Effects of Carbon Dioxide and Oxygen on Heart Contraction Rate of Navel Orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae)[1]

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Abstract Fifth instars of navel orangeworm, *Amyelois transitella* (Walker), were exposed to controlled atmospheres to measure the effects of elevated carbon dioxide and reduced oxygen concentrations and temperature on heart contraction rate. The atmospheres contained varying concentrations of oxygen, carbon dioxide and nitrogen and were produced by blending air with carbon dioxide or nitrogen. Carbon dioxide concentrations between 50% and 99.5% caused immediate cardiac arrest in *A. transitella,* but the heart contractions restarted after a transient delay. The length of this delay was directly proportional to the concentration of carbon dioxide, and contraction rate following restart was inversely proportional to the concentration of carbon dioxide. Cardiac arrest was irreversible in 100% carbon dioxide. Heart contraction rate of *A. transitella* was directly proportional to both temperature and oxygen content of the atmosphere through a range of 15°C to 50°C.

Key Words Controlled atmospheres, hypercarbia, hypoxia, anoxia, cardiac arrest, temperature, dried fruits and nuts, postharvest pest control

The navel orangeworm, *Amyelois transitella* (Walker), is a primary lepidopterous pest of almonds, walnuts and pistachios in California. By infesting the mature nut meats, it causes serious economic damage and is considered a major pest of these crops. It also infests a wide variety of decayed fruits such as orange, peach, apricot, plum, and fig. Control of this postharvest pest is most often achieved by fumigating with a conventional fumigant like methyl bromide or phosphine. Alternatively, controlled atmosphere (CA) fumigation can be used (Calderon and Barkai-Golan 1990). However, CA technology has not been widely accepted as a standard treatment because of the lengthy exposure times required to insure efficacy and the rigorous sealing of the fumigation structure required to contain the CA. There is an obvious need to shorten exposure periods of CA commodity treatments so that this environmentally friendly technology will become more acceptable. Most efforts aimed at shortening exposures of CA have involved increasing the treatment temperature (Navarro and Calderon 1980, Soderstrom et al. 1986, Soderstrom et al. 1992, Whiting et al. 1992, Donahaye et al. 1994, Soderstrom et al. 1996). These studies have shown

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that for any given atmospheric composition of CA, higher temperature and lower RH shorten the exposure time needed to achieve an equal level of control.

In addition to their direct toxicity (Banks et al. 1980), CAs cause a variety of effects in insects such as reduced egg production (Lum and Flaherty 1972), growth retardation (Brooks 1957), synergism of conventional insecticides and fumigants (Cotton 1932, AliNiazee and Lindgren 1969, Speirs and Zettler 1972, Kashi and Bond 1975, Zettler and Gill 1998), and altered metabolism (Friedlander and Navarro 1979, Friedlander and Navarro 1984, Friedlander et al. 1984, Donahaye 1991, Adler 1994, Donahaye and Navarro 2000). Additionally, cardiac physiology has been extensively studied in various insects. For example, asphyxia (Jones 1964, Buck and Keister 1955) and hypercarbia (Arnold 1964, Edwards and Patton 1965) can alter the heart contraction rate and can cause cardiac arrest. However, these physiological events have not been studied in postharvest pests. Therefore, the objective of this study was to determine the effects of CAs containing reduced oxygen (O_2) and elevated carbon dioxide (CO_2) concentrations and the influence of temperature on heart contraction rate of fifth instars of *A. transitella.*

Materials and Methods

Amyelois transitella were taken from laboratory cultures reared on fortified wheat bran diet at 27°C and 60% RH as described by Fries et al. (1989). On the day of testing, wandering fifth instars were removed from rearing medium with forceps and held in a Petri dish until testing. Prior to treatment, a larva was transferred to an exposure chamber and allowed to acclimate, unrestrained, for at least 1 h.

The atmospheres to which the insects were exposed were prepared according to the method described by Soderstrom et al. (1990). Air, nitrogen (N_2), and CO_2 gases were proportioned, mixed and humidified to provide two types of atmospheres and ranges of concentrations: 1.0% to 10% O_2 produced by blending air with N_2; and 15% to 99.5% CO_2 produced by blending air with CO_2. Anoxic atmospheres (0% O_2) were produced by using CO_2 alone.

