The metabolic responses of *Platynota stultana* pupae to reduced O₂, elevated CO₂, and their combinations were investigated using microcalorimetry, and mortality of pupae under elevated CO₂ atmospheres was correlated with metabolic responses. The metabolic heat rate decreased slightly with decreasing O₂ concentration until a critical O₂ concentration (Pc) below which the heat rate decreased rapidly. The Pc increased with temperature. The percentage decreases of metabolic heat rate were comparable to the percentage decreases of O₂ consumption rate (RO₂) at 10, 8, 6, and 4% O₂, but were smaller at 2 and 1% O₂. The metabolic heat rate decreased rapidly at 20% CO₂ relative to 0% CO₂, with little to no further decrease between 20 and 79% CO₂. The percentage decreases of RO₂ under 20 and 79% CO₂ at 20°C were comparable to the percentage decreases of metabolic heat rates. The additive effects of subatmospheric O₂ and elevated CO₂ levels on reducing metabolic heat rate were generally fully realized at combinations of ≤5% CO₂ and ≥4% O₂, but became increasingly overlapped as the O₂ concentration decreased and the CO₂ concentration increased. The high susceptibility of pupae to elevated CO₂ at high temperature was correlated with high metabolic heat rate. The metabolic responses of pupae to reduced O₂ concentrations included metabolic arrest and anaerobic metabolism. The net effect of elevated CO₂ on the pupal respiratory metabolism was similar to that of reduced O₂; however, mechanisms other than the decrease of metabolism were also contributing to the toxicity of CO₂. © 2000 Elsevier Science Ltd. All rights reserved.

**Keywords:** Carbon dioxide; Metabolic heat rate; Microcalorimetry; Oxygen; Respiration rate

### 1. Introduction

Controlled atmospheres (CA) with elevated CO₂, reduced O₂, or their combinations can be used to control insects (Carpenter and Potter, 1994; Mitcham et al., 1997b). Above 20%, CO₂ can cause significant insect mortality in proportion to CO₂ concentration. Reducing O₂ to less than 3% can be insecticidal, and efficacy increases as O₂ is reduced to lower concentrations. The combined effects of elevated CO₂ and reduced O₂ are less clear; some studies have shown additive effects while others have not (Fleurat-Lessard, 1990; Soderstrom et al., 1991). Temperature greatly affects the efficacy of CA; higher efficacy is usually achieved at higher temperatures (Banks and Annis, 1990; Carpenter and Potter, 1994). There have been few physiological or biochemical explanations for these mortality responses. Lack of such knowledge has rendered the development of CA treatments costly and time consuming (Carpenter et al., 1993). If we can understand the physiological and biochemical responses of insects to CA and can relate such responses to mortality, then we might be able to develop physiological or biochemical models to determine effective treatments instead of relying on empirical mortality tests.

Hypotheses have been proposed as to how invertebrates and higher animals respond to low O₂ environments (Herreid, 1980; Hochachka, 1986; Weyel and Wegener, 1996). An organism is described as a metabolic regulator if its O₂ consumption is independent of ambient O₂ concentrations and as a metabolic conformer if its O₂ consumption is dependent upon ambient O₂ concentrations. No species is a perfect regulator over the entire range of O₂ tensions; it becomes a conformer when the ambient O₂ concentration is below a critical level (Pc). A “good” regulator would have a low Pc. The
Reduced O₂ consumption leads to hypoxic/anoxic toxicity (Hochachka, 1986). Membrane permeability. The voltage-dependent Ca²⁺ gates are then opened, causing Ca²⁺ influx. The high concentration of Ca²⁺ in the cytosol activates phospholipases A₁, A₂, and C, leading to increased membrane phospholipid hydrolysis. The cell and mitochondrial membranes become more permeable, causing cell damage or death.

Do these general hypotheses apply to insects? If so, can some aspects of these hypotheses explain the effects of elevated CO₂? It has been proposed that the effects of hypercarbia on insects probably do not exclude the effects of hypoxia (Fleurat-Lessard, 1990) because high CO₂ can prevent insects from using O₂ (Navarro, 1975). However, this latter point is controversial because others have observed that the O₂ consumption rate of insects is not reduced by elevated CO₂ levels with 21% O₂ present (Edwards and Batten, 1973). As to the combined effects of elevated CO₂ and reduced O₂, it appears that the O₂ effect becomes marginal (Banks and Annis, 1990).

