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Original article (Orijinal araştırma)

Inhibition of egg development by hypercarbia and hypoxia in almond moth, *Ephestia cautella* (Walker, 1863) (Lepidoptera: Pyralidae)

İncir kurdu *Ephestia cautella* (Walker, 1863) (Lepidoptera: Pyralidae)'nın yumurta gelişiminin yüksek karbondioksit ve düşük oksijenle engellenmesi

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Summary

Hypercarbia-induced delay in the development of eggs was investigated in almond moth, *Ephestia cautella* (Walker, 1863) (Lepidoptera: Pyralidae), using two controlled atmospheres (CAs), 85% CO_2 + 3% O_2 (balance N_2) and 95% CO_2 + 1% O_2 (balance N_2) between 2012 and 2014 in Stored Products Pests Laboratory, Agricultural Faculty, Ankara University. Eggs of *E. cautella* (1-3 day-old) were exposed to both CAs for a wide a range of exposure periods of up to 104 h at three temperatures of 20±1, 25±1 and 30±1°C at 65±5% RH. In general, both CAs caused delay in egg development by 1 to 8 d. Inhibitory effects were more pronounced at lower temperatures. A maximum delay of 8 d was recorded at 20°C for the three-day-old eggs exposed to 95% CO_2 plus 1% O_2 for 88 h. Short exposure periods caused short term delays in development. Four h exposure caused 1d delay in three-day-old eggs exposed to 95% CO_2 plus 1% O_2 at 25°C. In practice, total egg hatch including delays lasted 5 d at 30°C, 8 d at 25°C, and 12 d at 20°C, which must be taken into account for successful CAs applications.

Keywords: Delayed development, egg hatching, *Ephestia cautella*, high carbon dioxide, low oxygen

Özet

İncir kurdu olarak bilinen *Ephestia cautella* (Walker, 1863) (Lepidoptera: Pyralidae) isimli zararlıya %85 CO_2 + %3 O_2 (denge gaz N_2) ve %95 CO_2 + %1 O_2 (denge gaz N_2) kompozisyonundaki iki farklı kontrollü atmosferin (KA) uygulanmasıyla yüksek karbondioksitli atmosferlerin yumurta gelişiminde oluşturduğu gecikme 2012-2014 yılları arasında Ankara Üniversitesi Ziraat Fakültesi Depolanmış Ürün Zararlıları Laboratuvarında yapılan çalışmada incelenmiştir. *Ephestia cautella*' nın 1-3 gün-yaşlı yumurtaları 20±1, 25±1 ve 30±1°C sıcaklık ve %65±5 orantılı nem koşullarında her iki KA kompozisyonuna 104 saate kadar varan değişik sürelerde maruz bırakılmıştır. Genel olarak, her iki KA 1-8 gün aralığında yumurta gelişiminde gecikmeye yol açmıştır. Gecikme düşük sıcaklıklarda daha dikkate değer bulunmuştur. Maksimum gecikme 20°C sıcaklıkta %95 CO_2 + %1 O_2 konsantrasyonuna 88 saat süreyle maruz kalan üç-gün-yaşlı yumurtalarda sekiz gün olarak tespit edilmiştir. Kısa uygulama süresi gelişmede kısa süreli gecikmeye neden olmuştur. Dört saatlik uygulama süresi %95 CO_2 + %1 O_2 konsantrasyonuna 25°C sıcaklıkta maruz kalan üç-gün-yaşlı yumurtada bir günlük gecikmeye neden olmuştur. Gecikmeyi de içeren yumurta açılımı toplam süresi 30°C de 5 gün, 25°C de 8 gün ve 20°C de 12 gündür. Dolayısıyla KA uygulamalarını başarıyla uygulayabilmek için bu sürelerin dikkate alınması önemlidir.