The atmospheres, conditioned to 60% RH, were metered through rotometers and valves to the exposure chambers. These were made of an acrylic plastic block (25 × 75 × 6 mm) into which a 9 × 3 mm well was milled to form a chamber (Fig. 1). The chamber was fitted with an O-ring set in a groove at the top of the well and then sealed with a glass microscope slide held in place with clips. Atmospheres were introduced through a 0.5-mm inside diam port and vented through an identical port on the opposite side of the chamber. Flow rates were maintained at about 3 ml/min with an Alltech digital flow-check meter (Alltech, Inc., Deerfield, IL). Each test atmosphere was preceded by a 2 min flush of air from a compressed gas cylinder. The concentrations of gases in the atmospheres were monitored with a Servomex® Model 570A O_2 analyzer (Servomex Co., Inc., Norwood, MA). The percentage of O_2 was measured directly. The percentage of CO_2 was monitored indirectly by first measuring the O_2 concentration and then calculating the concentration based on the ratio of O_2 to the other components in air.

Heart contraction in *A. transitella* larvae is visible through the cuticle and its response to the treatment CAs was recorded on a Mitsubishi HS-U760 VHS recorder using a Meiji Techno CK3800 TV video camera with an 18-108 mm Navitar TV zoom lens mounted above the exposure chamber. A Targa plus graphics board, release 4.0, (Truevision, Inc. 1993) and an Image-Pro Plus, version 1.3 for Windows®, (Media

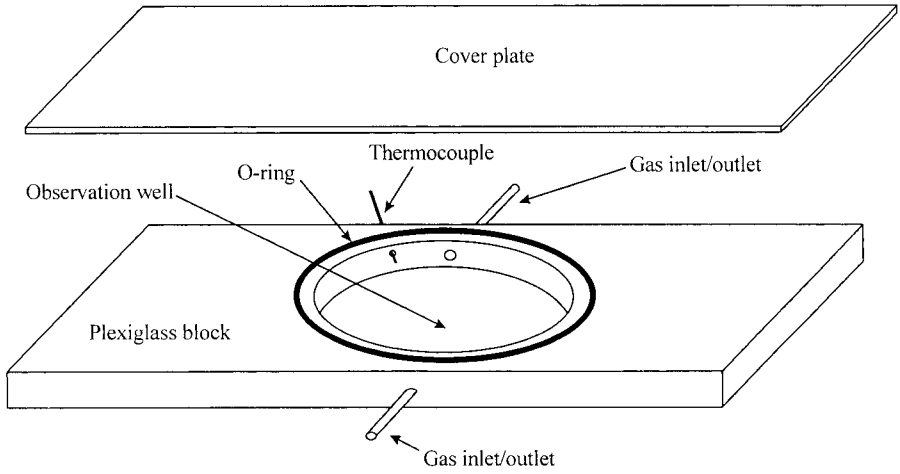

Fig. 1. Schematic view of exposure chamber used to expose individual insects to controlled atmospheres and to view resultant behavior.

Cybernetics 1994) were used for image processing. Gas mixing, humidifying, and insect treatment were done in a walk-in environmental chamber maintained at 25 ± 0.5°C.

CO_2 concentrations ranging from 15 to 100% (balance air) were tested against *A. transitella* for time to heart stoppage and subsequent contraction rate following restart. Air (0.03% CO_2) was included as a control treatment. Average contraction rate for each larva was based on an observation of 5 min. At least 24 individual larvae (range = 24 to 27), each tested individually, were observed for each treatment. The responses for contraction rate were fitted to a logistic curve (Nonlinear Least Squares) (SAS Institute 1997) from which predicted means and 95% confidence intervals were computed. The responses for time to restart were continuous, and the variances were unequal, and natural logs were used to both stabilize the variance (Levene's Test, $F = 74.76$, df = 1, $P = 0.001$) and linearize the response (GLM, SAS Institute 1997).

The effects of O_2 content and temperature of the CA on heart contraction rate were monitored by positioning the observation chamber above a laboratory hotplate coupled to a Powerstat voltage controller (Superior Electric Co., Bristol, CT) that facilitated ramping the temperature from 15°C to 50°C over 45 to 50 min. Temperature was monitored with an Omega model HH23 digital thermometer (Omega Engineers, Inc., Stamford, CT) using a type T thermocouple. Oxygen concentrations (balance N_2) were 1, 5, and 10%. Air (21% O_2) was included as a control treatment. Each treatment consisted of 3 to 6 replicates, each run on a separate day, with 5 insects per replicate. Data were subjected to GLM procedure (SAS Institute 1997), but different statistical methods were used among the treatments to accommodate the variance structures and the linearity of the responses. For the 1% O_2 treatment, the response to temperature was linear, but variances among replicates increased with temperature. This variance structure was modeled as follows: $\log_e(s) = -3.6 +$

0.063(temperature). A weighted regression with the reciprocals of the variances as weights was used, employing the above relationship between standard deviations and temperature to generate the variances. For the 5% and 10% O_2 treatments, square root transformation both stabilized the variances among replicates and linearized the responses. For the air treatment, the response was linear and the variances were homogeneous across temperatures. Predictions and their corresponding 95% confidence intervals were computed for each of the above cases. Overlapping confidence intervals served as means separator in all instances.