Our objectives were to address these questions by studying the metabolic responses of Platynota stultana pupae (an important pest on many horticultural commodities) to various levels of reduced O₂, elevated CO₂, and their combinations. Specifically, metabolic heat rates, indicative of the overall metabolic rates of an organism (Loike et al., 1981; Criddle et al., 1988), and respiration rates were measured under various atmospheres. In addition, mortality tests were performed under some atmospheres and the mortality responses were correlated with metabolic responses.

2. Materials and methods

2.1. Experimental insects

Platynota stultana was reared on a lima bean-based diet in an incubator at 27±0.5°C, 85% RH with a photoperiod of 16:8(L:D) h (Yokoyama et al., 1987). The 1–2 d old female pupae were selected for experiments because there was little variability in metabolic rate within this age group.

2.2. Calorimetry measurements

Rates of metabolic heat production were measured using differential scanning calorimeters with isothermal and temperature scanning capabilities (model 7707, Hart Scientific Inc., Provo, UT). The isothermal operating mode was used to measure metabolic heat rates at a given temperature. Each calorimeter has three measuring cells and one reference cell, allowing three samples to be measured simultaneously in one machine. Samples were placed in ampoules with an internal volume of 1.05 ml. The heat rates were measured continuously until they were stabilized to constant rates indicating that the samples and chamber had attained a steady state (approximately 45 min). The constant heat rates were corrected with baselines measured using empty ampoules. The corrected heat rates were the metabolic heat rates of the samples.

2.3. Controlled atmosphere set-up in the ampoules

Appropriate amounts of air, CO₂, N₂, and O₂ were mixed using metering valves to produce the desired atmospheres. The gas concentrations of the mixtures were analyzed by gas chromatography (model 211, Carle Instruments, Anaheim, CA). The gases flowed through a plastic bag (about 3 liters when fully inflated) at a constant rate of 2 liters/min after being first bubbled through water to obtain >90% relative humidity (RH). The plastic bag had an inlet, an outlet, and a sealable...
pupae were dried in an 80°C vacuum oven for at least 24 hours to obtain their dry weights. The percentage decrease of metabolic heat rate under an atmosphere was calculated.

2.5. Respiration measurement

The O$_2$ consumption rate (R$_{O_2}$) and CO$_2$ production rate (R$_{CO_2}$) of pupae under various O$_2$ concentrations were obtained by measuring the volume of O$_2$ (VO$_2$) consumed and the volume of CO$_2$ produced (VCO$_2$) at a given time in a closed syringe. Thirty pupae were weighed and placed in a 20 ml syringe without its needle. The syringe, along with the plunger and a small rubber septum, were placed in a plastic bag that was connected to a constant flow of a desired gas mixture as described above. When the correct concentration of the gas in the bag was established, the plunger was pushed into the syringe, leaving 18 ml of volume. Then the rubber septum was put on the tip of the syringe (where a needle is usually mounted) to seal it. These operations were performed through the plastic bag while it was sealed and the gas was flowing through it. Immediately after the sealed syringe was taken out of the bag, three 1-ml gas samples were taken from the syringe through the rubber septum and analyzed simultaneously for O$_2$ and CO$_2$ using an infra-red gas analyzer (model PIR-2000R, Horiba Instruments, Irvine, CA). The syringe, now having a volume of 15 ml, was placed in a temperature controlled room. The gas concentrations in the syringe were analyzed again after 2 hours. The VO$_2$ and VCO$_2$ were calculated from the change between the initial and final O$_2$ and CO$_2$ concentrations. The pupae were then dried in an 80°C vacuum oven for at least 24 hours to obtain pupal dry weight. The R$_{O_2}$s under various CO$_2$ concentrations were also measured as described above.

2.6. Mortality test

The mortality of pupae was tested under 20, 40, and 79% CO$_2$ at >90% RH (all added to 21% O$_2$, balance N$_2$) at 10, 20, and 30°C. Using flow boards, constant flow of gas mixtures at a rate of 150 ml/min passed through a 1 liter treatment jar where test pupae were placed. The gas concentrations inside jars were sampled daily during treatment and analyzed by gas chromatography.

Controlled atmosphere treatments were conducted in controlled temperature rooms maintained at 10, 20, and 30°C. Thirty pupae were placed in a cup with a mesh top. The cup was placed in a jar through which an atmosphere was passed. The range of treatment times for each atmosphere at each temperature was determined by preliminary tests, and corresponded with treatment times during which 10 to 100% mortality was expected. After treatments the pupae were transferred to an incubator at 27°C and 80–90% RH. Adult eclosion or lack thereof was observed after 2 weeks to determine mortality. All treatments were replicated at least 3 times.