Anahtar sözcükler: Gelişimde gecikme, yumurta açılımı, *Ephestia cautella*, yüksek karbondioksit, düşük oksijen

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Introduction

Almond moth, *Ephestia cautella* (Walker, 1863) (Lepidoptera: Pyralidae), is not only an important international pest of stored cereals and various food commodities, but also the most detrimental pest of dried figs, which are of significant export value for Turkey. Turkey is the biggest producer and exporter of dried figs in the world. Turkish dried fig export had revenue of 274 M USD (about 69 kt) in 2014-2015. After the ban on methyl bromide, which was the only fumigant used in the dried fig sector in Turkey, various alternative methods have been studied, primarily phosphine fumigation, sulfuryl fluoride, high CO_2, high CO_2 at elevated temperatures, high pressure CO_2, irradiation and ozonation (Tütüncü et al., 2004; Cetinkaya et al., 2006; Işıkber et al., 2006; Uslu et al., 2006; Sen et al., 2009; Akan & Ferizli, 2010; Tütüncü & Emekci, 2014). Also, high temperature, irradiation, controlled atmosphere, low pressure applications and numerous fumigants have been considered as alternatives in other studies (Fields & White, 2002; Baltaci et al., 2006; Navarro et al., 2006; Campabadal, 2007; Small, 2007; Ducom, 2012).

Among the methyl bromide alternatives, controlled atmospheres (CAs) have been increasingly adopted worldwide (Adler et al., 2000; Navarro, 2012). The major constraint of CAs applications for the disinfestation of dried figs in Turkey is the length of exposure periods. Exposure of 24 h to methyl bromide at normal atmospheric pressure was the typical disinfestation practice before its ban in 2015. Development of resistance to phosphine resulted with ineffective treatments at the label exposure periods of minimum 3 d (personal communication, S. Navarro), which means CA applications to compete with phosphine in term of exposure periods in dried fig pest management are now favored.

CAs in sublethal doses can cause several physiological and behavioral changes in insects. Among physiological responses; impaired metamorphosis (Ali-Niazee, 1971, 1972; Storey, 1977, 1978), reduced mating frequency and fecundity (Shorey, 1964; Lum & Flaherty, 1972), opening the spiracles continuously in hypercarbic conditions (Navarro, 2012), increased cell membrane permeability, (Hochachka, 1986; Zhou et al., 2001), as well as decreased respiration rate, metabolic rate and ATP production (Ali-Niazee, 1971; Friedlander & Navarro, 1979; Zhou et al., 2000; Carpenter et al., 2001) have been reported. For the behavioral ones, increasing in egg laying close to odor of wheat with in presence of CO_2 (Barrer & Jay, 1980), and immobilization (Ali-Niazee, 1972; Edwards & Batten, 1973) have also been reported.

CAs and toxic fumigants treatments against the arthropod pests of stored products can also lead to prolonged development in various life stages, including eggs and pupal stages of stored products pests (Ali-Niazee & Lindgren, 1970; Ali-Niazee,1971, 1972; Storey, 1977, 1978; Spratt, 1979; Rajendran, 2000; Nayak et al., 2003), and this is of practical importance in making right decision on the length of fumigation treatments to ensure maximum efficacy with the least chance of resistance development in insect pests. There is a limited number of studies available in regard to prolonged egg development caused by CAs treatments for *E. cautella*. Retardation in embryonal development as a sublethal effect of high CO_2 atmospheres in *E. cautella* was reported, especially when exposed to 100% CO_2 and 100% N_2 in CAs that cause 1-2 d of delay in hatching of *E. cautella* and *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) eggs (Bell et al., 1980).

The response of insects to modified atmospheres applications differs among life stages and even among age groups of the same life stages. Egg stage, in particular, has the ability to survive in hypoxic conditions and this ability gives insects an opportunity to extend their survivorship (Bell, 2012). Eggs and pupae of *E. cautella* are found to be more tolerant to hypercarbic or hypoxic environments than the larvae and adults (Storey, 1975; Jay, 1984). Thus, this study was undertaken to evaluate if there is any change in delayed hatching among different age groups of eggs of *E. cautella* which were exposed to combination of distinctive hypercarbic and anoxic atmospheres at various temperatures.

Materials and Methods

Age of eggs

Ephestia cautella cultures were reared in 1-L glass vials containing a mix of 200 g of coarsely grounded soft wheat grain, glycerin and brewer's yeast in a ratio of 14:2:1 (by wt) as described by Bell (1975) with the slight modifications in the rate of food ingredients. Insect cultures were held at 25°C and 65% RH in a constant temperature room. To obtain eggs of similar age, newly emerged adults were transferred to egg laying cages by suction apparatus made from PVC mesh.

