Bulletin of Entomological Research (2006) **96**, 213–222 DOI: 10.1079/BER2006424

REVIEW ARTICLE

The mode of action of insecticidal controlled atmospheres

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Abstract

Arthropods cope with reduced oxygen and elevated carbon dioxide atmospheres with a reduction in metabolic rate, also called metabolic arrest. The reduction in metabolism lessens the pressure on the organism to initiate anaerobic metabolism, but also leads to a reduction in ATP production. The natural permeability of cellular membranes appears to be important for the survival of the arthropod under low oxygen or high carbon dioxide atmospheres. Despite the similarities in response, arthropod mortality is generally greater in response to high carbon dioxide as apposed to low oxygen atmospheres. There appears to be a greater decrease in ATP and energy charge in arthropods exposed to high carbon dioxide as compared with low oxygen atmospheres, and this may be due to greater membrane permeability under carbon dioxide leading to an inefficient production of ATP. Reduced oxygen and elevated carbon dioxide atmospheres can have an additive effect in some cases, depending on the concentrations used. The effect of these atmospheres on arthropods depends also on temperature, species and life stage. Additional work is needed to fully understand the mode of action of controlled atmospheres on arthropod pests.

Keywords: carbon dioxide, disinfestation, insect, oxygen, temperature

Introduction

Phytosanitary measures to control insects in fresh and dried fruits, nuts and vegetables after harvest are critical to interstate (US domestic) and international marketing. Chemical fumigation, for example methyl bromide, was the most common method used for the control of arthropod pests in fresh products. However, methyl bromide fumigation has been phased from general usage in developed countries as of 2005 and will be phased out in developing countries by 2015. In addition, pressure from international consumer groups to reduce the chemicals used on food products makes a non-chemical insect control measure highly desirable. The development of insecticidal controlled atmosphere treatments is of interest as potential non-chemical alternatives to fumigants such as methyl bromide.

Low O_2 and elevated CO_2 atmospheres have been used for many years to control stored product pests in grains (De Lima, 1990). The terms controlled atmosphere and modified atmosphere are often used to describe these types of atmosphere modifications. The main difference between the two is the degree of precision in control of the gas concentrations. In controlled atmospheres, gas concentrations are controlled to within a few percent of setpoints through the addition of N_2 or air or scrubbing of CO_2. When the respiration of the commodity sealed in a container is used to alter the ratio of O_2 and CO_2 during storage, this is called modified atmosphere. For much of this paper, the experiments discussed tend to be laboratory studies where the ratio of O_2 and CO_2 is carefully controlled. Although controlled and modified atmospheres have been used for many years to store fresh fruits and vegetables, atmosphere

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treatments have not been commercially used for insect control in fresh fruit and vegetables. There has, however, been considerable research in this area in recent years.

Controlled atmospheres that are insecticidal generally contain $\geq 20\%$ CO_2 and/or $\leq 1\%$ O_2 depending on the temperature, with the remainder of the atmosphere composed of N_2 gas. These atmospheres are outside the optimum range for storage or transport of nearly all fresh fruits and vegetables, and generally induce stress in the commodity. In fact, product tolerance is generally the limiting factor in developing an effective insecticidal controlled atmosphere treatment. In spite of this, interest in insecticidal controlled atmosphere treatments is on the rise as more commodities are tested for tolerance and insect mortality due to controlled atmospheres is determined (Mitcham *et al*., 2001).

There is a significant amount of literature on the mortality of insect pests in response to various controlled atmosphere treatments (Mitcham *et al*., 2001). However, there is considerably less information on the mode of action of controlled and modified atmospheres on insects. The most recent review in this area was by Carpenter & Potter (1994), and a more thorough review can be found in Fleurat-Lessard (1990). This article will briefly review the information presented in Fleurat-Lessard (1990) and provide an update on new information in this area. The new information covers many different insects, including stored product insects, insects of quarantine significance, and some insects that may, during their normal life cycle, be exposed to anoxic, hypoxic, or hypercarbic conditions, such as fly larvae in carrion and beetle larvae in flooded underground burrows. The authors recognize that because many insect species live in different types of environments and have different life styles, the existence of more than one response to and mode of action of controlled atmospheres is plausible.