2.7. Statistical analysis

The data for the percentage decrease of metabolic heat rate, respiration rates, and respiration quotient (RQ) were analyzed by ANOVA (GLM, SAS Institute, 1989). Means for significant effects were separated by t-test (LSD). Response surfaces were fitted for the percentage decrease of metabolic heat rates under the combinations of reduced O$_2$ and elevated CO$_2$. A separate probit curve for each treatment level was fitted with mortalities as the dependent variables and treatment duration as the independent covariates (PROC PROBIT, SAS Institute, 1989). The fitted probit curves were used to calculate LT$_{99}$ values.

3. Results

3.1. Temperature

The metabolic heat rate under air (21% O$_2$/0.03% CO$_2$) was 1.2, 3.7, and 7.5 μW/mg at 10, 20, and 30°C, respectively. The Q$_{10}$ between 10 and 20°C was approximately 3, and was 2 between 20 and 30°C.
3.2. Reduced O₂

The metabolic heat rate decreased with decreasing O₂ concentration (Fig. 1). At all three temperatures, the decrease was slight until a critical O₂ concentration below which the decrease became rapid. The critical O₂ concentrations were higher at higher temperatures, being 6% O₂ at 10°C, 8% O₂ at 20°C, and 10% O₂ at 30°C (Fig. 2(A)). Temperature had slight but significant effects on the heat rate decreases (Fig. 2(A)). The percentage decreases were slightly higher at higher temperatures at 6, 4, 2, and 1% O₂. But at 10 and 8% O₂ the percentage decreases at 10°C were higher than at 20 or 30°C. ANOVA results for reduced O₂ concentrations were highly significant (P<0.0001) for O₂ concentration, temperature and O₂ concentration×temperature.

The O₂ consumption rate (R₀₂) at 20°C decreased slightly with decreasing O₂ concentration down to 8% O₂, and then decreased rapidly (Fig. 3). The percentage decreases of R₀₂ at 20°C, which were 11, 15, 25, 41, 77, and 83 at 10, 8, 6, 4, 2, and 1% O₂, respectively, were comparable to the percentage decreases of metabolic heat rate under various O₂ concentrations at 20°C except at 2 and 1% O₂, where the percentage decreases of R₀₂ were about 10% higher (Fig. 2(A)). The percentage decreases of CO₂ production rate (R_CO₂), which were 9, 19, 21, 33, 58, and 70 at 10, 8, 6, 4, 2, and 1% O₂, respectively, were comparable to those of metabolic
heat rate under all O₂ concentrations (Fig. 2(A)). The respiratory quotient (RQ) showed no significant change between 21 and 4% O₂, with values between 0.65 and 0.80. However, the RQ increased significantly to about 1.3 when O₂ concentration was reduced to 2 and 1% (Fig. 3). ANOVA results for various O₂ concentrations were highly significant (P<0.0001) for RO₂, RCO₂, and RQ.

3.3. Elevated CO₂

The metabolic heat rate decreased rapidly between 0 and 20% CO₂ (Fig. 1), with a 60% decrease at 10 and 20°C and a 40% decrease at 30°C under 20% CO₂ (Fig. 2(B)). Further decrease of metabolic heat rate between 20 and 79% CO₂ was slight. At 10°C, there was no further decrease of metabolic heat rate between 20 and 79% CO₂. At 20°C, the metabolic heat rate further decreased under 60 and 79% CO₂, with a 72% decrease at 79% CO₂. At 30°C, the metabolic heat rate continued to decrease from 20 to 79% CO₂, but at a much slower rate than that from 0 to 20% CO₂. The percentage decreases of metabolic heat rate at a certain CO₂ concentration were generally lower at 30°C than at 10 and 20°C, which were mostly similar except at the two ends of the CO₂ spectrum (Fig. 2(B)). ANOVA results for elevated CO₂ concentrations were highly significant (P<0.0001) for CO₂ concentration, temperature and CO₂ concentration·temperature.

The RO₂ at 20°C (660 μl•h⁻¹•g⁻¹) decreased by 62% at 20% CO₂ (250 μl•h⁻¹•g⁻¹) and by 73% at 79% CO₂ (185 μl•h⁻¹•g⁻¹). The percentage decreases of RO₂ were comparable to the percentage decreases of metabolic heat rate under the same CO₂ concentrations (Fig. 2(B)).