Twenty-four h after the adults were transferred to egg laying cages, eggs of 0-24 h old were collected from the cages. To obtain eggs 24-48 and 48-72 h old, eggs 0-24 h old were kept at insect rearing room for additional 24 and 48 h, respectively. Studies were conducted between 2012 and 2014 in laboratory of Stored Products Pests, Plant Protection Department, Faculty of Agriculture, Ankara University.

Gas composition

In the experiments, cylinders containing 85% CO_2 + 3% O_2 +12 % N_2 and 95% CO_2 + 1% O_2 + 4% N_2 supplied by Linde Gas (Ankara) were used as the gas source. For brevity, these gas compositions are called 85% CO_2 and 95% CO_2 below.

Experimental equipment and design

Plexiglass vials (10mL) were used to contain eggs of different age groups. A 10-mm round hole was drilled in the lid of each vial, and a piece of wire mesh (125 micron) was hot glued to the inner surface of the lid to allow air passage. Eggs counted under stereomicroscope at 20 X magnification and transferred to plexiglass vials as 50 eggs per vial. A small amount of food was also introduced into each vial as the food source for the emerging larvae.

The CA treatments were performed in Dreshel flasks of 550 mL capacity as described by Hashem et al. (2012). The flasks inlet valves were connected to the premixed gas cylinder and outlet valves connected with oxygen meter (OxyCheq Expedition O2 Analyzer, OA-01-01, OxyCheck, Marianna, FL, USA).

Gas application

Plexiglass vials containing eggs of different ages were put into Dreshel flask, and 15 min of gas purging period with flow rate of 100 mL/min was applied to reach the desired gas composition inside the flask. The humidity inside the Dreshel flask was controlled using plexiglass vials containing 50 mL of KOH solution (70% RH) inside the flask. During the whole gas purging period O_2 concentration inside the flask was continuously monitored by O_2 meter connected to the outlet tube of the Dreshel flask by a short hose. After the end of gas purging process, inlet and outlet valves were closed and the Dreshel flasks were put into incubators (Binder KB-720, Tuttlingen, Germany). Vials containing control groups were treated similarly except they were exposed to normal atmospheric air only.

Experiments were conducted at 20, 25 and 30°C at 65±5% RH at different exposure periods ranging from 4 to 104 h with three or more replicates.

Post treatment observations

After the end of each exposure periods, vials containing eggs were taken from Dreshel flasks and transferred to insect rearing room adjusted at 25°C temperature and 65±5% RH. Egg hatching were then checked under the stereomicroscope twice daily for 20 d.

Statistical analysis

Differences in exposure periods were statistically evaluated using the general linear model procedure for one way ANOVA (Stat Soft Inc., Tulsa, OK, USA). Duncan's multiple range test was used to compare daily hatching eggs against the control group. A 95% confidence level was applied for all statistical analysis.

Results

At 30°C, eggs of different age groups exposed to 85% CO_2 for short exposure times such as 4-12 h started hatching at the same day as the controls. However, in comparing with controls, the hatch of eggs 24-48 h and 48-72 h-old was statistically different ($P \leq 0.05$). Mostly, the delayed hatching in all of the three age groups were statistically different at various exposure periods when compared to control (Table 1).

At 30°C, disregarding exposure periods or age groups, 85% CO_2 exposure caused 1-3 d delays in egg hatch. With exposure of 16 and 20 h, a 1-d delay in hatch occurred in eggs 0-24 and 24-48 h old, whereas in eggs 48-72 h old, a 2-d delay in hatch was observed. At 95% CO_2 and 30°C, similar results were obtained at 85% CO_2 exposures. At these two CO_2 levels, there were extended delays of hatching simultaneously with exposure periods (Tables 1 & 2).

Table 1. Hatch of eggs of *Ephestia cautella* exposed to 85% CO_2 + 3% O_2 + 12% N_2 at 30°C

	Daily egg hatch (number)														
	eggs 0-24 h old				eggs 24-48 h old					eggs 48-72 h old					
Exposure period (h)	n	R	D3[a]	D4	n	R	D2[a]	D3	D4	n	R	D1[a]	D2	D3	D4
Control	546	7	284	92	646	10	430	129	0	445	6	213	172	0	0
4	300	5	209	46	250	4	164	54	0	260	3	64*	103	29*	0
8	347	5	182	76	250	4	97*	111*	0	241	3	0*	100	24*	0
12	380	5	123*	36	210	3	0*	117*	32*	210	3	0*	0*	61*	35*
16	396	5	0*	119*	387	6	0*	245*	39*	150	3	0*	0*	21*	2*
20	452	6	0*	42	266	4	0*	79	11*	280	4	0*	0*	36*	7*
24	-	-	-	-	211	3	0*	38	10*	206	3	0*	0*	10*	4*
28	-	-	-	-	262	4	0*	0*	10*	258	4	0*	0*	0	2*
32	-	-	-	-	260	4	0*	0*	1*	-	-	-	-	-	-

