USING MICROCALORIMETRY TO BETTER UNDERSTAND INSECT RESPONSE TO CA

Shijun Zhou[1], Richard S. Criddle[2] and Elizabeth J. Mitcham[1]*
[1]Dept. of Pomology, University of California, Davis, CA 95616
[2]Dept. of Molecular and Cellular Biology, University of California, Davis, CA 95616

The mode of action of controlled atmospheres on insects was investigated. Specifically, we investigated the effects of a host of factors such as temperature, atmosphere compositions, and treatment duration on the energy metabolism of omnivorous leafroller (*Platynota stultana*) female pupae using differential scanning calorimeters. In addition, the relationship between the pupal metabolic response and their susceptibility to controlled atmosphere treatments was investigated.

Temperature Effects
The normal metabolic heat rate, indicative of total metabolic rate, tripled from 10 to 20°C and doubled from 20 to 30°C, reflecting the huge impact of temperature on insect metabolism. Temperature also had a slight but significant effect on the metabolic response of insects to both reduced O_2 and elevated CO_2. The percent decrease of metabolism by a certain reduced O_2 concentration was higher at higher temperatures, whereas the percent decrease of metabolism by a certain elevated CO_2 concentration was lower at higher temperatures. However, the response patterns with varying O_2 or CO_2 concentrations at different temperatures were similar. The critical O_2 concentration, at which the pupae's O_2 consumption became dependent on atmospheric O_2 concentration, increased with temperature, with 6% O_2 at 10°C, 8% O_2 at 20°C, and 10% O_2 at 30°C.

Immediate Response to Low Oxygen and High Carbon Dioxide Concentrations
The metabolic heat rate decreased slightly with decreasing O_2 concentration until a critical O_2 concentration below which the heat rate decreased rapidly. The percent decreases in metabolic heat rate at 20°C were comparable to the percent decreases in O_2 consumption rate at 10, 8, 6, and 4% O_2, but were smaller at 2 and 1% O_2. Respiratory quotient showed no significant change (0.65 – 0.80) at 21– 4% O_2, but increased to 1.3 at 2 and 1% O_2. The metabolic heat rate decreased rapidly between 0 and 20% CO_2 (by 40-60% at 20% CO_2). There was no or slight further decrease in metabolic heat rate between 20 and 79% CO_2, depending on temperature, and O_2 consumption rates were comparable. The additive effects of reduced O_2 and elevated CO_2 on reducing metabolic heat rate were generally fully realized at combinations with ≤ 5% CO_2 or ≥ 4 % O_2. The combined effects of reduced O_2 and elevated CO_2 became increasingly overlapped as the O_2 concentration decreased and the CO_2 concentration increased. The high susceptibility of pupae to elevated CO_2 at high temperature was correlated with high metabolic heat rate. The metabolic responses of pupae to reduced O_2 concentrations were metabolic arrest and anaerobic metabolism. Since elevated CO_2 prevented the pupae from using O_2, the net effect of elevated CO_2 on the pupal respiratory metabolism was similar to that of reduced

O_2; however, mechanisms other than the decrease of metabolism were also contributing to the toxicity of CO_2.

We came to the following hypothesis about the metabolic response of *Platynota stultana* pupae to reduced O_2 concentrations. When O_2 tension is above the critical O_2 concentration, the insects can regulate their metabolism at close to normal levels by accelerated ventilation. This O_2 range does not affect the insects except that high ventilation may cause water loss at high temperature and low humidity. However, at O_2 tensions below the critical O_2 concentration when sufficient O_2 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 O_2 range between the critical O_2 concentration and the anaerobic compensation point, the reduced oxidative respiration is probably sufficient to satisfy the reduced energy demand and thus anaerobic metabolism is not necessary. This O_2 range would probably not threaten the insects' survival. At O_2 tensions below the anaerobic compensation point, the reduced oxidative respiration is not sufficient to satisfy the reduced energy demand, and 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. The O_2 range below the anaerobic compensation point appears to be the insecticidal range. This appears to agree with empirical data which has shown that the O_2 level needs to be below 3% to be toxic and in most cases, it needs to be below 1% for rapid kill.

Assuming that the decrease of metabolism is the main mode of toxicity of controlled atmospheres, 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 very low O_2 concentrations, such as < 1%. This information should reduce the amount of empirical testing required for development of controlled atmosphere treatments.

Long Term CA Effects

The metabolic heat rate of omnivorous leafroller pupae remained reduced and did not recover over time under elevated CO_2 or reduced O_2 atmospheres. The pupae developed under reduced metabolism and emerged under 6% O_2 or 5% CO_2, which decreased metabolism by 20 to 27%. Under 40 and 79% CO_2, which decreased metabolism by 60 and 70%, respectively, the pupae did not develop and the metabolic heat rate decreased further after 5 days.

Mortality of pupae under these controlled atmospheres showed that with a similar decrease in metabolic heat rate due to elevated CO_2 or reduced O_2, the insecticidal efficacy of elevated CO_2 was greater than that of reduced O_2. These findings indicate that the mode of action of CO_2 on insects was more than just by reducing metabolism. It is likely that the increased susceptibility to energy shortage under elevated CO_2 is attributable to higher permeability of cell membranes.

Table 1: Major findings and significance.

1. The metabolic responses of pupae to reduced O_2 concentrations were metabolic arrest and anaerobic metabolism.

2. The O_2 range below the anaerobic compensation point appears to be the insecticidal range.

3. 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 very low O_2 concentrations, such as < 1%.

4. Mortality of pupae showed that with a similar decrease in metabolic heat rate due to elevated CO_2 or reduced O_2, the insecticidal efficacy of elevated CO_2 was greater than that of reduced O_2.

5. The mode of action of CO_2 on insects was more than just by reducing metabolism.

6. It is likely that the increased susceptibility to energy shortage under elevated CO_2 is attributable to higher permeability of cell membranes.

7. Additional work is needed to determine if general response to CA varies significantly by insect species.

8. This knowledge should reduce the need for empirical testing to develop quarantine treatments.