Effectiveness of carbon dioxide under reduced pressure against some insects infesting dried fruit

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Abstract

The effectiveness of CO_2 at different temperatures (20, 25 and 30°C) and exposure times (6, 9, 8, 24, 36, 48, 60 hours) in a vacuum autoclave (34.6–44 kPa) has been tested against the life stages (eggs, I and II instar larvae and mature larvae) of *Ephestia cautella* (Walk.), *Plodia interpunctella* (Herbst) and *Tribolium castaneum* Hbn. (eggs, larvae, pupae and adults).

Tests were carried out in a vacuum chamber ($3m^3$) and the samples were placed in the middle of 1 m^3 pallet of almonds.

Ninety-five and 99% mortalities were achieved after 55 and 64 hours at 20°C, 41 and 48 hours at 25°C, and 31 and 37 hours at 30°C. Pupae proved to be most resistant to the treatment. The I and II instar larvae of *Plodia interpunctella* and *Ephestia cautella* were less resistant than mature larvae. The LT_{99} at 20°C for the early and mature larvae of *Plodia interpunctella* are 40 and 64 hours, 27 and 48 hours at 25°C and 12 and 34 hours at 30°C respectively.

Introduction

Several species of insect are very common pests of dried fruit. These include, particularly, the moths *Ephestia cautella* (Walker), *E. elutella* Hubner, *E. figulilella* Greg. and *Plodia interpunctella* (Hübner), (Phycitidae) and the beetles, *Tribolium* spp. (Tenebrionidae) and *Oryzaephilus* spp. (Cucujidae). These polyphagous species live on a variety of foodstuffs ranging from flour to finished products, from tobacco to cocoa beans and almond meal. However, raisins, hazelnuts and almonds are most frequently infested.

Temperatures in warehouses are usually sufficient to permit the development of these species. They are often present in raw materials, but they can establish in processing premises and then infest finished products. For this reason it is important to control infestation of raw materials in storage.

Low temperature storage is usually used as a preventative measure against development of infestation as the activity and rate of reproduction of the insects is limited. High temperature treatment is used for products that can tolerate the treatment and, if there is a substantial infestation, fumigation at atmospheric pressure or under vacuum may be required (Bond, 1984).

Although there are fumigants currently available for treatment of dried fruit, there is an increasing preference for non-chemical methods that are intrinsically safe for humans. In the authors' opinion, it is better to develop alternative methods of pest control, such as controlled atmospheres (Jay et al. 1970; Gaunce et al. 1982; Soderstrom et al. 1991).

The present study was undertaken to see if a combination of factors: low pressure, high temperature and exposure to CO_2, could be used successfully to disinfest dried fruit.

Materials and Methods

The experiments were carried out on *E. cautella*, *P. interpunctella* and *T. castaneum*. Samples of the insects, at different stages of development, were treated with a high CO_2 atmosphere at low pressure inside a vacuum chamber (3 m^3). Their survival was observed. The chamber was the one previously used to assess control of rice pests (Locatelli and Daolio, 1993).

Test material was reared in incubators at 26 ± 1°C, 70 ± 5% r.h.

Samples of insects to be exposed were placed in gauze bags (0.2 mm mesh). For *T. castaneum*, samples of 20 adults, 20 mixed-age larvae or 20 pupae were used. For *P. interpuntella* and *E. cautella* samples of 20 first and second instar larvae, 20 mature larvae or 20 pupae were tested. Adults were not exposed as they are notoriously susceptible to the treatment.

Eggs of *T. castaneum* for testing were obtained by allowing 100 adults to lay for 5 days on 50 g samples of food substrate (wheat bran, whole wheat flour and wheat germ).

Eggs of the moths were obtained by allowing 50 gravid females to lay in a special plexiglass cylinder (15 cm diam., 40 cm high) fitted with a gauze base (18-mesh) through which the eggs could fall. The cylinders were held at 26 ± 1°C, 70 ± 5% r.h. on a 12 hours light: 12 hours dark cycle. Tests were carried out on 30–40-hour-old eggs, held under the laying conditions.

The test samples in the gauze bags were placed in the middle of 1 m^3 pallet loads of almonds held in jute sacks (50 kg). This is the form in which they are usually traded.