[a] First day of hatch after exposure; R, replicate; D1-D4: Days 1 to 4;
* In the same column differences in comparing with control is significant $p \leq 0.05$ (Duncan).

At 25°C and 85% CO_2, delayed hatching occurred with exposure of 4 h and longer. Delayed hatch of eggs 0-24 h old was observed with exposures lasting longer than 1 d. The increase of exposure periods did not cause any delay of hatching in eggs 24-48 and 48-72 h old. For eggs 24-48 h old exposed for 4-42 h, a 2-d delay in hatch occurred, and a 3-d delay in egg hatch for eggs 48-72 h old exposed for 4-36 h (Table 3).

At 95% CO_2 and 25°C, a hatching delay of 1-3 d occurred in eggs 0-24 h old as the exposure period increased. For short exposures, such as 2 and 4 h, egg hatch started at the same day as the control, while other exposure times starting from 8 h caused hatching delays. Exposures lasting longer than 12 h caused delays in egg hatch in eggs 24-48 h old, and up to a 2-d delay occurred following 32 h exposure. Unlike eggs 0-24 h and 24-48 h old, egg hatching delays in eggs 48-72 h old occurred with the exposure of 4 h and longer, and with a delay of up to 2 d as exposure time increased (Table 4).

At 20°C and 85% CO_2, egg hatch started 4-7 d later than controls with long exposures of 80, 88 and 96 h. Delayed hatch was positively correlated with increased in exposure period. Hatching delay in eggs 0-24 h old started after 24 h of exposure, but started at 8 h for eggs 24-48 and 48-72 h old (Table 5).

Table 2. Hatch of eggs of *Ephestia cautella* exposed to 95% CO_2 + 1% O_2 + 4% N_2 at 30°C

	Daily egg hatch (number)															
	eggs 0-24 h old					eggs 24-48 h old					eggs 48-72 h old					
Exposure period (h)	n	R	D3[a]	D4	D5	n	R	D2[a]	D3	D4	n	R	D1[a]	D2	D3	D4
Control	547	9	322	115	0	644	10	387	152	0	590	9	294	217	0	0
8	-	-	-	-		-	-	-	-	-	200	3	0*	129	31*	0
12	250	4	22*	99*	5*	250	4	5*	155*	10*	250	4	0*	98	47*	0
16	314	5	0*	109	37*	250	4	0*	73	22*	200	3	0*	0*	49*	22*
20	443	7	0*	114	50*	759	11	0*	74	46*	379	6	0*	0*	39*	16*
24	402	5	0*	0*	16*	257	4	0*	0*	19*	242	3	0*	0*	0	26*
28	365	6	0*	0*	3*	369	6	0*	0*	4*	611	6	0*	0*	0	3*
32	271	5	0*	0*	6*	265	5	0*	0*	3*	-	-	-	-	-	-

[a] First day of hatching after exposure; R, replicate; D1-D5: Days 1 to 5;
* In the same column differences in comparing with control is significant $p \leq 0.05$ (Duncan).

Table 3. Hatch of eggs of *Ephestia cautella* exposed to 85% CO_2 + 3% O_2 + 12% N_2 at 25°C

