Susceptibility of *Rhyzopertha dominica* to high CO_2 modified atmospheres in packaged chickpeas

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ABSTRACT

Three MAs with 50%, 70% and 90% CO_2 in air was tested at different exposure times on all the developmental stages of *R. dominica* using chickpeas packed with 4% and 96% filling ratio in which there was excess of CO_2. At 4% filling ratio the loss of the gas was negligible. The exposure time to reach 50% mortality (LT_{50}) was estimated for each developmental stage and CO_2 concentration, ranging from 7 hours for larvae with 90% to 2 days for pupae with 50% CO_2. At 96% filling ratio and for the intervals of the estimated exposure times (LT_{50}) from the 4% filling ratio, mortality decreased significantly for eggs and adults at the three MAs tested, while for the internal developmental stages, larvae at 50% and 70% CO_2 and pupae in all concentrations of CO_2, the susceptibility remained the same as with a 4% filling ratio. This decline in mortality of the external developmental stages was possible due to the sorption of CO_2 by the chickpeas, which caused a loss of intergranular levels of CO_2 between 17% and 29%. This sorption ranged from 0.1955 to 0.3285 g of CO_2 per kg of chickpeas and produced a negative pressure of 77.12 to 60.65 kPa. In conclusion, when chickpeas are packed with high CO_2 MAs, a decrease in the mortality of eggs and adults of *R. dominica* could be expected due to pulse sorption.

Keywords: Pests, grain, packaging, filling ratio, CO_2 sorption

1. INTRODUCTION

Rhyzopertha dominica (F.) (Col. Bostrichidae) is a primary pest of stored grain that is widely distributed globally, especially in warmer regions between latitude 40º N and latitude 40º S of the equator. Females deposit eggs on grain kernels, and the newly hatched larvae bore into the kernels and develop within until they reach the adult stage. The mature adult bores out of the kernel by a large exit hole; the kernel is then referred to as an "insect-damaged kernel" (IDK) (Potter, 1935; Arbogast, 1991; Rees, 1995). *Rhyzopertha dominica* attacks a wide range of grains, such as rice, wheat, corn, and others containing starch (Chujo, 1958; Rees, 1995), as well as to chickpeas (Wong-Corral et al., 1987; Batta, 2005). The most common method of controlling *R. dominica* is through the use of organosphosphate, pyrethroid and juvenile hormone analogue pesticides, and fumigants such as phosphine. However, *R. dominica* has been shown to have developed resistance to both methodologies (Bengston et al., 1975; Zettler and Cuperus, 1990; Collins, 1998; Opit et al., 2012; Jagadeesan and Nayak, 2016; Daglish and Nayak, 2018). The problem of resistance to insecticides and the consumer concern over the use of pesticides in food has resulted in the search for alternative control methods.

An alternative control measure is the treatment of grains with modified atmospheres (MAs), which has the advantage of avoiding pesticide residues in food commodities (Navarro, 2012). MAs with high carbon dioxide (CO_2) and low oxygen (O_2) contents can be applied to stored raw materials, to semi-processed commodities, and/or to final food products (Fleurat-Lessard, 1990; Adler et al., 2000; Navarro, 2006; Riudavets et al., 2007). Previous studies with high CO_2 MAs showed that larvae and adults of weevil pests are susceptible stages, while eggs and pupae are more tolerant (Riudavets et al., 2009; Wong-Corral et al., 2013). These studies were done in flexible packages with a low proportion of the commodity in relation to the total volume of the package (low filling ratio), and the loss of CO_2 content in the package was negligible. However, in practical situations (e.g., packages of grains and pulses), the proportion of the total volume of the package occupied by the commodity is much higher (high filling ratio), causing gas sorption that decreases the concentration of CO_2 in the headspace. This sorption produces a vacuum effect inside the package, and most of the sorption occurs during the first hours of exposure to CO_2 (Cofie-Agblor et al., 1995, 1998; Navarro, 1997). The higher the volume occupied by the commodity (filling ratio), the greater the vacuum and the loss of CO_2 in the headspace of the package (Iturralde-García et al., 2019). This loss of CO_2 in packages headspace after gas sorption by the commodity might affect pest control efficacy, and this effect remains to be tested.