The exposure chamber was initially at 20°C, but a special heating system could be used to give the required temperature (20, 25 or 30°C) for each run. At the start of the exposure, the atmosphere within the chamber was modified by a vacuum pump to give an absolute pressure of between 34.6 and 37.3 kPa and by introduction of CO_2 to give a composition of 98% CO_2 by volume. At this composition the oxygen content was 0.5% or less. These conditions were maintained for test periods of 6 to 60 hours. At the end of the tests the internal pressure ranged from 38.7 to 44.0 kPa. It was necessary to maintain a high degree of vacuum throughout the entire exposure period to obtain the most rapid control.

Survival in the test samples was assessed immediately after completion of the exposure period and also 24 hours later, to check on delayed mortality. Surviving larvae and pupae were incubated until adult emergence. Each test was repeated four times, usually with four untreated controls. In some cases an estimate of control mortality was obtained from preliminary experiments. No appreciable mortality was recorded in untreated control batches of all larvae of *E. cautella* and *P. interpunctella*, and of larvae and adults of *T. castaneum*. Control survival in pupae of *P. interpunctella* in preliminary

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experiments was found to be 17 ± 1.0 and was assumed to be so for later tests.

Statistical analysis of the results was carried out using probit transformation to estimate lethal times for 95 and 99% mortality.

Results

Average survival times and standard deviations are given in Tables 1, 2, 3 for the different test species and stages, and different temperatures. Regression parameters from the probit analyses of these results and estimted LT_{95} and LT_{99} are given in Table 4. Goodness of fit and adequacy of the model are indicated by chi-square and significance levels (P).

The estimates for complete control (LT_{99}) can be used to indicate the most resistant stages and species to the treatment. Control regimens can be sent on the basis of these values and the best conditions for the foodstuff to be treated.

For *E. cautella*, the most resistant stages were the pupae and mature larvae. At 20°C, pupae and mature larvae required 63 and 58hours respectively. These times are much longer than required to kill first and second instar larvae (40 hours). At 25 and 30°C the LT_{99} of mature larvae and pupae were very similar, 45 and 43 hours and 31 hours each, respectively. First and second instar larvae and eggs were much more susceptible.

For *P. interpunctella*, mature larvae and pupae were also the most tolerant, with estimates of LT_{99} at 20, 25 and 30°C of 64, 60, 48, 48 and 34, 37 hours, respectively, and again, first and second instar larvae and eggs were more susceptible.

For *T. castaneum*, eggs and pupae were the most tolerant stages, with estimates of LT_{99} at 20, 25 and 30°C of 46, 46, 44, 44 and 17, 33 hours, respectively. The low value for the egg tolerance at 30°C is based on only two observations and has a large range of confidence limits.

There was no particular difference in the species in their response to the low pressure controlled atmosphere treatment. Eggs of the three species are the only stage to show markedly different tolerances.

Figures 1, 2 and 3 give the mortality data for *E. cautella*, *P. interpunctella* and *T. castaneum* as a plot of probit mortality against time. For most species and stages, the slope of the line doubles for increase in temperature from 20 to 30°C. Exceptions are the pupal stages of *P. interpunctella* and *T. castaneum*, mature larvae in *E. cautella* and adults of *T. castaneum*, all of which show lesser sensitivity to change in temperature.

Discussion

The probit transformation of the mortality data allows extrapolation to give the time required to give complete control, here taken to be the LT_{99}. It is also possible to extrapolate the LT_{99} expected to other temperatures, (Fig. 4) though this must be done continuously as the data values were only obtained for a narrow range of temperatures and the relation between LT_{99} and temperature may not be linear.

Table 1. Average survival (±SD) rate of different stages of *Ephestia cautella* (Walk.) and of control with the different temperatures and exposure times at chamber atmosphere of 98% CO_2 and $O_2 < 0.5\%$; absolute pressure 34.6–37.3 kPa.