Results

When exposed to increasing CO_2 concentrations up to 40% in air, the heart of *A. transitella* continued to contract uninterrupted, and contraction rates were not statistically different from the control (Table 1). Treatment with ≥50% CO_2 caused immediate (within 1 s) but momentary stoppage of the heart. When O_2 was present in the CA, contractions resumed. However, at 100% CO_2 the heart abruptly stopped contracting and did not restart. Time required for the contractions to resume increased gradually from about 3 to 5 s to about 22 to 36 s as the CO_2 concentration increased from 50% to 99.5% in air. Contraction rate after restart was inversely proportional to CO_2 concentrations. For example, in air, heart contraction rate of mature larvae averaged about 1.7 beats/s. As the CO_2 concentration in the atmosphere increased,

Table 1. Time to heart stoppage of larval *Amyelois transitella* after exposure to controlled atmospheres containing different concentrations of carbon dioxide (in air balance) and time to subsequent restart of heart contractions

CO_2 concentration (percent in air)	Time (sec) to:		Contractions (beats/sec) after restart**
	Heart stoppage	Heart restart*	
100	<1	>4 hr†	0†
99.5	<1	36.4 (24.9–53.2)	0.61 (0.47–0.76)
90	<1	22.5 (16.8–30.2)	0.77 (0.65–0.89)
80	<1	13.6 (10.7–17.2)	0.94 (0.84–1.05)
60	<1	4.9 (3.6–6.7)	1.29 (1.17–1.41)
50	<1	3.0 (2.0–4.4)	1.43 (1.32–1.55)
40	None	—	1.56 (1.46–1.66)
30	None	—	1.66 (1.55–1.76)
15	None	—	1.76 (1.59–1.93)
0.03(air)	None	—	1.71 (1.60–1.83)

* Backtransformed predictions and 95% confidence intervals from linear regressions using $\log_e$ of time.
** Predictions for logistic model and 95% confidence intervals.
† Experiment terminated at 4 h.

the contraction rate decreased concomitantly, falling to an average of about 0.6 to 0.8 beats/s at 99.5% CO_2, just prior to cardiac arrest.

The effects of O_2 concentration and temperature on heart contraction rate in larval *A. transitella* are shown in Table 2. When observed in air, contraction rate increased from about 0.5 beats/s at 15°C to about 3 beats/s at 50°C, a 6-fold increase. When O_2 concentrations were reduced in the N_2 atmosphere, contraction rate increased proportionally with temperature but did so at rates less than in air. For example, when the O_2 concentration was reduced to 1%, contraction rate increased from about 0.5 beats/s to about 2 beats/s between 15°C and 50°C, a 4-fold increase. Contraction rates in 5% and 10% O_2 atmospheres were intermediate between those of air and 1% O_2 throughout the temperature range, although they were significantly reduced only at concentrations below 10% O_2 and above 20°C.

Discussion

The frequency with which the insect heart contracts varies in different species and also with the stage of development and physiological condition of the individual insect (Jones 1964, Jones 1977). CO_2 arrests the hearts of many insects (Arnold 1964). Edwards and Patton (1965) showed that the heart contraction rate of *Acheta* is reduced by increasing CO_2 concentrations and eventually stops at 100%. Asphyxia caused by submersion in water caused cardiac arrest immediately in *Culex* larvae and within 7 min in *Periplaneta* (Jones 1964). Asphyxia with either N_2 or helium also caused immediate cardiac arrest in *Phormia* (Buck and Keister 1955) and, with CO_2, in *Anopheles* larvae within 3 min (Jones 1964).

Results of our study support these findings by showing that cardiac arrest occurred immediately in larvae of *A. transitella* upon exposure to ≥50% CO_2 (Table 1). There are, however, some insects that are resistant to the effects of CO_2 whereby their hearts contract long after the insect is immobilized (Jones 1964). Such was not the

Table 2. Effect of temperature and oxygen content on heart contraction rate of larval navel orangeworm, *Amyelois transitella,* in a controlled atmosphere maintained with nitrogen