The hypothesis of the present study was that the control efficacy of the CO_2 MAs on the developmental stages of *R. dominica* will decrease as the volume of the commodity increases inside the package due to sorption and loss of CO_2 in the headspace. To test this hypothesis, we evaluated the mortality of *R. dominica* eggs, larvae, pupae, and adults in chickpeas packaged with different CO_2 concentrations and with different filling ratios.

2. MATERIALS AND METHODS

Chickpeas (cv. Blanco Lechoso) were purchased free of pesticides and insect infestation. The pulse had a water activity of 0.650 measured with Aqualab pre (Labferrer, Cervera, Spain), and consisted of 57% carbohydrates, 6.8% fat, and 23% protein (properties provided by the supplier).

A stock colony of *R. dominica* was reared on chickpeas and maintained at 28 ± 2ºC, 70 ± 10% r.h., and a photoperiod of 16:8 hours of Light:Dark. Experiments were performed in the same environmental conditions. To daily collect fresh eggs, fifty 3-day-old adults were offered 200 g of chickpeas in ventilated cages during 24 h. Some of these newly deposited eggs were confined with 50 g of chickpeas and kept for 9–15 days to obtain mature larvae or kept 34–39 days to obtain pupae. The presence of penetration holes on the surface of the pulse was an indication that they held an immature stage of *R. dominica*.

2.1 <u>Mortality of *R. dominica* when chickpeas were packaged at a 4% filling ratio</u>

Mortality of *R. dominica* was assesed by exposing each developmental stage inside a cage (7 cm diameter x 4 cm high) with 4% filling ratio to three MAs at different exposure intervals. For treating eggs, 1–3-day-old eggs of *R. dominica* were placed inside a gelatin capsule (three loose eggs per capsule), and 5 of these capsules were deposited in a small ventilated cage. For treating larvae or pupae, 15 chickpeas infested with a minimum of one individual of *R. dominica* were deposited in each cage. For treating adults, 15 three-day-old individuals were placed in each cage. Each cage was placed inside a 300 x 210 mm (59 micrometer-thick) plastic bag (Cryovac BB4L, Sealed Air, Elmwood Park, NJ, USA). The plastic bags had permeabilities to O_2 and CO_2 of 30 and 150 $cm^3\ m^{-2}\ day^{-1}\ bar^{-1}$ respectively, measured under conditions standardised at 23ºC and 0% r.h. Bags were filled with three CO_2 concentrations prepared using a gas mixer (Witt KM 100-3M/MEM, Witt Gasetechnick, Witten, Germany), and filled with a vacuum packaging machine (EVT-7-TD, Tecnotrip, Terrassa, Spain). The MAs tested were: i) 50% CO_2, with a residual of 10% O_2 and a 40% balance of N_2; ii) 70% CO_2, with a residual of 6% O_2 and a 24% balance of N_2; and iii) 90% CO_2, with a residual of 3% O_2 and a 7% balance of N_2. An aliquot of 6 ml of the headspace gas was collected with a gas analyser (OXYBABY®, Witt Gasetechnick, Witten, Germany) to verify the CO_2 and O_2 contents inside the plastic bags at the beginning and at the end of the different periods of exposure tested. A foam rubber seal (Witt Gasetechnick, Witten, Germany) was used when punching the package to avoid introducing any outside atmosphere. Five different exposure times were tested for eggs (6, 24, 48, 72 and 96 h), larvae (6, 10, 18, 24 and 48 h), pupae (24, 72, 120, 168, 240 h) and adults (24, 30, 48, 72 and 96 h). Three replicates were done for each CO_2 concentration and exposure time. Sets of cages with the same number of individuals as in the tests were maintained without MAs for the same exposure intervals in order to determine control mortality .

Adult mortality was assessed 24 hours after opening the bags. Cages with eggs were kept up to 10 days to allow their hatching, while cages with larvae and pupae were kept until the emergence of adults.