Stage	Temp. (°C)		Exposure times (hours)						
			6	9	18	24	36	48	60
Eggs									
	20	treated	26 ± 4.2	21 ± 3.0	16 ± 1.7	10 ± 2.2	3.5 ± 2.6	0.2 ± 0.5	0
		control	41 ± 1.8	43 ± 3.9	42 ± 3.7	39 ± 2.2	38 ± 4.1	39 ± 3.3	31 ± 4.1
	25	treated	18 ± 2.6	12 ± 1.3	7.0 ± 0.8	1.3 ± 1.5	0		
		control	39 ± 2.1	41 ± 1.8	42 ± 3.4	36 ± 7.0	42 ± 3.9		
	30	treated	7.0 ± 2.6	6.0 ± 1.8	1.3 ± 1.5	0			
		control	41 ± 2.6	44 ± 3.7	41 ± 2.6	43 ± 4.2			
I and II instar larvae									
	20	treated	19 ± 1.0	18 ± 1.0	11 ± 1.0	6.5 ± 1.3	1.0 ± 1.4	0	
	25	treated	12 ± 1.3	7.5 ± 1.3	2.8 ± 1.0	0			
	30	treated	8.8 ± 1.0	5.3 ± 1.0	0				
Mature larvae									
	20	treated	20 ± 1.0	18 ± 0.5	16 ± 0.8	13 ± 1.0	7.3 ± 1.0	0.8 ± 1.0	0
	25	treated	19 ± 1.3	17 ± 1.3	13 ± 1.3	9.5 ± 1.3	0		
	30	treated	17 ± 1.0	11 ± 1.5	6.8 ± 1.0	1.0 ± 1.4	0		
Pupae									
	20	treated	17 ± 0.8	16 ± 0.6	13 ± 0.8	11 ± 0.8	8.5 ± 0.6	0.8 ± 1.5	0
		control	18 ± 0.8	18 ± 0.6	18 ± 1.0	18 ± 1.0	18 ± 1.0	18 ± 1.0	18 ± 0.8
	25	treated	16 ± 1.0	15 ± 1.0	12 ± 2.2	7.8 ± 1.9	0.8 ± 1.0	0	
		control	18 ± 1.0	18 ± 1.0	17 ± 1.0	17 ± 1.0	18 ± 0.8	17± 0.5	
	30	treated	16 ± 0.8	13 ± 1.3	6.3 ± 1.0	1.0 ± 1.2	0		
		control	18 ± 1.0	18 ± 0.8	16 ± 1.0	19 ± 0.6	18 ± 0.8		

Notes: The number or survivors in the control of I and II instar larvae and mature larvae was assumed to be 20, since no appreciable mortality was recorded in preliminary tests.