3.4. Combinations of elevated CO₂ and reduced O₂

Reducing O₂ concentration at 20°C decreased metabolic heat rate further at all CO₂ concentrations (Fig. 4(A) and Table 1). However, the effects of reduced O₂ were smaller at higher CO₂ concentrations (Table 2). The effects of elevated CO₂ on metabolic heat rates varied with O₂ and CO₂ concentrations (Fig. 4(B), Table 1). At 4% O₂ or higher, metabolic heat rate decreased rapidly between 0 and 20% CO₂ and there was little further decrease between 20 and 79% CO₂. At 1% O₂, only 20 and 79% CO₂ decreased the metabolic heat rate further. The additional percentage decreases in metabolic heat rate contributed by elevated CO₂ were generally smaller at lower O₂ concentrations (Table 3). At 10% O₂, all CO₂ concentrations showed their full effects, with the additional percentage decreases similar to those at 21% O₂. At 1% O₂, however, there was little additional effect of CO₂ (Table 3).

The response surface of the percentage decrease of metabolic heat rate fitted with a polynomial of term 3 showed that the full additive effects on reducing metabolism mostly occurred at combinations of ≤5% CO₂ and ≥4% O₂ (Fig. 5). The combined effects of reduced O₂ and elevated CO₂ became increasingly overlapped as the O₂ concentration decreased and the CO₂ concentration increased.

3.5. Mortality responses to elevated CO₂

Temperature had a dominant impact on the mortality responses; the higher the temperature, the more susceptible the pupae (Fig. 6, Table 4). However, the effect of temperature varied with CO₂ concentration. At 20% CO₂, lowering temperature from 20 to 10°C increased LT₉₉ greatly (Table 4). At 79% CO₂, however, lowering temperature from 20 to 10°C did not change LT₉₉ significantly. CO₂ concentrations affected mortality, but the specific effects were dependent on temperature. Forty and 79% CO₂ were more effective than 20% CO₂ at all three temperatures; however, 79% CO₂ was not more
Table 1
Percentage decrease of metabolic heat rate of 1–2d old Platynota stultana female pupae under combinations of CO₂ and O₂ at 20°C.

<table>
<thead>
<tr>
<th>% O₂</th>
<th>% CO₂ 0</th>
<th>5</th>
<th>20</th>
<th>40</th>
<th>79</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>0.0 d, E</td>
<td>25.8 d, D</td>
<td>58.8 d, B</td>
<td>52.3 d, C</td>
<td>69.0 d, A</td>
</tr>
<tr>
<td>10</td>
<td>5.7 c, E</td>
<td>34.2 c, D</td>
<td>68.1 c, B</td>
<td>57.9 c, C</td>
<td>75.2 c, A</td>
</tr>
<tr>
<td>4</td>
<td>29.6 b, D</td>
<td>61.3 b, C</td>
<td>77.8 b, A</td>
<td>68.3 b, B</td>
<td>79.5 b, A</td>
</tr>
<tr>
<td>1</td>
<td>76.6 a, C</td>
<td>77.4 a, C</td>
<td>80.1 a, B</td>
<td>74.2 a, D</td>
<td>84.7 a, A</td>
</tr>
</tbody>
</table>

* Within each column, mean differences are indicated by lower case letters (t-test). Within each row, mean differences are indicated by upper case letters.

Table 2
The additional percentage decrease of metabolic heat rate caused by reduced O₂ when 1–2d old Platynota stultana female pupae were under various concentrations of CO₂ at 20°C.

<table>
<thead>
<tr>
<th>% O₂</th>
<th>% CO₂ 0</th>
<th>5</th>
<th>20</th>
<th>40</th>
<th>79</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
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<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>10</td>
<td>5.7</td>
<td>8.4</td>
<td>9.3</td>
<td>5.6</td>
<td>6.2</td>
</tr>
<tr>
<td>4</td>
<td>29.6</td>
<td>35.5</td>
<td>19.0</td>
<td>16.0</td>
<td>10.5</td>
</tr>
<tr>
<td>1</td>
<td>76.6</td>
<td>51.6</td>
<td>21.3</td>
<td>21.9</td>
<td>15.7</td>
</tr>
</tbody>
</table>

Table 3
The additional percentage decrease of metabolic heat rate caused by elevated CO₂ when 1–2d old Platynota stultana female pupae were under various concentrations of O₂ at 20°C.