2.2 Mortality of *R. dominica* when chickpeas were packaged at a 96% filling ratio

Mortality of developmental stages was assessed at time intervals of the LT_{50} that was previously calculated with the three modified atmospheres for a filling ratio of 4%. Each set of individuals was deposited in a semi-rigid plastic container (710 mL capacity, 500 μm thickness, polyethylene terephthalate [PET]) filled with 500 g of chickpeas (bulk density of 0.74 g/cm^3), which occupied a filling ratio of 96%. The lid was sealed with hot glue and the MAs tested were flushed through an inlet hole at the top of the container removing the air from inside package. Gas concentrations were measured at the start and at the end of the exposure time. Test performed with the same CO_2 concentrations and developmental stages using the same intervals for mortality measurement as previously describe at 4% filling ratio. The hole procedures repeated ten times (10 replications).

2.3 Data analysis.

Percentage of mortality was calculated using the Abbott´s formula (1925). Data were analysed with Probit analysis (Poloplus, LeOra Software) and estimated exposure times to achieve 50% and 90% mortality were compared using the fiducial limits as the criterion to determine significant differences: when fiducial limits did not overlap between modified atmospheres for each developmental stage, the comparison was significant (Robertson et al., 2007). A chi-square (X^2) test was used to compare mortality of each developmental stage obtained when in chickpeas packed at high filling ratio with that of chickpeas packed at a low filling ratio at time intervals of the estimated LT_{50}. The available gas volume in the semi-rigid package, the volume of CO_2 sorbed by chickpeas, and the negative pressure (vacuum) produced at the filling ratio of 96% were calculated according to the methodology described in Iturralde-García et al. (2019):

-CO_2 sorption using the equation from Jian et al. (2014):

$$S = (\rho_{CO2}\ V_S) / M_{chickpea} \quad (1)$$

where.

S = sorption of CO_2 (g) per mass of chickpea (kg);

ρ_{CO2} = CO_2 density of 0.00182952176 g/mL, according to the equation of the density of gases (Chang and College, 2002);

V_S = volume of CO_2 sorbed by the chickpeas (mL);

$M_{chickpeas}$ = chickpeas mass (kg).

-Negative pressure using Cofie-Agblor et al. (1995) equation:

$$P_f = ((m R T) / (V_{gas} M_{CO2})) - P_i \quad (2)$$

where.

P_f = final pressure (kPa);

R = universal gas constant (8.314472 L kPa/K mol);

T = temperature (K);

V_{gas} = gas volume available in the container (L);

M_{CO2} = molar mass of the CO2 (g/mol);

P_i = initial pressure (Kpa).

m = total mass of CO_2 sorbed in the container (g).

-Total mass of CO_2 sorberd (m) was previously calculated as follows:

$$m = S M_{chickpeas} \quad (3)$$

3. RESULTS

3.1. Mortality of *R. dominica* when chickpeas were packaged at a 4% filling ratio

Percentage of CO_2 content within the sealed plastic bags during the exposure to three MAs are shown in Fig. 1. Carbon dioxide declined slowly during exposure time: after 240 h of dosing, the

CO_2 content in the bags filled with 50%, 70%, and 90% CO_2 were 46.6 ± 0.2%, 62.4 ± 1.1 %, and 84.5 ± 0.9%, respectively.

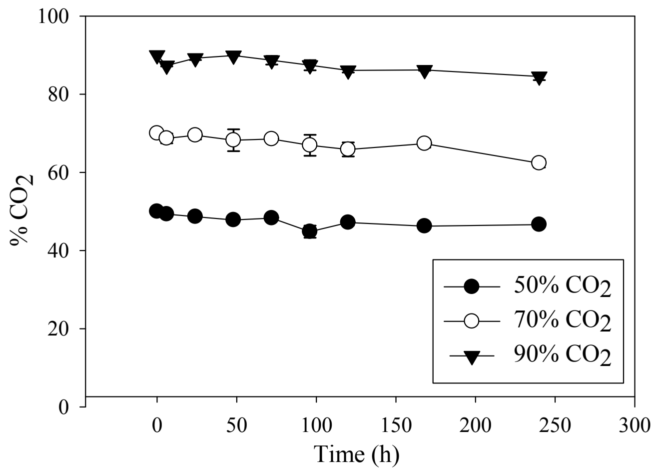

Figure 1. Percentage of CO_2 in the plastic bags with a 4% filling ratio of chickpeas for the three CO_2 concentrations and at the different exposure times tested.