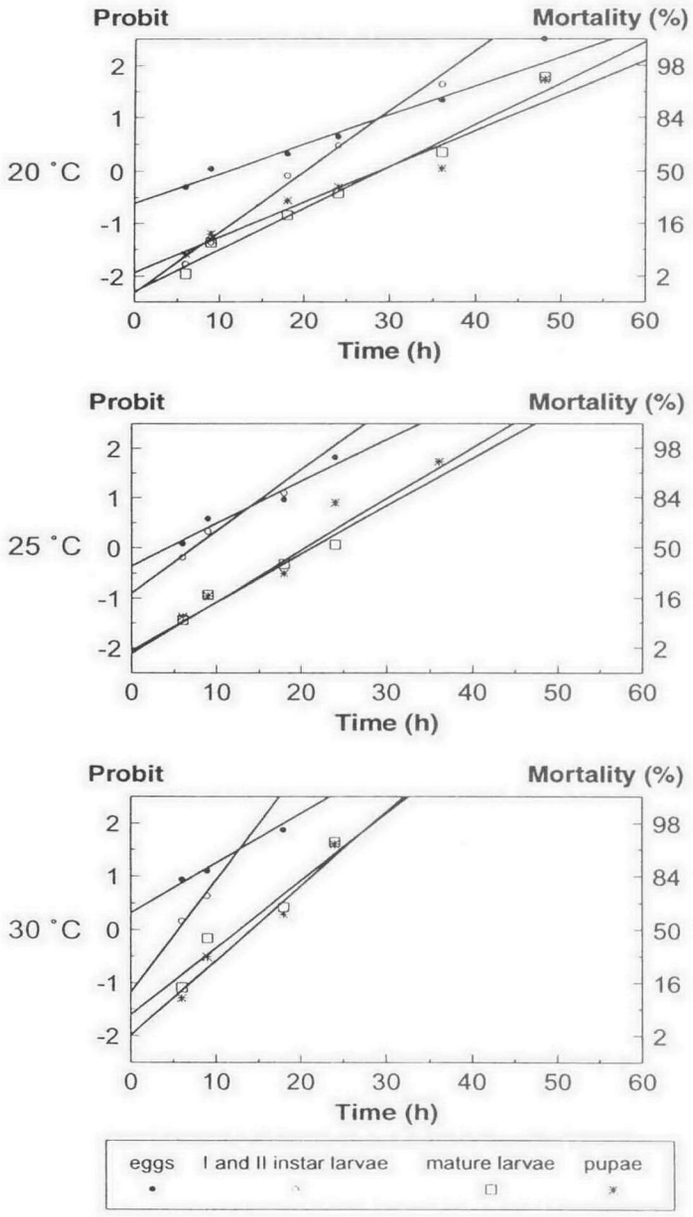

Fig.1. Mortality of *Ephestia cautella* (Walk.) eggs, I and II instars larvae, mature larvae and pupae with different temperatures (20, 25 and 30°C) and exposure times at chamber atmosphere 98% CO_2; O_2< 0.5%; absolute pressure 34.6–37.3 kPa.

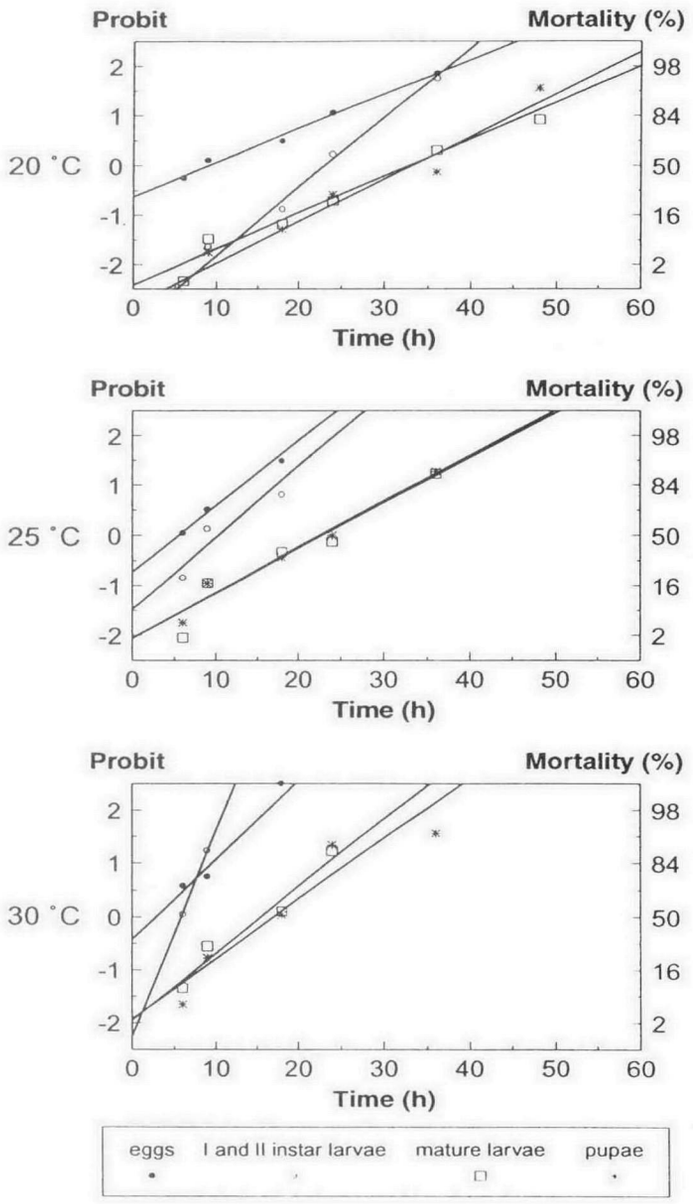

Fig. 2. Mortality of *Plodia interpunctella* (Hbn.) eggs, I and II instars larvae, mature larvae and pupae with different temperatures (20, 25 and 30°C) and exposure times at chamber atmosphere 98% CO_2; O_2< 0,5%; absolute pressure 34.6–37.3 kPa.