<table>
<thead>
<tr>
<th>% CO₂</th>
<th>% O₂ 21</th>
<th>10</th>
<th>4</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>5</td>
<td>25.8</td>
<td>28.5</td>
<td>31.7</td>
<td>0.8</td>
</tr>
<tr>
<td>20</td>
<td>58.8</td>
<td>62.4</td>
<td>48.2</td>
<td>3.5</td>
</tr>
<tr>
<td>40</td>
<td>52.3</td>
<td>52.2</td>
<td>38.7</td>
<td>−2.4</td>
</tr>
<tr>
<td>79</td>
<td>69.0</td>
<td>69.5</td>
<td>49.9</td>
<td>8.1</td>
</tr>
</tbody>
</table>

effective than 40% CO₂ at 20 and 30°C. Increasing CO₂ concentration from 20 to 79% CO₂ greatly improved efficacy at 10°C, but not at 20°C.

An atmosphere of 40% CO₂+21% O₂ at 20°C caused high mortality at short treatment durations, e.g., above 40% mortality with only 3 hours of exposure (Fig. 6). This atmosphere also caused the pupal body fluid to leak out immediately during exposure. This body fluid leakage phenomenon did not occur under 20 and 79% CO₂ (+21% O₂), 0 to 21% O₂, or even 40% CO₂+1% O₂.

4. Discussion

4.1. Reduced O₂ concentrations

The O₂ consumption rate of Platynota stultana pupae decreased slightly with decreasing O₂ concentration until a critical concentration point (Pₖ) below which the decrease became rapid. This O₂ consumption pattern is typical of that of invertebrates in response to decreasing environmental O₂ concentrations (Herreid, 1980). The pupae regulated their O₂ consumption between 21 and 8% O₂ at 20°C, probably by increasing ventilation. The pupae were metabolic regulators at this O₂ range. However, the pupae became metabolic conformers at below 8% O₂ when increased ventilation could not compensate for O₂ insufficiency. It is interesting to note that the pupae’s Pₖ was lower at a lower temperature (6% O₂ at 10°C) and higher at a higher temperature (10% O₂ at 30°C). This is in accordance with the generalization that Pₖ is higher at higher metabolic demand (Herreid, 1980).

When the pupae could not compensate for the O₂ insufficiency in their tissues at below Pₖ, they started to experience hypoxia. Of the two strategies, metabolic arrest and anaerobic metabolism, that an organism uses to cope with hypoxia (Herreid, 1980; Hochachka, 1986; Weyel and Wegener, 1996), it seemed that metabolic arrest was the main strategy used by Platynota stultana pupae. With the decreasing O₂ consumption at below Pₖ, the pupal total metabolism, as indicated by metabolic heat rates, decreased accordingly; the percentage decreases of metabolic heat rate were comparable to the percentage decreases of O₂ consumption rates at 10, 8, 6, and 4% O₂. The RQ at these O₂ concentrations, with a range of 0.65 to 0.80, did not differ significantly with each other and with that at 21% O₂, suggesting that the pupae were still using lipids as their metabolic substrates.
and that the pupae did not initiate anaerobic metabolism. When O₂ concentration was reduced to 2 or 1%, the percentage decrease of metabolic rate was less than the percentage decrease of O₂ consumption, suggesting that the metabolic arrest could not match the decrease of oxidative phosphorylation. Anaerobic metabolism must be initiated to compensate for the shortage of energy. This was confirmed by the increased RQ (1.3) at 2 or 1% O₂. This O₂ concentration at which anaerobic metabolism was initiated can be denoted as Pₐ (called anaerobic compensation point in plant literature).

That insects use metabolic arrest to cope with hypoxia and anoxia was also observed on Locusta migratoria and Manduca sexta adults by Wegener and Moratzky (1995). The metabolic heat rates of L. migratoria and M. sexta did not change between 21 and 2% O₂ at 20°C, but decreased by 30–40% at 1% O₂, 60–75% at 0.5% O₂, and 95–96% at 0% O₂. The initiation of anaerobic metabolism by insects at very low O₂ tensions was also observed by Navarro and Friedlander (1975), who found that the lactate levels in Ephesia cautella pupae (6 mg/100 ml hemolymph) did not change when the O₂ concentration was reduced from 20 to 3% at 26°C, but rose suddenly at below 3% and reached 288 mg/100 ml hemolymph at 1% O₂.