Mortality of *R. dominica* developmental stages produced by the three CO_2 MAs are shown in Fig. 2. Larvae was the most sensitive developmental stage, reaching total mortality after 48 h of exposure to all CO_2 concentrations tested. In contrast, pupae was the most tolerant, needing 240 h of exposure to 70 and 90% CO_2 concentrations for reaching a 100% mortality; a 96.4% mortality was obtained at 50% CO_2 with the same exposure time. Eggs and adults had an intermediate sensitivity needing 96 h of exposure to all CO_2 concentrations tested to obtain complete mortality.

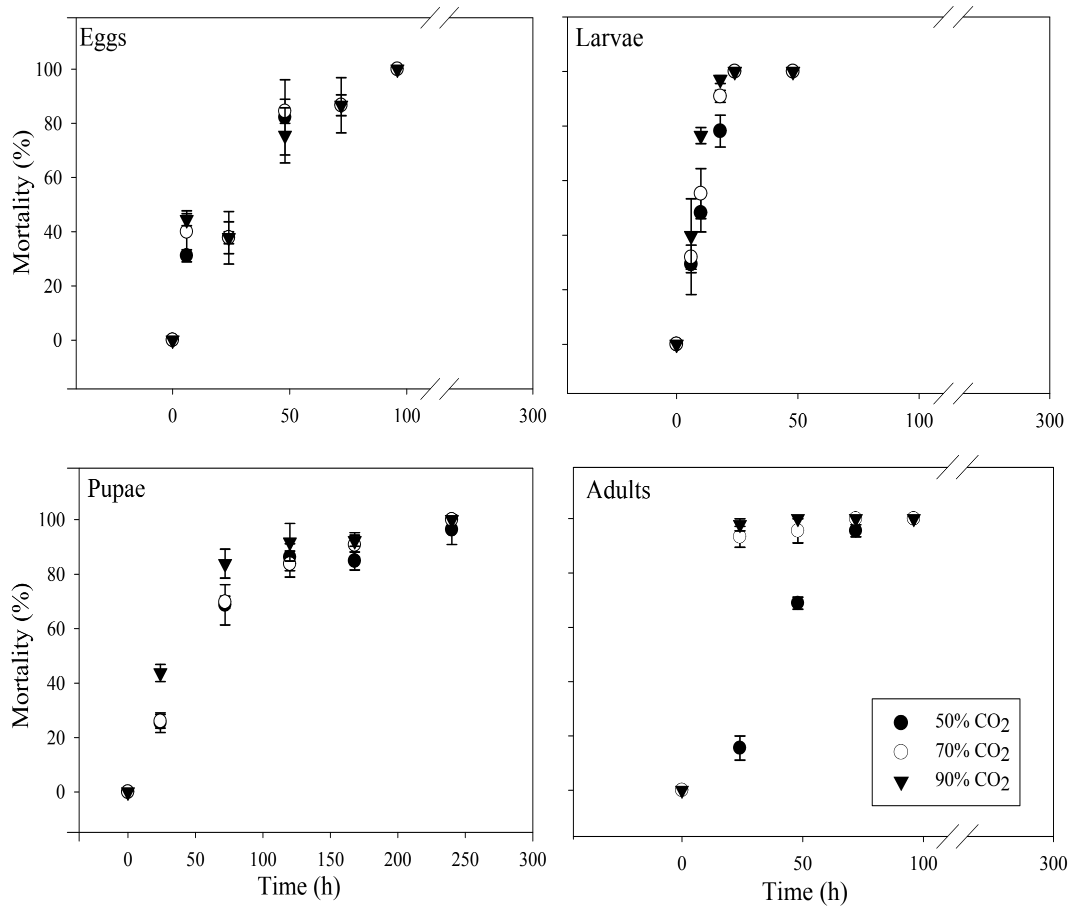

Figure 2. Percentage mortality of the developmental stages of *R. dominica* exposed to 50%, 70%, and 90% CO_2 concentrations during different exposure intervals.

Parameters of the Probit analysis for eggs, larvae, pupae, and adults with three CO_2 concentrations tested are shown in Table 1. Mortality in the control treatments were 0% (adults), 11.1 ± 3.8% (eggs), 16.4 ± 6.6% (larvae) and 16.0 ± 3.7% (pupae). Mortality of larvae and adults had a positive dose-response and fiducial limits of the two extreme CO_2 concentration tested (50% and 90%) did not overlap, while intermediate values were obtained with 70% CO_2, both at LT_{50} and LT_{90}. However, mortality of eggs and pupae was not significantly different among the three CO_2 concentrations tested, both at LT_{50} and LT_{90}.