	Daily egg hatch (number)																						
	eggs 0-24 h old								eggs 24-48 h old							eggs 48-72 h old							
Exposure period (h)	n	R	D3[a]	D4	D5	D6	D7	D8	n	R	D2[a]	D3	D4	D5	D6	n	R	D1[a]	D2	D3	D4	D5	
Control	506	8	330	89	12	0	0	0	403	7	248	94	3	0	0	404	7	222	126	14	0	0	
4	250	4	0*	112*	5	0	0	0	240	4	0*	0*	191*	0	0	189	3	0*	0*	0*	130*	0	
12	250	4	0*	54	54*	0	0	0	150	3	0*	0*	99*	0	0	157	3	0*	0*	0*	100*	0	
16	150	3	0*	10	99*	2*	0	0	150	3	0*	0*	99*	0	0	150	3	0*	0*	0*	67*	0	
20	375	5	0*	35	17	3*	0	0	220	4	0*	0*	118*	18*	0	319	5	0*	0*	0*	91*	10*	
24	469	6	0*	3*	5	2*	0	0	193	3	0*	0*	115*	18*	0	458	4	0*	0*	0*	136*	25*	
28	405	6	0*	0*	0*	7*	11*	1*	210	4	0*	0*	83*	43*	0	208	3	0*	0*	0*	73*	3*	
32	150	3	0*	0*	0*	1*	6*	1*	250	3	0*	0*	42*	49*	0	197	3	0*	0*	0*	17*	0	
36	422	8	0*	0*	23	49*	2*	0	251	4	0*	0*	61*	10*	0	381	6	0*	0*	0*	8*	0	
42	150	3	0*	0*	0*	0	17*	0	361	5	0*	0*	11	14*	2*	-	-	-	-	-	-	-	
48	-	-	-	-	-	-	-	-	219	4	0*	0*	0**	1*	0	-	-	-	-	-	-	-	

[a] First day of hatching after exposure; R, replicate; D1-D8: Days 1 to 8;
* In the same column differences in comparing with control is significant $p \leq 0.05$ (Duncan).

Table 4. Hatch of eggs of *Ephestia cautella* exposed to 95% CO_2 + 1% O_2 + 4% N_2 at 25 ^{0}C

	Daily egg hatch (number)																			
	eggs 0-24 h old							eggs 24-48 h old							eggs 48-72 h old						
Exposure period (h)	n	R	D3[a]	D4	D5	D6	D7	n	R	D2[a]	D3	D4	D5	D6	n	R	D1[a]	D2	D3	D4	D5
Control	401	6	235	94	3	0	0	587	8	245	259	27	0	0	591	8	195	314	28	0	0
2	150	3	43*	35*	0*	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	150	3	11*	94*	7	0	0	150	3	1*	108*	9	0	0	150	3	0*	110*	28	0	0
8	150	3	0*	03*	9	1*	0	192	3	12*	130*	10	3*	0	434	6	0*	256	106*	11*	0
12	287	6	0*	72*	50*	2*	0	475	7	20*	248	98*	0	0	243	4	0*	31*	90*	1*	0
16	250	5	2*	16*	72*	2*	0	290	6	0*	167	94*	3*	0	331	5	0*	8*	122*	57*	0
20	350	7	0*	99	110*	4*	0	505	9	0*	174	13*	14*	1*	322	5	0*	0*	102*	7*	0
24	257	5	0*	13	84*	12*	0	223	4	0*	18*	05*	9*	3*	303	6	0*	0*	86*	15*	0
28	216	4	0*	0*	31	7*	0	212	4	0*	0*	45*	16*	0	220	4	0*	0*	19	9*	0
32	158	3	0*	0*	9	5*	0	155	3	0*	0*	24	2*	0	200	3	0*	0*	3	12*	1*
36	720	9	0*	0*	12	34*	7*	-	-	-	-	-	-	-	424	7	0*	0*	5	0	0

[a] First day of hatching after exposure; R, replicate; D1-D7, Days 1 to 7;
* In the same column differences in comparing with control is significant $p \leq 0.05$ (Duncan).

Table 5. Hatch of eggs of *Ephestia cautella* exposed to 85% CO_2 + 3% O_2 + 12% N_2 at 20°C