From the above analysis we make the following hypothesis about the metabolic response of P. stultana pupae to reduced O₂ concentrations. When O₂ tension is above Pₐ, the insects can regulate their metabolism at
close to normal levels by accelerated ventilation. This O₂ range does not affect the insects except that high ventilation may cause water loss at high temperature and low humidity. However, at O₂ tensions below Pₐ when sufficient O₂ cannot be supplied to the tissues and thus ATP generation is reduced, the insects lower their metabolism; that is, they reduce metabolic demands. At the O₂ range between Pₐ and Pₐ, the reduced oxidative respiration is probably sufficient to satisfy the reduced energy demand and thus anaerobic metabolism is not necessary. This O₂ range would probably not threaten the insects’ survival. At O₂ tensions below Pₐ, the reduced oxidative respiration is not sufficient to satisfy the reduced energy demand. Anaerobic metabolism must be initiated to supplement the energy demand. Both the accumulated anaerobic end products and the very low metabolism impose stress on the insects (Hochachka, 1986). This O₂ range (below Pₐ) appears to be the insecticidal range.

Recent reviews of the use of controlled atmospheres for the control of insect pests (Banks and Annis, 1990; Mitcham et al., 1997b; Carpenter and Potter, 1994) have concluded that the O₂ level needs to be below 3% to be effective; and in most cases, it needs to be below 1% for rapid kill. These O₂ levels (below 3%) seem to coincide with Pₐ; the O₂ level at which anaerobic metabolism is initiated. It appears that empirical data support our proposition about the relationship between Pₐ and the toxic O₂ level.

It is important to point out that this relationship should not imply that anaerobic metabolism is the sole cause of hypoxic toxicity. The very low energy supply is probably the main cause of hypoxia toxicity, as proposed by Hochachka (1986). The low energy supply under hypoxia/anoxia has been confirmed by ATP measurements. The ATP concentration of the whole tissues of *Ephestia cautella* pupae decreased by 30% after exposure to 1% O₂ for 24 hours at 26°C (Friedlander and Navarro, 1979). The contents of ATP in the flight muscle of *L. migratoria* adults dropped to 1% of normal during 2 hours of anoxia; the ADP contents was also decreased to levels below normal while AMP accumulated 20 fold (Weyel and Wegener, 1996).

### 4.2. Elevated CO₂ concentrations

Our data clearly showed that elevated CO₂ concentrations prevented insects from using O₂ even with 21% O₂ present. The O₂ consumption rate of *Platynota stultana* pupae decreased by 62% in 20% CO₂+21% O₂ and by 73% in 79% CO₂+21% O₂ at 20°C. Similar observations have been made with other insect species. The O₂ consumption by *Ephestia cautella* pupae was significantly reduced by hypercarbia (Navarro, 1975). In crickets it was indicated that high CO₂ pushed respiration into anaerobic pathways (fermentative metabolism) even with 20% O₂ present (Kerr et al., 1993). The rate of respiration of *Tribolium confusum* adults, as measured by CO₂ output, was severely depressed during initial hours of exposure to elevated CO₂ concentrations (Aliniazee, 1971). However, there seem to be exceptions to this generalization. Edwards and Batten (1973) observed that the O₂ consumption rate of house flies did not decrease in 33% CO₂+21% O₂ compared with that in air. But this observation is in contradiction Edwards (1968) that high CO₂ inhibited in vitro succinic dehydrogenase in the gut tissues of *Heliothis zea* larvae, which suggests that O₂ consumption should be depressed by high CO₂ because the main metabolic pathway of oxidative respiration is inhibited.

Because elevated CO₂ prevents insects from using O₂, it appears that the net effect of elevated CO₂ on the insect respiratory metabolism is similar to that of reduced O₂. Both reduce oxidative phosphorylation even though the target sites of the two types of atmospheres may be different; reduced O₂ limits a substrate (O₂) of respiratory metabolism, whereas elevated CO₂ inhibits respiratory enzymes such as succinic dehydrogenase (Edwards, 1968). Reduced oxidative phosphorylation leads to reduced ATP generation. This has been demonstrated by Friedlander and Navarro (1979), who found that high CO₂ causes a decrease in ATP levels and the energy charge in insect tissues. It is likely that insects use the same strategies to cope with energy shortages caused by hypercarbia as those used to cope with energy shortages caused by hypoxia: metabolic arrest and/or anaerobic metabolism (Hochachka, 1986; Weyel and Wegener, 1996). That the strategy of metabolic arrest is used by insects in response to hypercarbia is supported by our observation that the total metabolism of *Platynota stultana* pupae decreased at elevated CO₂ concentrations and that the percentage decrease of metabolism, as indicated by metabolic heat rate, was comparable to the percentage decrease of O₂ consumption rate at various CO₂ levels. The insects probably reduce or cease most growth-related biosynthetic activity and limit their energy use to survival needs such as maintaining membrane potentials. That high CO₂ reduces NADPH production (Friedlander et al., 1984) and inhibits the biosynthesis of glutathione (Friedlander and Navarro, 1984) seems to support this notion. Although it was not clear from our data that the pupae initiated anaerobic metabolism under elevated CO₂, this effect has been shown by other researchers. Kerr et al. (1993) suggested that in crickets high CO₂ atmospheres induced anaerobiosis even with 20% O₂ present. Navarro and Friedlander (1975) observed that lactate rose in *Ephestia cautella* pupae exposed to 80% CO₂+20% O₂.