Stage	CO_2 (%)	Slope (SE)	LT_{50} (h)[a]	95% fiducial limits	LT_{90} (h)[a]	95% fiducial limits	X^{2b}
Eggs	50	1.96 (0.22)	16.8 a	9.0-25.2	76.0 a	49.2-162.7	43.9
	70	1.72 (0.21)	14.6 a	7.8-21.6	81.1 a	53.1-166.5	31.76
	90	1.55 (0.20)	14.3 a	5.8-23.3	96.4 a	56.6-289.7	42.72
Larvae	50	3.78 (0.42)	9.4 a	7.6-11.1	20.4 a	16.6-28.7	22.94
	70	4.27 (0.48)	7.8 ab	6.8-8.6	15.5 ab	13.6-18.5	5.46
	90	4.58 (0.61)	6.7 b	5.8-7.5	12.8 b	11.3-15.5	12.37
Pupae	50	2.27 (0.21)	45.6 a	35.0-55.4	166.9 a	134.8-225.8	18.04
	70	2.61 (0.24)	44.3 a	35.1-53.0	137.2 a	115.1-172.1	14.73
	90	2.04 (0.23)	29.7 a	17.6-40.9	125.9 a	97.3-179.4	20.13
Adults	50	4.28 (0.55)	32.6 a	28.1-36.7	64.9 a	55.4-82.9	13.10
	70	5.25 (0.73)	16.2 ab	11.1-50.5	28.4 b	22.1-52.4	43.54
	90	4.83 (0.51)	10.5 b	8.2-13.4	19.4 b	15.0-30.4	36.38

[a]Values with different letters in a column for each developmental stage are significantly different ($P < 0.05$, confident interval for the ratio of LTs).

[b]Eggs, df = 4; Larvae, df = 4; Pupae, df = 4; Adults, df = 4. Chi-square testing the linearity of dose-dependent mortality indicated that normal distribution provided an adequate fit for the model in all cases.

Table 1. Parameters of the Probit analysis with modified atmospheres of 50%, 70%, and 90% CO_2 for the eggs, larvae, pupae, and adults of *R. dominica*.

3.2. Mortality of *R. dominica* when chickpeas were packaged at a 96% filling ratio

When the rigid plastic containers were filled at the 96% of their capacity with chickpeas and exposed to three CO_2 concentrations for the LT_{50} calculated for each developmental stage, sorption of CO_2 was took place together with a negative pressure (Table 2). Mortality in the control treatments were 0% (adults), 11.7 ± 3.0% (eggs), 18.0 ± 2.1% (larvae) and 0% (pupae).

Initial CO_2 (%)	Exposure time of LT_{50} (h)	Loss of CO_2 (%)	CO_2 sorption (g/Kg chickpea)	Negative pressure (kPa)

50	9.4 (larvae)	17.19	0.1955	77.12
	16.8 (eggs)	17.58	0.1965	76.99
	32.6 (adults)	17.68	0.2010	76.43
	45.6 (pupae)	18.08	0.2057	75.86
70	7.8 (larvae)	23.44	0.2665	68.32
	14.6 (eggs)	24.62	0.2800	66.66
	16.2 (adults)	25.50	0.2900	65.42
	44.3 (pupae)	25.80	0.2934	64.99
90	6.7 (larvae)	28.29	0.3217	61.49
	10.5 (adults)	27.25	0.3099	62.96
	14.3 (eggs)	28.88	0.3285	60.65
	29.7 (pupae)	27.31	0.3106	62.87

Table 2. Gas changes that occurred inside the rigid plastic containers with a filling ratio of 96% by the application of different CO_2 modified atmospheres at the exposure time at which the stages of *R. dominica* reached the LT_{50}.