Temperature (°C)	Contraction rate* (beats/sec)			
	1% O_2	5% O_2	10% O_2	21% O_2 (Air)
15	0.49 (0.32–0.62)	0.45 (0.26–0.65)	0.48 (0.39–0.58)	0.50 (0.09–0.92)
20	0.71 (0.68–0.73)	0.63 (0.60–0.67)	0.70 (0.62–0.79)	0.85 (0.77–0.93)
25	0.94 (0.92–0.97)	0.83 (0.80–0.87)	0.97 (0.91–1.04)	1.21 (1.15–1.26)
30	1.18 (1.15–1.21)	1.06 (1.04–1.09)	1.29 (1.23–1.35)	1.56 (1.52–1.60)
35	1.42 (1.37–1.46)	1.32 (1.29–1.35)	1.64 (1.56–1.72)	1.92 (1.88–1.95)
40	1.65 (1.59–1.71)	1.60 (1.56–1.65)	2.04 (1.91–2.18)	2.27 (2.22–2.32)
45	1.89 (1.82–1.96)	1.92 (1.85–1.99)	2.49 (2.29–2.69)	2.63 (2.56–2.69)
50	2.13 (2.04–2.21)	2.30 (1.80–2.82)	2.95 (2.30–3.75)	2.98 (2.90–3.07)

* Predicted means (GLM) and 95% confidence intervals.

case with *A. transitella* in our study. In addition, asphyxiated insects can resume normal heart contractions in the presence of O_2 (Abdoul-Nasr 1960, Jones 1964) and recovery times vary up to 1 h. Our results showed that, following cardiac arrest, heart contractions of larval *A. transitella* recovered in the presence of as little as 0.1% O_2 (0.5% air containing 21% O_2) and that the time to recovery was directly related to the concentration of CO_2 in the atmosphere that initiated heart stoppage (Table 1). These effects, attributed to increasing levels of CO_2, are partly due to decreasing levels of O_2, however, because each of the CAs blended with air contained some O_2. Indeed, reduced O_2 levels alone affect heart contraction rate of *A. transitella* (Table 2).

It is not known if the arrested heart of *A. transitella* in the anoxic environment would resume contracting prior to death (our experiments were terminated at 4 h). However, some insects can survive for many days with their hearts either in a state of cardiac arrest or surgically removed (Jones 1964). Even if the heart restarted prior to death, it is unlikely its contractions would contribute to longer survival times because significantly high mortality occurs in *A. transitella* populations within about 2 d under similar anoxic conditions (Brandl et al. 1983).

Rates of biochemical reactions and physiological functions usually double or triple (temperature coefficient = 2 to 3) with each 10°C rise in temperature (Wigglesworth 1965). In our study, contraction rate of *A. transitella* followed this pattern by increasing directly with temperature (Table 2), although it was only in air at the lower temperatures that heart contraction rate approximated a temperature coefficient of about 2. Likewise, as the O_2 concentration of the CA increased to normal atmospheric levels, heart contraction rate concomitantly increased. However, there was little difference between the rates under different O_2 atmospheres. This is not surprising because insect respiration, stimulated by increased levels of CO_2 (Hazelhoff 1928) and reduced levels of O_2 (Cotton 1932), is effectively uncoupled from the circulatory system because the hemolymph is not concerned with gaseous transport. Thus, the composition and temperature of a CA would have a smaller effect on heart rate than on respiration. Nevertheless, CO_2 concentrations of 7 to 10% can cause ordinary respiratory movements of the abdomen (Hazelhoff 1928), presumably a mechanism for increasing aeration in the insect under the influence of such atmospheres.

Increased respiration caused by higher temperature can shorten the exposure time required for a CA to achieve equivalent insect mortality. For example, an increase in temperature from about 16° to 27°C of a low O_2 atmosphere at 60% RH reduced the LT_{95} of *A. transitella* larvae by about 2 to 4 times (Storey and Soderstrom 1977, Soderstrom et al. 1986). Also, increased respiration caused by elevated levels of CO_2 and reduced levels of O_2 can have a significant impact on the toxicity of respiratory fumigants. By forcing the spiracles to remain open longer than normal (Burkett and Schneiderman 1974, Miller 1974), these atmospheric gases permit the movement of more fumigant into the tracheae and thus render the insect more susceptible to it (Hazlehoff 1928, Cotton 1932, Jones 1938, AliNiazee and Lindgren 1969, Williams 1985, Scheffrahn et al. 1995). In addition, with the spiracles open, the insect cannot regulate its water balance and is subject to dehydration (Lighton 1996). Indeed, moisture loss has been implicated in the mortality of insects treated with CA (Jay et al. 1971).

Because CAs exert salient effects on the insect respiratory system, further studies should investigate their effect on insect respiration in relation to insect toxicity. In addition, CO_2 by virtue of its synergistic effects, should be studied in combination with conventional fumigants with the view that fumigant mixtures containing CO_2 can be

developed that require less dose and/or shorter exposure times than are required for the fumigants alone.

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