The metabolism of *Platynota stultana* pupae decreased rapidly as the environmental CO₂ concentration was elevated to 20%, with a 60% decrease at 20°C. Further decrease was slight when CO₂ concen-
tration was elevated from 20 to 79%. Since respiratory enzymes are inhibited by CO₂ (Edwards, 1968), this quantitative response seemed to indicate that the capacity of respiratory enzymes was increasingly inhibited by increasing concentrations of CO₂, but after a point more CO₂ did not further inhibit the capacity. It is interesting to note that empirical mortality data have shown that toxic levels of CO₂ are generally above 20% (Banks and Annis, 1990; Mitcham et al., 1997a; Carpenter and Potter, 1994).

4.3. Temperature

The normal metabolic rate of Platynota stultana pupae tripled from 10°C to 20°C and doubled again from 20°C to 30°C, reflecting the huge impact of temperature on insect metabolism. Temperature also has a slight but significant effect on the metabolic response of insects to both reduced O₂ and elevated CO₂, but the effect seemed to differ between reduced O₂ and elevated CO₂. The percentage decrease of metabolism by a given low O₂ concentration was higher at higher temperatures, whereas the percentage decrease of metabolism by a certain elevated CO₂ concentration was lower at higher temperatures. However, it is interesting to note that the response patterns with varying O₂ or CO₂ concentrations at different temperatures were similar.

4.4. Relationship between metabolic response and mortality response to elevated CO₂

Three trends have been observed regarding the mortality response of Platynota stultana pupae to elevated CO₂: (1) the pupae were more susceptible to CO₂ treatment at higher temperatures; (2) the effects of temperature varied with individual CO₂ concentration; and (3) CO₂ concentration (above 20%) affected mortality, but the specific effects were temperature dependent. The higher susceptibility at higher temperatures seemed to correlate with higher metabolism. However, it is interesting to note that the metabolic response to elevated CO₂, as indicated by the percentage decrease of metabolism, was only slightly different at 10, 20 and 30°C. In fact, the percentage decreases at 30°C were less than the percentage decreases at 20 and 10°C. It appeared that it is not the relative percentage decrease of metabolism but the absolute decrease of metabolism that was related to susceptibility. To illustrate, if we accept that metabolism can be represented by the unit of metabolic heat rate, then the absolute decrease of metabolism by 20% CO₂ was 2.2 μW/mg at 20°C (a 60% decrease of the normal metabolism of 3.7 μW/mg). The absolute decreases of metabolism were 3.1 at 30°C and 0.7 at 10°C. It is likely that it is the absolute decrease of metabolism that causes energy shortage, which would have to be compensated from the same ATP pool. Because the absolute decrease of metabolism is much lower at 10°C than at 20 or 30°C, it would take longer to use up the ATP pool at 10°C than at 20 or 30°C. Therefore, it seems that the insect susceptibility is related to the absolute decrease of metabolism. However, this correlation cannot explain the observation that the efficacy of 79% CO₂ differed little at 10 and 20°C.