For the pupae, the most tolerant stage, initial concentrations of 50%, 70%, and 90% lost a total of 18.08%, 25.80%, and 27.31% of CO_2, respectively. The rate of CO_2 sorption follow a logarithmic curve: at 50%, CO_2 sorption was of 0.1955 g/Kg chickpea at 9.4 h and of 0.2057 g/Kg chickpea at 45.6 h; at 70%, CO_2 sorption was of 0.2665 g/Kg chickpea at 7.8 h and of 0.2934 g/Kg chickpea at 44.3 h; and at 90%, CO_2 sorption was of 0.3217 g/Kg chickpea at 6.7 h and of 0.3285 g/Kg chickpea at 14.3 h. The negative pressure produced inside the containers was proportional to the CO_2 sorption, increasing as the CO_2 concentration increases. Vacuum ranged between 77.12–75.86, 68.32–64.99, and 62.96–60.65 kPa for 50%, 70%, and 90% CO_2 concentrations, respectively. Mortality data of *R. dominica* developmental stages in the containers with a filling ratio of 96% of chickpeas are shown in Fig. 3 and Table 3. Maximum egg and adult mortality observed in this assay reach up to 19.8% and 12.7%, respectively, and were significantly lower than the 50% mortality estimated on the flexible package trials at the same exposure time (LT_{50}). However, mortality of larvae with 50% and 70% CO_2 concentrations and of pupae with all CO_2 concentrations was not significantly different from the 50% calculated on the flexible packages (LT_{50}) with the Probit model. Surprisingly, mortality of larvae with a 90% CO_2 concentration was significantly greater (64.1% of mortality) than that estimated on the flexible package trials at the LT_{50} (Fig. 3, Table 3).

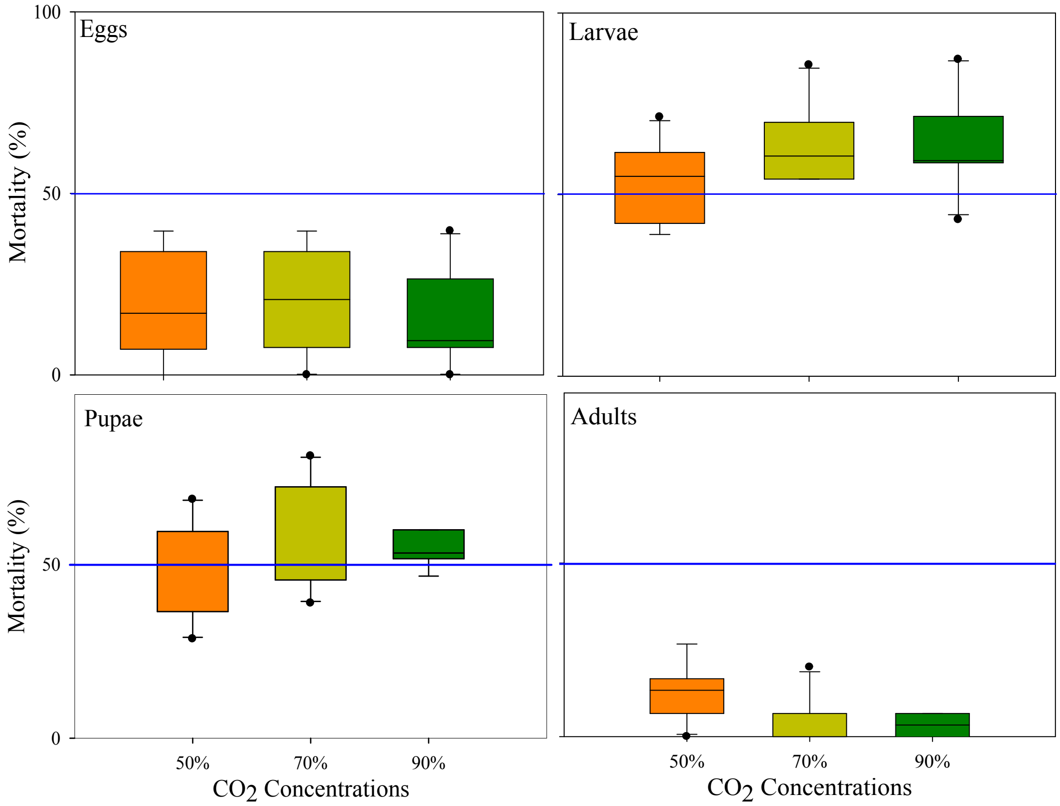

Figure 3. Mortality (%) of the four developmental stages of *R. dominica* when treated in semi-rigid packages (96% filling ratio) with different modified atmospheres at the LT_{50} calculated with the flexible package (4% filling ratio). Boxplot with median (solid line), the inter-quartile range (box length), and the minimum and maximum values (whiskers).