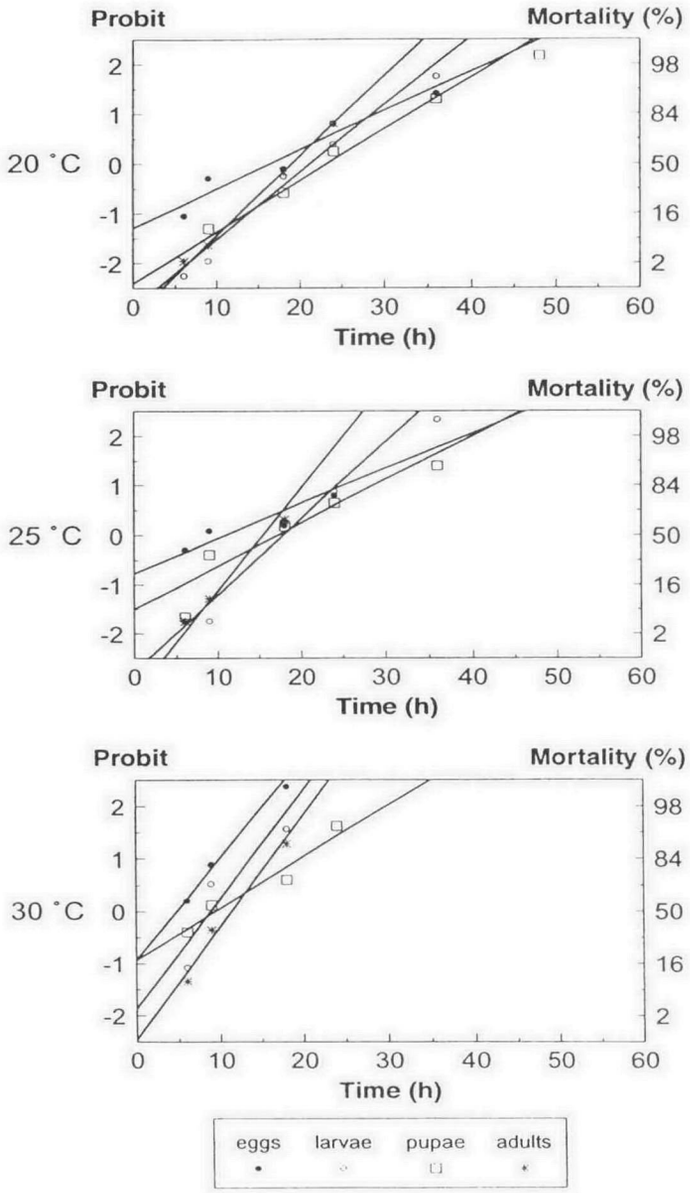

Fig. 3. Mortality of *Plodia interpunctella* (Hbn.) eggs, I and II instars larvae, mature larvae and pupae with different temperatures (20, 25 and 30°C) and exposure times at chamber atmosphere 98% CO_2; O_2< 0,5%; absolute pressure 34.6–37.3 kPa.

However, the general co clusion is that there is a need to adjust the time of treatment for the temperature of the foodstuff treated.

Table 5 gives the LT_{99} for the most tolerant stage of each of the three species tested for each of the three exposure temperatures. Complete kill (LT_{99}) of all stages of all the species was reached in 37 hours at 30°C and 64 hours at 20°C.

Our results indicate that carbon dioxide under reduced pressure is less toxic at lower temperatures, as shown by many authors for controlled atmospheres at ambient pressure. If the temperature is below the optimum for insect development, its metabolism is slower and thus less oxygen is required and the effect of the controlled atmosphere is lessened (Bailey and Banks 1980; Jay 1986).

The experiments described here show the different stages of the phycitid moths to be very tolerant at all temperatures compared with those of *T. castaneum*. Also, the mature larvae and pupae were found to be the most tolerant stages.

Generally, the last larval instar is reported to be the most tolerant stage to CO_2 (Jay 1984). It also has been observed with other species exposed to CO_2 that the pupae are very tolerant. Pupae, in the presence of unfavourable ambient conditions, can modify their metabolism to assist survival (Lindgren and Vincent 1970; Childs and Overby 1983).

The tolerance of *P. interpunctella* in the experiments described here was higher than observed previously (Locatelli and Daolio 1993). However, in the present case the test insects were placed in the middle of a pallet of bagged almonds where they were more protected. Under these conditions the rate of access of the CO_2 to the insects is slower and it takes a longer time to heat the whole mass of tested product to the test temperature.

