-8.47)	11.00	b (8.82-13.73)	20.89	b (15.01-29.06)
12	9	7.55	a (5.17-11.03)	16.53	a (13.25-20.63)	36.19	a (26.01-50.36)
17	9	8.31	a (5.69-12.15)	21.44	a (17.19-26.75)	55.31	a (39.76-76.96)
27	9	1.12	c (0.77-1.64)	8.05	c (6.45-10.04)	57.58	a (41.39-80.12)

Within a column means followed by the same letter are not significantly different.

Table 3. Eggs laid by *R. dominica* adults exposed to different temperatures.

Temperatures (°C)	adults emerged within 1-3 days		adults emerged within 4-5 days		adults emerged within 6-7 days	
	Petri dishes with eggs	Petri dishes without eggs	Petri dishes with eggs	Petri dishes without eggs	Petri dishes with eggs	Petri dishes without eggs
3	0	3	1	2	1	2
7	3	0	3	0	3	0
12	3	0	3	0	3	0
17	3	0	3	0	2	1
27	3	0	3	0	3	0

exception of those laid at the control temperature (27 °C), were hatched after 50 days of incubation at 27 °C. In contrast, at the temperature of 3 °C, the oviposition was strongly affected, mainly for the younger insects tested (table 3).

Conclusions

The exposure of *R. dominica* adults to low temperatures apparently caused a delay of mortality compared to the insect maintained at optimal temperature of 27 °C. This effect was probably due to a general slowing down of the metabolic activity of the insect which affected also the rate of mortality. This effect was observed also for the mortality level of fifty percent of the insects. In fact this level of killed insects was obtained faster at the control temperature than at the temperatures of 17, 12 and 7 °C. Only the lowest temperature of 3 °C caused an increase of mortality rate of the beetles. In any case, for the purposes of a cold treatment is more interesting to discriminate the temperatures that can cause the faster decline of an adult population of insects. In our experiments the death of the 95% of the *R. dominica* adults was significantly faster only at 3 or 7 °C but not at higher temperatures. In fact at 7 °C we should expect a quite complete decline of the population after about 21 days.

The low temperatures affected also the ability of the lesser grain borer to reproduce. In fact, with the assumption that the unsexed adult tested were at a sex ratio of 50%, as shown by Faroni and García-Mari (1992), we mainly noted a reduction in egg survival or egg vitality rather than in the ability to oviposit. In fact in our experiments we emphasized that the females of *R. dominica* can lay eggs also at a temperature of 3 °C, well below the thermic threshold of 16 °C observed for egg deposition by Golebiowska (1962). In any case none of the eggs hatched, so the ability to reproduce was completely inhibited at temperatures equal or under 17 °C. This happens when the insect is not subjected to cold acclimation. On the contrary the lesser grain borer is able to reproduce until 5 °C (Hagstrum and Flinn, 1994).

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