Response to low O_2

Metabolic effects

The definition of a hypoxic atmosphere loosely applies to any atmosphere containing an O_2 concentration of less than 21%. Anoxic atmospheres have a total absence of O_2. In general, arthropods are hypoxia-tolerant organisms, but their mortality increases with lower O_2 concentrations (Hoback & Stanley, 2001). Insect development under low O_2 slows and typically ceases under anoxia. A reduction in the metabolic rate, also called metabolic arrest, has been proposed as a major strategy used by animals to cope with hypoxia (Herreid, 1980; Hochachka, 1986; Weyel & Wegener, 1996). The reduction in metabolism lessens the pressure on organisms to initiate anaerobic metabolism, which would require very high rates of anaerobic glycolysis and thus lead to rapid exhaustion of carbohydrate reserves while toxic end products accumulate (Hochachka, 1986; Weyel & Wegener, 1996; Ofuya & Reichmuth, 2002). However, metabolic arrest, when decoupled from membrane functions, has been thought to be the cause of hypoxic/anoxic toxicity (Hochachka, 1986). According to Hochachka, reduced O_2 consumption leads to a decreased rate of ATP production. As a result of energy insufficiency, the membrane ion pumps fail, leading to K^+ efflux, Na^+ influx, and membrane depolarization. The voltage-dependent Ca^{2+} gates are then opened, causing Ca^{2+} influx. The high concentration of Ca^{2+} in the cytosol activates phospholipases A1, A2, and C, leading to increased membrane phospholipid hydrolysis. The cell and mitochondrial membranes become more permeable, leading to cell damage or death (Hochachka, 1986). Some evidence for this physiological pathway was reported in experiments with *Schistocerca gregaria* (Forskål) (Orthoptera: Acrididae). Minutes after treatment with anoxia, ATP levels declined and ADP, AMP, and IMP (inosine monophosphate) levels increased (Hoback & Stanley, 2001).

Zhou *et al*. (2000) found that *Platynota stultana* Walsingham (Lepidoptera: Tortricidae) pupae used metabolic arrest as a major response to hypoxia. The O_2 consumption rate and metabolic heat rate of the pupae decreased slightly with decreasing O_2 concentration until a critical concentration point below which the decrease became rapid. The critical concentration points were 10, 8, and 6% at 30, 20, and 10°C, respectively. Although their metabolism decreased quickly below the critical concentration points, the pupae did not initiate anaerobic metabolism until the O_2 concentration was below 2% at 20°C. Zhou *et al*. (2001) also found that under 6 or 4% O_2 at 20°C, the pupae developed to eclosion with a reduced metabolic rate, but could not develop under 2 or 1% O_2. The metabolic heat rate of pupae was decreased by about 40% at 4% O_2 and by 60% at 2% O_2.

A similar response was observed by Wegener & Moratzky (1995) with adults of *Locusta migratoria* (Linnaeus) (Orthoptera: Acrididae) and *Manduca sexta* (Johannsen) (Lepidoptera: Sphingidae). At 20°C, the metabolic heat rates of *L. migratoria* and *M. sexta* did not change between 21 and 2% O_2, but decreased by 30–40% at 1% O_2, 60–75% at 0.5% O_2, and 95–96% at 0% O_2. Alder beetle adults, *Agelastica alni* (Linnaeus) (Coleoptera: Chrysomelidae), showed a 5% reduction in metabolic heat rates at 21.7°C as measured using a calorimeter when air was changed to pure N_2. Metabolism decreased as the atmosphere became more hypoxic until it reached a stable rate at anoxia (Kölsch *et al*., 2002). Metabolic effects of hypoxia were also observed in *Schistocerca americana* (Drury) (Orthoptera: Acrididae). Metabolic rates of grasshoppers recovering in air after exposure to hypoxic conditions were 45% higher compared to grasshoppers that were not exposed to hypoxic atmospheres. The authors suggested that the higher metabolic rate was a sign of classic O_2 debt related to lactate removal (Greenlee & Harrison, 1998). Oxygen debt was also observed in the desert locust, *S. gregaria*. During recovery in air after treatment for 8 h in an anoxic atmosphere, the metabolic rate increased above the metabolic rate measured before treatment. Metabolism was reduced by 6% during treatment compared to the control (Hoback & Stanley, 2001). In *A. alni*, the metabolic heat rate increased by 50% above normoxic levels within 15 min after treatment with pure N_2 gas. Even after 5 h of recovery in air, the metabolic heat rate remained higher than in normoxia (Kölsch *et al*., 2002).