Time required to obtain disinfestation with carbon dioxide under reduced pressure is considerably shorter than for normal controlled atmospheres (Annis 1987), but longer than required for vacuum fumigation with methyl bromide (Bond 1984).

Though the increase in temperature results in a higher mortality and could be useful in reducing the treatment period, some foodstuffs could be subject to changes in organoleptic quality and the use of increased temperature for reducing treatment time will be restricted to some products only. Undoubtedly, when assessing the utility of this method for other commodities, the possible organoleptic and quality changes will have to be considered. However, the use of this method is particularly appropriate, despite the technical difficulties, where the traditional disinfestation methods result in a high level of residues of the active material.

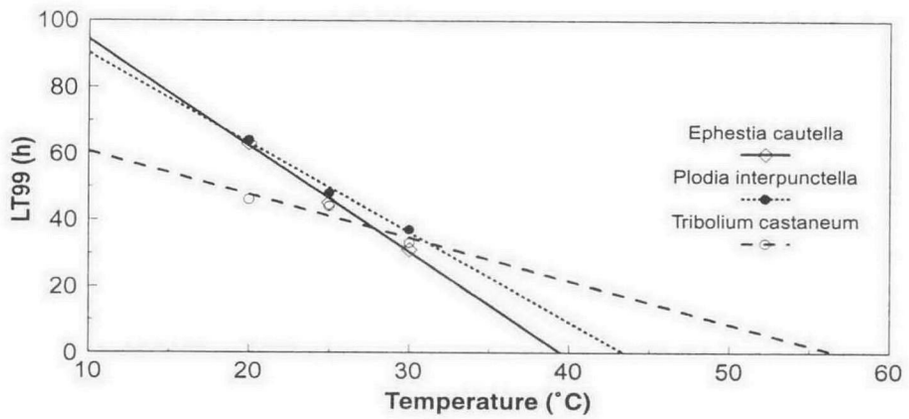

Fig. 4. Plot of the regression lines of LT_{99} and temperature for the *Ephestia cautella* (Walk.), *Plodia interpunctella* (Hbn.) and *Tribolium castaneum* Herbst.

Table 2. Average survival (±SD) rate of different stages of *Plodia interpunctella* (Hbn.) and of control with the different temperatures and exposure times at chamber atmosphere of 98% CO_2 and $O_2 < 0.5\%$; absolute pressure 34.6–37.3 kPa.

Stage	Temp. (°C)		Exposure times (hours)						
			6	9	18	24	36	48	60
Eggs									
	20	treated	23 ± 2.9	18 ± 2.2	12 ± 2.8	5.5 ± 1.3	1.3 ± 1.5	0	
		control	39 ± 3.9	39 ± 2.9	38 ± 1.7	38 ± 4.2	40 ± 2.9	40 ± 2.8	
	25	treated	17 ± 2.2	13 ± 2.1	2.8 ± 2.2	0			
		control	35 ± 4.6	42 ± 3.9	40 ± 2.9	40 ± 6.8			
	30	treated	11 ± 2.2	8.8 ± 1.7	0.3 ± 0.5	0			
		control	40 ± 3.3	39 ± 2.6	41 ± 1.7	41 ± 1.9			
I and II instar larvae									
	20	treated	20 ± 0	19 ± 1.2	16 ± 1.0	8.3 ± 1.7	0.8 ± 1.0	0	
	25	treated	16 ± 1.8	9.0 ± 0.8	4.3 ± 1.7	0			
	30	treated	9.5 ± 1.3	2.3 ± 1.5	0				
Mature larvae									
	20	treated	20 ± 0.5	19 ± 0.6	18 ± 0.6	15 ± 1.0	7.5 ± 0.6	3.5 ± 0.6	0
	25	treated	20 ± 0.6	17 ± 1.3	13 ± 1.3	11 ± 1.2	2.3 ± 1.3	0	
	30	treated	18 ± 0.5	14 ± 1.7	9.3 ± 1.0	2.3 ± 1.5	0		
Pupae									
	20	treated	17 ± 0.8	16 ± 1.0	15 ± 0.5	12 ± 1.0	9.3 ± 0.5	1.0 ± 2.0	0
	25	treated	16 ± 1.0	14 ± 0.8	11 ± 1.0	8.5 ± 1.3	1.8 ± 1.6	0	
	30	treated	16 ± 0.8	13 ± 1.3	8.3 ± 1.0	1.5 ± 1.7	1.0 ± 1.4	0	

Notes: The number or survivors in the control of I and II instar larvae and mature larvae was assumed to be 20, since no appreciable mortality was recorded in preliminary tests. The number of survivors in the control of pupae was measured only in the first part of the experiment and the same mortality was assumed as constant throughout the whole experiment. The number of survivors in this case was 17 ± 1.0.