E (h)	Daily egg hatch (number)																									
	eggs 0-24 h old									eggs 24-48 h old										eggs 48-72 h old								
	n	R	D6[a]	D7	D8	D9	D10	D11	D12	n	R	D4[a]	D5	D6	D7	D8	D9	D10	D11	n	R	D3[a]	D4	D5	D6	D7	D8	D9	D10
C	200	4	54	98	27	2	0	0	0	350	7	36	131	130	12	1	1	0	0	402	8	34	109	128	100	1	1	0	0
8	150	3	4*	54	39	7	0	0	0	250	5	0*	118	56	31	5	1	0	0	250	5	0*	113	73	23	1	0	0	0
16	150	3	1*	45	59*	16	1*	1*	0	250	5	0*	81	81	22	4	1	0	0	250	5	0*	79	57	26	4	0	0	0
24	150	3	0*	7*	45	19	1*	0	0	250	5	0*	13*	136	43	19	2	0	0	250	5	0*	68	66	37	9	0	0	0
32	150	3	0*	0*	18	28*	4*	0	0	300	6	0*	0*	117	81*	26	16	0	0	250	5	0*	38	84	43	15	1	0	0
40	150	3	0*	1*	23	47*	4*	1*	0	250	5	0*	0*	77	71*	21	15	0	1*	250	5	0*	0*	42	34	23	2	0	0
48	150	3	0*	0*	0*	14	4*	0	0	250	5	0*	0*	0*	70*	35	23	6*	0	250	5	0*	0*	60	38	25	7	2*	0
56	150	3	0*	0*	0*	10	12*	5*	0	250	5	0*	0*	0*	69*	39	34*	8*	1*	150	3	0*	0*	1	19	31*	7	9*	0
64	150	3	0*	0*	0*	1	16*	12*	0	150	3	0*	0*	0*	22	13	20*	9*	0	150	3	0*	0*	0*	0*	18	17*	4*	0
72	150	3	0*	0*	0*	0*	1*	1*	0	150	3	0*	0*	0*	1	27*	4	4*	0	150	3	0*	0*	0*	0*	10	20*	5*	0
80	150	3	0*	0*	0*	0*	1*	7*	1*	150	3	0*	0*	0*	0*	0*	0*	28*	0	150	3	0*	0*	0*	0*	0*	0*	0	14*
88	150	3	0*	0*	0*	0*	1*	0	2*	150	3	0*	0*	0*	0*	0*	0*	13*	0	150	3	0*	0*	0*	0*	0*	0*	0	3*
96	-	-	-	-	-	-	-	-	-	200	4	0*	0*	0*	0*	0*	11	9*	1*	200	4	0*	0*	0*	0*	0*	0*	5*	1*

[a] First day of hatching after exposure; D3-D12, Days 3 to 12; E (h), Exposure period (h); C, control; R, replicate;
* In the same column differences in comparing with control is significant $p \leq 0.05$ (Duncan).

At 95% CO_2 and 20°C, hatching delays at short exposures, such as 8 h onwards, occurred in eggs 0-24 and 48-72 h old, but from 24 h onwards in eggs 24-48 h old. Delays in egg hatch increased to 7 d for eggs 0-24 h old, and 6 and 8 d for eggs 24-48 and 48-72 h old, respectively (Tables 6 & 7).

Table 6. Hatch of eggs of *Ephestia cautella* 0-24 h old exposed to 95% CO_2 + 1% O_2 + 4% N_2 at 20°C

Exposure period (h)	n	Replicate	Daily egg hatch (number)							
			D5[a]	D6	D7	D8	D9	D10	D11	D12
Control	256	6	5	98	103	43	4	0	0	0
8	150	3	0*	3*	27	59*	6	0	0	0
16	150	3	1	19	24	53*	19	0	0	0
24	150	3	0*	0*	21	20	31*	10*	1*	1*
32	150	3	0*	0*	1*	13	31*	10*	2*	0
40	150	3	0*	0*	2*	33	32*	7*	0	0
48	150	3	0*	0*	0*	3	23	7*	0	1*
56	150	3	0*	0*	0*	0*	9	10*	2*	3*
64	150	3	0*	0*	0*	0*	4	5*	3*	1*
72	150	3	0*	0*	0*	0*	0*	5*	0	1*
80	150	3	0*	0*	0*	0*	0*	0	8*	7*
88	150	3	0*	0*	0*	0*	0*	0	0	1*

[a] First day of hatching after exposure; D5-D12, Days 5 to 12;
* In the same column differences in comparing with control is important $p \leq 0.05$ (Duncan).