Developmental stage	Initial CO_2 concentration (%)	Exposure time (h)	Mortality ± ET (%)	X^2 (df)	P*

Eggs	50	16.9	19.6 ± 4.8	24.33 (9)	<0.01
	70	14.6	19.8 ± 4.6	20.60 (9)	<0.05
	90	14.3	16.0 ± 4.2	22.47 (9)	<0.01
Larvae	50	8.3	53.5 ± 3.4	10.74 (9)	0.294
	70	7.7	63.3 ± 3.4	15.05 (9)	0.090
	90	6.7	64.1 ± 4.1	19.39 (9)	<0.05
Pupae	50	45.6	49.2 ± 4.2	4.72 (9)	0.858
	70	44.3	56.9 ± 4.8	8.56 (9)	0.479
	90	29.5	54.7 ± 1.7	1.40 (9)	0.998
Adults	50	33.1	12.7 ± 2.7	43.80 (9)	<0.001
	70	15.9	4.0 ± 2.0	64.60 (9)	<0.001
	90	10.6	3.3 ± 1.1	65.67 (9)	<0.001

* The *P* value > 0.05 indicates that the observed assay data are not significantly different from the expected data for the Probit model.

Table 3. Mortality (%, mean ± ET) of the developmental stages of *R. dominica* when treated in semi-rigid packages with a filling ratio of 96% of chickpeas and different modified atmospheres at exposure times equivalent to reach 50% of mortality (LT_{50}) in flexible packages with a filling ratio of 4%.

4. DISCUSSION

While the decrease of CO_2 in flexible packages (4% filling ratio) was negligible (Fig. 1), it was significant in the semi-rigid containers with a 96% filling ratio (Table 2). Similar losses of CO_2 have been reported for semi-rigid packages with a filling ratio of 96% with chickpeas, in which the CO_2 content decreased around 45%, 40%, and 25% from the initial concentrations of 90%, 70%, and 50%, respectively, after 10 d of exposure (Iturralde-García et al, 2019). These differences in CO_2 losses between low and high filling ratio were due to the sorption of CO_2 caused mainly by the diffusion of the gas into the kernel pores by the van der Waals adsorption and by the formation of carbamate when reacting with the functional groups of proteins in the kernel (Brunauer, 1943; Mitsuda et al., 1973; Yamamoto and Mitsuda, 1980).

The sorption of CO_2 observed in this study were in the range of 0.19–0.32 g/Kg of chickpea at the three concentrations of CO_2 tested (Table 2). Similar sorptions were obtained in corn and coffee beans (0.306 g/kg and 0.203 g/kg); other grains such as rice, wheat, and red beans had lower sorption values (0.126 g/kg, 0.135 g/kg, and 0.115 g/kg), while peanuts, soybeans, and sesame seeds had higher sorptions (1.008 g/kg, 0.792 g/kg, and 0.414 g/kg) (Mitsuda et al, 1973). Iturralde-García et al. (2019) reported the equilibrium sorption of CO_2 to be of 0.47 g/Kg of chickpea at 49.5 h in semi-rigid packages with a filling ratio of 96% and 90% of CO_2. In comparison, the CO_2 sorption recorded in the present study represented 68% of the total capacity to sorb CO_2 of the chickpeas.

The negative pressure was mainly influenced by the filling ratio and the initial concentration of CO_2. The main increase in vacuum occurs during the 14.3 h of interaction at 90% CO_2 (60.65 kPa) (Table 2), which corresponded to the 70% of vacuum reported by Iturralde-García et al. (2019) at the equilibrium (49.5 h of exposure) in a similar study with chickpeas at 96% filling ratio and same initial concentration of CO_2.