The efficacy of 40 and 79% CO₂ was higher than that of 20% CO₂ at all three temperatures. But there was no difference between 40 and 79% at 20 and 30°C, while 79% was more effective than 40% at 10°C. Similar findings have been obtained with other insect species. The LT₉₅% for codling moth eggs at 25°C were 3.6, 1.3, 1.4, and 1.6d at 20, 40, 60, and 80% CO₂ in air (Soderstrom et al., 1991), suggesting that the efficacy was not enhanced above 40% CO₂. The mortality of New Zealand thrips adults did not increase when CO₂ concentration was increased from 40 to 60% at 24°C (Carpenter et al., 1998). Recent reviews have concluded that there was no enhancement of insect mortality above 40–60% CO₂ (Banks and Annis, 1990; Carpenter and Potter, 1994). Our data show that this conclusion is mostly applicable to temperatures such as 20 and 30°C. At 10°C, increasing CO₂ concentration from 40 to 79% increased mortality of Platynota stultana pupae. The increased efficacy of CO₂ concentrations above 40–60% at low temperatures was also observed at 0°C on Pacific spider mites (Zhou and Mitcham, 1998). The metabolism of Platynota stultana pupae decreased rapidly from 0 to 20% CO₂, but further decreases were slight between 20 and 79% CO₂. The minor enhancement of mortality between 40 and 79% CO₂ at 20 or 30°C could be related to the slight further decrease of metabolism. However, the higher efficacy of 40% CO₂, compared with that of 20% CO₂, was not correlated with a similar percentage decrease of metabolic rate. In addition, although the efficacy of CO₂ increased greatly from 20 to 79% CO₂ at 10°C, the percentage decrease of metabolism showed no difference at this concentration range. It seems that mechanisms other than the decrease of metabolism were contributing to the toxicity of CO₂. For example, 40% CO₂ at 20°C caused Platynota stultana pupae’s body fluid to leak out, suggesting that the insects’ membrane systems were affected. Because CO₂ can increase intracellular Ca²⁺ by decreasing pH (Lea and Ashley, 1978), it is likely that although the metabolism cannot be further reduced by CO₂ concentration above 40%, elevating CO₂ concentration can further decrease pH and thus cause intracellular Ca²⁺ to rise more and faster, leading to cell damage or death (Hochachka, 1986). The greater efficacy of higher concentrations of CO₂ at low temperatures could be related to the higher solubility of CO₂ in tissues at low temperatures (Yacee, 1986).

It is important to point out that the metabolic responses presented in this report were immediate responses, which do not necessarily reflect the responses
under extended exposure to reduced $O_2$ or elevated $CO_2$ concentrations. It is interesting to note that the percentage decreases of metabolism are comparable between 2% $O_2$ and 20 or 40% $CO_2$ and between 1% $O_2$ and 79% $CO_2$. If other modes of action in addition to the decrease of metabolism are contributing to $CO_2$ toxicity, then the elevated $CO_2$ concentrations should be more effective than their comparable reduced $O_2$ concentrations.

4.5. Combinations of elevated $CO_2$ and reduced $O_2$

Empirical studies on the additive effects of combinations of elevated $CO_2$ and reduced $O_2$ on insect mortality have yielded mixed results. Some observed additive effects (Calderon and Navarro, 1979; Krishnamurthy et al., 1986) while others did not (Soderstrom et al., 1991; Mitcham et al., 1997a). However, it seems that these different results are probably, in most part, attributable to the different ranges of gases used; additive effects were mostly observed at milder gas combinations such as 5–15% $CO_2$+2% $O_2$, while absence of additive effects was mostly observed at more severe gas combinations, such as >40% $CO_2$+0 to 0.5% $O_2$. These mixed results in mortality are probably related to metabolic responses. The additive effects of combinations of elevated $CO_2$ and reduced $O_2$ on the decrease of metabolism of *Platynota stultana* pupae were almost fully realized at combinations of ≤5% $CO_2$ and ≥4% $O_2$. However, the combined effects became increasingly overlapped as $O_2$ concentration decreased and $CO_2$ concentration increased.

Assuming that the decrease of metabolism is the main mode of toxicity, the observations that the additional decreases of metabolism contributed by reduced $O_2$ were smaller at higher $CO_2$ concentrations and that the additional decreases of metabolism contributed by elevated $CO_2$ were smaller at lower $O_2$ concentrations suggest that reducing $O_2$ concentrations at high concentrations of $CO_2$, such as 40–79%, would not enhance mortality nor would elevating $CO_2$ concentrations at <1% $O_2$ concentrations. This information should reduce the amount of empirical testing required for development of insecticidal controlled atmosphere treatments.

Acknowledgements

We thank Veronique Bikoba, James Shannon, and William Biasi for their technical assistance in the research. We thank John Church and Lisa Neven for consultations and Alan Carpenter and Adel Kader for reviewing the manuscript. This project was supported by USDA NRI grant # 58-5352-8-011.

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