Table 7. Hatch of eggs of *Ephestia cautella* exposed to 95% CO_2 + 1% O_2 + 4% N_2 at 20°C

	Daily egg hatch (number)																				
	eggs 24-48 h old										eggs 48-72 h old										
Exposure period (h)	n	R	D4[a]	D5	D6	D7	D8	D9	D10	D11	n	R	D2[a]	D3	D4	D5	D6	D7	D8	D9	D10
Control	406	8	29	143	160	29	3	0	1	0	253	5	8	57	68	31	75	4	0	0	0
8	150	3	9	41	25	41	9	0	0*	0	150	3	0*	37	34	18	39	2	0	0	0
16	150	3	5	45	31	25	18	2*	0*	0	150	3	0*	4	67	5	36	11	1*	0	0
24	150	3	0*	15	40	18	28	7*	3	0	200	4	0*	0*	50	37	27	22	3*	0	0
32	200	4	0*	1*	49	37	37	22*	2	1*	150	3	0*	0*	18	43	13	31	7*	2*	0
40	150	3	0*	0*	32*	24	10	19*	3	0	150	3	0*	0*	0*	25	22	18	16*	3*	0
48	150	3	0*	0*	8*	38	7	18*	7	2*	150	3	0*	0*	0*	8	29	12	18*	4*	0
56	150	3	0*	0*	6*	24	10	8*	6	1*	150	3	0*	0*	0*	5	10	12	13*	8*	2*
64	150	3	0*	0*	0*	9	1	2*	2	1*	200	4	0*	0*	0*	0*	0*	11	18*	6*	0
72	150	3	0*	0*	0*	1	9	3*	0*	1*	150	3	0*	0*	0*	0*	1	12	13*	5*	2*
80	200	4	0*	0*	0*	0*	0*	0	31*	0	150	3	0*	0*	0*	0*	0*	0*	0	0	9*
88	150	3	0*	0*	0*	0*	0*	0	15	0	150	3	0*	0*	0*	0*	0*	0*	0	0	3*
96	150	3	0*	0*	0*	0*	0*	6*	8	0	150	3	0*	0*	0*	0*	0*	0*	1*	0	1*
104	250	5	0*	0*	0*	0*	0*	3*	0*	0	-	-	-	-	-	-	-	-	-	-	-

[a] First day of hatching after exposure; D2-D11, Days 2 to 11; R, replicate;
* In the same column differences in comparing with control is important $p \leq 0.05$ (Duncan).

Discussion

As found in the earlier studies, both low O_2 and high CO_2 cause mortality by disrupting the metabolic balance (Banks & Annis, 1990; Fleurat-Lessard, 1990). However, there are numerous metabolic factors that lead to mortality (Mitcham et al., 2006). The reason that the insects react to high CO_2 more than to low O_2 lies in the difference in the permeability of insect tissues to these gases. The permeability to CO_2 is six times more than that to O_2. Regulation of respiration which is largely dependent on the brain receptors is another reason, since the brain receptors are more susceptible to increasing CO_2 than to low O_2 levels (Fleurat-Lessard, 1990).

In CA applications, 3% O_2 is the critical concentration and any CO_2 atmosphere containing O_2 below this concentration should not be considered as a high CO_2 atmosphere (Banks & Annis, 1990). On the other hand, some researchers believe that low O_2 and high CO_2 act together synergistically in low O_2 atmospheres (2-5%) containing some amount of CO_2 (5-40%) (Ali-Niazee, 1971; Calderon & Navarro, 1979; Calderon & Navarro, 1980; Krishnamurthy et al., 1986). Gas compositions used in this study fall into both low O_2 and high CO_2 categories and therefore they are considered to act synergistically to induce mortality.

Egg hatch in *E. cautella* is completed in 5 d and most embryonic development in the first 2 d (Bell, 1975). Embryonic development in insects starts with the first formative period (larval development phase) and lasts for 1 d in most insects. In this period, metabolic rate is slow. This slow period is followed by an increased metabolic rate (Fink, 1925). It is reported that during anoxia development is arrested and the longevity is largely dependent on the capacity of both accumulating glycolytic products and lowering the metabolic demands (Fleurat-Lessard, 1990). Both low O_2 and high CO_2 cause depleted metabolic rate and elevated cell permeability. Decreased metabolic rate means reduced ATP production, which implies insufficient energy supply to the tissues (Hochachka, 1986; Carpenter et al., 2001; Ofuya & Reichmuth, 2002). In these conditions delayed embryonal development is an inevitable result.