Table 3. Average survival (±SD) rate of different stages of *Tribolium castaneum* (Herbst) and of control with the different temperatures and exposure times at chamber atmosphere of 98% CO_2 and $O_2 < 0.5\%$; absolute pressure 34.6–37.3 kPa.

Stage	Temp. (°C)		Exposure times (hours)						
			6	9	18	24	36	48	60
Eggs									
	20	treated	25 ± 4.2	19 ± 1.7	17 ± 1.7	6.0 ± 1.8	2.3 ± 1.7	0	
		control	29 ± 2.8	31 ± 3.4	31 ± 2.6	28 ± 2.8	28 ± 1.7	30 ± 1.3	
	25	treated	19 ± 2.5	13 ± 2.8	12 ± 2.5	6.8 ± 1.3	0		
		control	31 ± 4.0	29 ± 2.1	28 ± 2.2	32 ± 2.4	30 ± 1.7		
	30	treated	13 ± 2.8	5.5 ± 2.1	0.3 ± 0.5	0			
		control	32 ± 2.6	29 ± 2.2	29 ± 2.8	29 ± 2.2			
Larvae									
	20	treated	20 ± 0.5	20 ± 1.0	12 ± 2.2	7.0 ± 0.8	0.8 ± 1.0	0	
	25	treated	19 ± 1.0	19 ± 1.0	7.8 ± 1.7	3.8 ± 2.6	0.3 ± 0.5	0	
	30	treated	17 ± 1.0	6.0 ± 2.2	1.3 ± 1.5	0			
Pupae									
	20	treated	16 ± 1.3	14 ± 1.4	11 ± 1.8	6.0 ± 1.4	1.5 ± 1.9	0.3 ± 0.5	0
		control	16 ± 1.3	16 ± 1.3	15 ± 1.0	15 ± 0.8	16 ± 1.3	16 ± 1.0	16 ± 0.6
	25	treated	15 ± 1.3	9.8 ± 1.0	6.5 ± 1.3	4.0 ± 0.8	1.3 ± 1.5	0	
		control	15 ± 1.2	15 ± 0.9	15 ± 0.8	15 ± 1.0	16 ± 0.9	16 ± 1.0	
	30	treated	10 ± 0.8	7.0 ± 0.8	4.3 ± 1.0	0.8 ± 1.0	0		
		control	15 ± 1.3	15 ± 0.8	15 ± 1.0	15 ± 1.5	16 ± 0.6		
Adults									
	20	treated	20 ± 0.6	19 ± 0.8	11 ± 1.0	4.3 ± 1.0	0		
	25	treated	19 ± 0.5	18 ± 1.4	7.5 ± 1.9	0			
	30	treated	18 ± 0.5	13 ± 2.2	2.0 ± 2.5	0			

Notes: The number or survivors in the control of larvae and adults was assumed to be 20, since no appreciable mortality was recorded in preliminary tests.

Table 4 Regression parameters of the probit lines (intercept, slope and observed significance level of chi-square statistic, P) and lethal times to kill 95 and 99% (LT_{95} and LT_{99}) estimated for different stages of *Ephestia cautella* (Walk.), *Plodia interpunctella* (Hbn.), and *Tribolium castaneum* (Herbst) with different temperatures at chamber atmosphere of 98% CO_2 and $O_2 < 0.5\%$; absolute pressure 34.6–37.3 kPa.