Decreased respiration can be used as a measurement of reduced metabolism. Respiration decreased by 50% of the normal rate in larval *Phormia regina* (Meigen) (Diptera: Calliphoridae) in 2% O_2 and in *Calliphora vomitoria* (Linnaeus) (Diptera: Calliphoridae) larvae at 1% O_2. In *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) immature stages and eggs, respiration rates decreased proportional to the amount of O_2 in the atmosphere (Emekci *et al*., 2004). Egg respiration was suppressed the most at less than 3% O_2. Respiration rates were lowered for *R. dominica* pupae, but this stage was not as affected as immature and egg stages. Increased respiration in adults was observed in 3% and 5% O_2 levels at 30°C and 70% relative humidity, suggesting respiratory stress. Additionally, respiration quotient values were higher than respiration quotient values measured in air, suggesting resource type changes (carbohydrates vs. amino acids vs. lipids) potentially indicating a mechanism insects use for acclimating to controlled atmospheres and metabolic stress (Emekci *et al*., 2004). Similar results were observed in *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) exposed to reduced O_2 concentrations (Emekci *et al*., 2002). Respiration rates were reduced in atmospheres containing less than 5% O_2 in eggs, larvae and pupae (*T. castaneum* pupae, as with *R. dominica* pupae, were not as affected). Similar to *R. dominica* adults, respiration rates also increased at 3 and 5% O_2 with *T. castaneum* adults. Emekci *et al*. (2002) suggested increased respiration at these O_2 concentrations compensated for the reduced amount of O_2 in the atmosphere. At O_2 levels of less than 3%, the authors suggest that survival was attributed to suppressed respiration (Emekci *et al*., 2002).

The intertidal root aphid, *Pemphigus trehernei* Foster (Hemiptera: Aphididae), is subjected to periodic immersion and copes with anoxic conditions by maintaining low metabolic rates (Hoback & Stanley, 2001). Diapausing pupae of the flesh fly, *Sarcophaga crassipalpis* Macquart (Diptera: Sarcophagidae), with lower metabolism, survive longer in anoxia than non-diapausing pupae. Interestingly, the development of diapausing pupae is stimulated rather than repressed by anoxia. At low temperatures, eggs of *Bombyx mori* (Linnaeus) (Lepidoptera: Bombycidae) survived more than 50 days of anoxia due to a low metabolic rate during diapause. Similarily, tiger beetle larvae, *Cicindela togata* Casey (Coleoptera: Cicindelidae), survive flooding of their underground burrows by reducing their metabolic rates by 97%. Anaerobic pathways further support reduced metabolism if anoxia continues (Hoback & Stanley, 2001). More about the initiation of the anaerobic pathway follows below.

Anaerobic respiration

Zhou *et al*. (2000) hypothesized that when O_2 tension is above the critical concentration point (P_c), *P. stultana* pupae can regulate their metabolism at close to normal levels by accelerated ventilation, similar to increased respiration observed in *T. castaneum* and *R. dominica* at 3 and 5% O_2 (Emekci *et al*., 2002, 2004). This O_2 range does not affect the insects except that high ventilation may cause water loss at high temperature and low humidity (Mbata & Phillips, 2001). However, at O_2 tension below P_c, when sufficient O_2 cannot be supplied to the tissues and thus ATP generation is reduced, the insects lowered their metabolic rate; that is, they reduced metabolic demands. At the O_2 range between P_c and the anaerobic compensation point (P_a), 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 tension below P_a, 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). The initiation of anaerobic metabolism by insects at very low O_2 tensions was observed by Navarro & Friedlander (1975), who found that the lactate levels in *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae) pupae (6 mg per 100 ml haemolymph) did not change when the O_2 concentration was reduced from 20 to 3% at 26°C, but rose suddenly at below 3% and reached 288 mg per 100 ml haemolymph at 1% O_2. This O_2 range (below P_a) appears to be the lethal range. Abnormal quantities of various end products accumulate during anoxia. The survival of insects under anoxia depends on the ability of their nervous systems to tolerate lethal quantities of metabolic end products (Kennington & Cannel, 1967).

Montane carabid beetles, *Pelophila borealis* (Paykull) (Coleoptera: Carabidae), are periodically encased in ice and survive anoxia by anaerobic respiration (Hoback & Stanley, 2001). Lactate accumulates but mechanisms must exist to tolerate or rid the body of lactate because 100% of the beetles can survive anoxia for 127 days at 0°C. Lactate and alanine are typical anaerobic end-products in terrestrial insects (Hoback & Stanley, 2001). When treated with pure N_2 gas for 10 h at 20°C, alder leaf beetles, *A. alni*, had a highly significant increase in lactate compared to control beetles. Alanine was also increased (Kölsch *et al*., 2002). Bot fly larvae, *Gasterophilus intestinalis* (De Geer) (Diptera: Gasterophilidae), were found to have high concentrations of succinate in their haemolymph suggesting that these flies use anaerobic metabolism to survive anoxia. The well developed succinate-oxidase system of the larvae may rid them of excess succinate once the bot flies return to aerobic conditions. In aquatic chironomid larvae (*Chironomus*), anaerobic metabolism by alcoholic fermentation maintains high levels of ATP for 14 h in anoxic conditions. The end product of alcoholic fermentation is ethanol, which easily diffuses into the aquatic environment. Other aquatic midges (*Chaoborus*) typically phase between aerobic and anaerobic conditions. With anoxia, malate is used as a substrate for anaerobic metabolism and the end product is succinate. During normoxia, succinate is recycled back to malate (Hoback & Stanley, 2001).