The susceptibility to CO_2 of *R. dominica* developmental stages in flexible packages with a low filling ratio showed variations in sensitivity among them for the MAs tested (Fig. 2). Larvae were the most sensitive to hypercarbia and hypoxia, needing 1 day of exposure to obtain complete mortality, while the pupae presented the greatest tolerance, since they only reached complete mortality after 10 days of exposure to concentrations of 70% and 90% of CO_2. Other studies have reported the differences susceptibility of the developmental stages of *R. dominica* in wheat and rice. For wheat, with 50% CO_2 and a low filling ratio in the package, a total of 5 days are needed to obtain complete mortality of 1st instar larvae and 15 days to control pupae (Gonçalves et al., 2000). For rice, with 50% and 90% CO_2 and a low filling ratio, the oldest larvae and pupae were killed after 12 days and 8 days, respectively (Riudavets et al., 2009). Our results show similar mortalities of *R. dominica* developmental stages with other type of grains. For example the control with MAs of the common pest of the chickpea *Callosobruchus maculatus* (F.) (Col. Bruchidae) and *R. dominica* can be achieved together, since a total of 10 days were needed for reaching total mortality of *R. dominica* with 70% and 90% CO_2 (present study), and a total of 9 days are needed for killing all developmental stages of *C. maculatus* with 50%, 70%, or 90% CO_2 (Wong-Corral et al., 2013; Iturralde-García et al., 2016).

The fact that the susceptibility of larvae and adults of *R. dominica* were affected by the different concentrations of CO_2 while eggs and pupae were not can be related to the metabolic activity of each developmental stage. Active stages are more sensitive to hypoxia and hypercarbia than inactive stages, such as eggs and pupae (Hoback and Stanley, 2001; Navarro, 2006). Active stages, such as the young larvae and adults, present a high respiratory activity and have the ability to change their metabolism from aerobic to anaerobic in response to hypoxia and hypercarbia (Emekci et al., 2004). During the first 24 h, they try to adapt to the low oxygen concentrations by decreasing their metabolic activity and, therefore, the oxidation of the substrates decreased. But after 24 h, the process stops impeding the utilization of the O_2 and they became susceptible to MAs (Blomberg and Siegbahn, 2014; Levy-de la Torre et al., 2018).

Eggs have a different sensitivity to CO_2 during their developmental period, with the young and old eggs being more susceptible than intermediate developed eggs. Recently laid eggs lose more water or oxygen through the soft chorion than unexposed eggs, while in older eggs, the susceptibility to MAs is due to the high respiratory activity of the pre-emergent larvae (Mbata et al., 2004). Finally, the pupae are less disturbed by the reduction of the O_2 concentrations than the other developmental stages (Emekci et al., 2004).

The mortality of the developmental stages located outside the pulse in the treatments with a filling ratio of 96% were significantly lower than those of the filling ratio of 4%, reaching up to 20% (eggs) and 13% (adults) of the expected 50% mortality in the different concentrations of CO_2. This reduction was caused by the decline in the concentration of CO_2 in the headspace of the package caused by the sorption of the gas by the chickpeas. Mortality of the internal development stages (larvae and pupae) in the semi-rigid container was not significantly different from the expected 50% mortality on the flexible package, with exception of the larvae with a mortality of 64% at 90% of CO_2 that was significantly greater. This similitude between the mortality obtained and expected was due to the fact that the amount of CO_2 sorbed by each chickpea was similar in the flexible and in the semi-rigid packages. In the case of the larvae at 90% of CO_2, the greater mortality was probably due to the increase of the negative pressure in the package combined with the higher CO_2 concentration. Mbata et al. (2005) also has shown that the active larval stage of *C. maculatus* is sensitive to negative pressures.

In conclusion, and in agreement with our hypothesis, mortality of eggs and adults was lower in packages with a high filling ratio than in those with a low filling ratio. This reduction in mortality of the external developmental stages was due to the decline of CO_2 in the headspace of the package caused by its sorption on the chickpeas. However, and in disagreement with our hypothesis, mortality of larvae at concentration of 50% and 70% CO_2 and of pupae at all CO_2 concentrations tested were similar in packages with low and high filling ratios. This was due to the fact that all internal developmental stages were similarly exposed to CO_2 since the amount sorbed by each chickpea was similar at any filling ratio. The increment of the larval mortality at 90% of CO_2 in packages with high filling ratios could be due to the combined effect of the modified atmospheres and the negative pressure produced in the packages.

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