Species	Stage	Temp. (°C)	Intercept	Slope ± SE	P	LT_{95} (h) (95% CL)	LT_{99} (h) (95% CL)
Ephestia cautella (Walk.)							
	Eggs						
		20	–0.61	0.06 ± 0.008	0.916	41 (35–50)	53 (44–67)
		25	–0.35	0.08 ± 0.017	0.854	24 (20–32)	32 (26–45)
		30	0.32	0.09 ± 0.031	0.993	14 (11–24)	22 (16–44)
	I and II instar larvae						
		20	–2.31	0.11 ± 0.018	0.989	34 (30–42)	40 (34–51)
		25	–0.89	0.12 ± 0.030	0.960	20 (17–30)	26 (2041)
		30	–1.17	0.21 ± 0.088	0.999	13 (10–39)	16 (12–56)
	Mature larvae						
		20	–2.29	0.08 ± 0.011	0.930	50 (44–59)	58 (51–71)
		25	–2.11	0.10 ± 0.017	0.685	36(31–45)	43 (36–55)
		30	–1.60	0.12 ± 0.024	0.739	26 (22–34)	31 (2642)
	Pupae						
		20	–1.93	0.07 ± 0.010	0.603	53 (46–65)	63 (54–79)
		25	–2.05	0.10 ± 0.016	0.903	38 (33–48)	45 (38–59)
		30	–1.98	0.14 ± 0.026	0.915	26 (22–34)	31 (26–41)
Plodia interpunctella (Hbn.)							
	Eggs						
		20	–0.62	0.07 ± 0.011	0.973	33 (28–41)	43 (36–55)
		25	–0.72	0.13 ± 0.027	0.986	18 (15–24)	23 (19–32)
		30	–0.41	0.15 ± 0.044	0.962	14 (11–22)	18 (14–33)
	I and II instar larvae						
		20	–3.22	0.14 ± 0.024	0.953	35 (31–42)	40 (3449)
		25	–1.47	0.14 ± 0.029	0.660	22 (18–29)	27 (22–37)
		30	–2.24	0.38 ± 0.150	.999	10 (8.5–22)	12 (9.6–30)
	Mature larvae						
		20	–2.41	0.07 ± 0.010	0.887	55 (49–65)	64 (56–67)
		25	–2.05	0.09 ± 0.014	0.760	41 (35–50)	48 (41–61)
		30	–1.94	0.13 ± 0.022	0.872	28 (24–36)	34 (2844)
	Pupae						
		20	–2.83	0.08 ± 0.013	0.726	52 (46–63)	60 (53–74)
		25	–2.05	0.09 ± 0.015	0.934	40 (35–51)	48 (40–62)
		30	–1.92	0.11 ± 0.020	0.495	31 (27–40)	37 (31–49)
Tribolium castaneum (Herbst)							
	Eggs						
		20	–1.29	0.08 ± 0.011	0.515	37 (32–46)	46 (39–58)
		25	–0.76	0.07 ± 0.012	0.367	34 (29–44)	44 (36–59)
		30	–0.92	0.19 ± 0.065	0.997	13 (11–24)	17 (13–35)
	Larvae						
		20	–2.88	0.14 ± 0.023	0.968	33 (2940)	38 (33–48)
		25	–2.75	0.15 ± 0.026	0.802	28 (25–34)	33 (28–41)
		30	–1.86	0.21 ± 0.049	0.345	17 (14–24)	20 (16–29)
	Pupae						
		20	–2.41	0.10 ± 0.018	0.924	39 (34–49)	46 (39–59)
		25	–1.50	0.09 ± 0.017	0.698	36 (30–48)	44 (36–60)
		30	–0.93	0.10 ± 0.025	0.960	26 (21–40)	33 (26–54)
	Adults						
		20	–3.05	0.16 ± 0.028	0.999	29 (26–36)	33 (2942)
		25	–3.20	0.21 ± 0.034	0.911	23 (21–28)	27 (23–33)
		30	–2.44	0.21 ± 0.040	0.979	19 (16–24)	22 (19–29)

Notes: SE= standard error; CL=confidence limits; h=hours

Table 5. Time needed to kill the 99% (LT_{99}) of individuals of the most resistant stage of the three species investigated, at different experimental temperatures in chamber atmosphere conditions of 98% CO_2 and O_2 < 0.5%; absolute pressure 34.6 – 37.3 kPa

Temp. (°C)	LT_{99} (hours)		
	Ephestia cautella	*Plodia interpunctella*	*Tribolium castaneum*
20	63	64	46
25	45	48	44
30	31	37	33

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