In the present study, eggs 0-24, 24-48 and 48-72 h old were exposed to various conditions that caused delayed hatch in response to both high CO_2 and low O_2 atmospheres. In 85% CO_2 atmosphere hatching delay in eggs 0-24 h old was found to be 1 d with short exposure periods and ≥2 d in long exposure periods (Tables 1, 3 & 5). Due to the lowered metabolic rate in eggs 24 h old, it is thought that the inhibitory effect of high CO_2 atmospheres for short exposures is minimal and its inhibitory effects can only be marked during long exposure periods. Fink (1925) proposed that metabolic rate increases when eggs are at an age closer to hatching. In the present study, in agreement with Fink (1925), there was a greater delay in eggs 48-72 h old (2-6 d at 25 and 20°C, respectively) than in eggs 24-48 h old (3-7 d at 25 and 20°C, respectively) (Tables 3 & 5). Due to the increased metabolic rate, we assume that older eggs when compared to younger ones respire more and absorb greater amounts of CO_2, which presumably resulted in an increased hatching delays in older eggs. Similarly, at 30°C, egg hatching delay extended to 3 d in eggs 48-72- h old, which was 2 d in eggs 24-48 h old (Table 1).

In a 95% CO_2 atmosphere, delays in eggs 48-72 h old were seen earlier than the two other age groups. Delays at different temperatures that occurred after 4-8 h exposures in eggs 48-72 h old started with 16 or 24 h of exposure in the other two age groups (Tables 2, 4, 6 & 7).

High temperatures cause an increase in insect metabolism which accelerates the toxic effect of CO_2 (White et al., 1995). In the present study at 85% CO_2 atmosphere, delays observed in different age groups and in different exposure periods were between 1-3 d at 30°C, 1-4 d at 25°C and 1-7 d at 20°C (Tables 1, 3 & 5).

At 30°C, delay in egg hatch increased to 3 d. Decreasing temperature to 25 and 20°C, increased the hatching delay to 4 and 7 d, respectively. These differences in hatching demonstrated the effect of temperature on the metabolic rate under elevated CO_2 and depleted O_2 conditions.

The respiration rate of low O_2 conditions reported to be decreased similarly with high CO_2 atmospheres (Zhou et al., 2000). In *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae), egg and adult respiration rate showed a significant decrease at O_2 below 5% (Yang et al., 2008a). In another study with *T. castaneum*, $O_2 \leq 5\%$ was found to be effective in lowering the respiration rate, particularly in eggs and young larvae (Emekci et al., 2002). Similarly, Guiqiang et al. (2008) reported that 10% O_2 caused delay in development in eggs and larvae of *T. castaneum*. In *T. castaneum, Oryzaephilus surinamensis* (Linnaeus, 1758) (Coleoptera: Silvanidae) and *Sitophilus zeamais* (Motschulsky), 1855 (Coleoptera: Curculionidae), a low O_2 atmosphere (15%) was reported to increase the egg development period (Yang et al., 2008b). In *S. zeamais*, egg development increased by up to 10-11 d after exposure to a modified atmosphere consisting of O_2, CO_2 and N_2 gases in a ratio of 1:1:8 (by vol.) (Spratt, 1979). In *Rhyzopertha dominica* (Fabricius, 1792) (Coleoptera: Bostrichidae), respiration of one-day-old eggs was suppressed by O_2 below 2% (Emekci et al., 2004). *Callosobruchus maculatus* (Fabricius, 1775) (Coleoptera: Chrysomelidae) eggs exposed to a gas composition of 10% CO_2 + 10% O_2 completed their development more slowly than the control (Cheng et al., 2012). According to Ali-Niazee & Lindgren (1970), high CO_2 in *Tribolium confusum* Jacquelin du Val, 1863 (Coleoptera: Tenebrionidae) and *T. castaneum* causes delayed or failed egg development through interfering with the embryo's nervous system by narcosis and with egg growth. Another important issue relating with synergistic effect of high CO_2 and low O_2 conditions is that threshold concentration of susceptibility for high CO_2 toxicity generally decreases in hypoxic conditions (Fleurat-Lessard, 1990). In the present study, delayed egg hatch was found to be statistically significant and longer exposure times caused greater delays, which is in agreement with previous studies. In the present study, there were eggs that hatched at 20°C after 12 d following an 88-h treatment. Similarly, at 25 and 30°C, live larvae were found after 8 d following 42-h exposure and after 5 d following 32-h exposure. In practice, potential delays in insect development in CA applications should be considered carefully when making decision on suitable fumigation times for dried fruits. Fumigation time should be extended until there are no live insects, otherwise infestation by storage pests can easily build up.

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