Concentrations of O_2 below the anaerobic compensation point appear to be in the insecticidal range. Recent reviews of the use of controlled atmospheres for the control of insect pests (Banks & Annis, 1990; Carpenter & Potter, 1994; Mitcham *et al*., 2001) have concluded that the O_2 level needs to be below 3% to be effective; and in most cases, it needs to be below 1% for rapid kill. These O_2 levels (below 3%) seem to coincide with P_a, the O_2 level at which anaerobic metabolism is initiated.

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 initiation of anaerobic metabolism may just be an indication of low energy supply. The ATP concentration of the whole tissues of *E. cautella* pupae decreased by 30% after exposure to 1% O_2 for 24 h at 26°C (Friedlander & Navarro, 1979). The content of ATP in the flight muscle of *L. migratoria* adults dropped to 1% of normal during 2 h of anoxia; the ADP content was also decreased to levels below normal while AMP accumulated 20-fold (Weyel & Wegener, 1996).

Other direct effects

Relative humidity (RH) is assumed to play no role in mortality at high humidity levels, while at lower levels RH plays an important role in affecting treatment efficacy (Jay *et al*., 1971). At low humidity, reduced O_2 is lethal causing rapid water loss through opened spiracles (Navarro, 1978; Jay & Cuff, 1981; Ofuya & Reichmuth, 2002). At high RH levels, mortality is attributed to the inability of the insect to maintain aerobic respiration leading to anaerobic respiration, which produces less energy and toxic end products accumulate, rather than to desiccation from water loss (Donahaye & Navarro, 2000; Ofuya & Reichmuth, 2002).

Research with *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) embryos demonstrated a direct effect of hypoxia on the cell cycle by inducing S phase arrest before DNA replication. Douglas *et al*. (2001) suggested that low O_2 directly affected the cell cycle rather than first affecting energy stores due to the rapidity of the response. Arresting before DNA replication acts as a safeguard against DNA replication errors. One possible affect of hypoxic atmospheres could be an inability to properly coordinate the arrest of this process (Douglas *et al*., 2001).

Other direct effects include increased haemolymph pH, as was observed during exposure to hypoxic atmospheres in the grasshopper, *S. americana*, and the inability to develop tolerance to high or low temperatures observed in adult flesh flies (Hoback & Stanley, 2001). A 30 min anoxic exposure reduced tolerance that typically develops to 45°C temperature exposure after several brief exposures at sublethal temperatures. Cold tolerance down to -10°C normally occurs when flies are exposed to 0°C for 2 h, but this effect was diminished after exposure to anoxia (Hoback & Stanley, 2001). The inability to develop such tolerance is significant for the development of combination treatments with cotrolled atmosphere and temperature (high or low).

Another theory on the mode of action of anoxia is that reactive oxygen species (e.g. hydroxyl radicals or superoxide anions) form after exposure to anoxic conditions, which induced oxidative stress (Hoback & Stanley, 2001) causing death during recovery. However, experiments with two gall insects, *Eurosta solidaginis* (Fitch) (Diptera: Tephritidae) and *Epiblema scudderiana* (Clemens) (Lepidoptera: Tortricidae), did not show oxidative stress, possibly because low metabolism protected the insects from damage (Hoback & Stanley, 2001).

Coping mechanisms

Hochachka (1986) suggested that increased cell membrane permeability is the mode of action of low O_2 atmospheres. If the initial membrane permeability is low, the failure of membrane function does not occur at all, or develops slowly. Therefore, Hochachka stressed that the real survival tool for organisms under hypoxia/anoxia is the coupling between metabolic arrest and low permeability of cell membranes. Ionic concentration gradients do not fall to their thermodynamic equilibrium in tissues of ectothermic anaerobes at lower ATP turnover rates under hypoxia; however, the ion concentration gradients are rapidly lost in hypoxia-sensitive tissues. Therefore, it has been proposed that the higher tolerance of ectothermic anaerobes to hypoxia, as compared with that of higher animals, is attributable to the lower membrane permeability of ectothermic anaerobes (Hochachka, 1986).

Hochachka (1991) defined two classes of response to hypoxia in insects. In the regulating class, energy flow is maintained as O_2 is depleted. The energy charge remains constant and an increased glycolytic flux provides for energy needs. The penalty is that this class of insect must use substrate at a higher rate to avoid energy deficiency (Storey & Storey, 1990). In the conforming class, energy consumption declines with the O_2 supply, as does respiration rate and substrate use. Conformers avoid activation of glycolysis and reduce energy demanding cell functions such as that of the ion channel pumps. Both classes adapt to O_2 deficit, but the conformers make an adaptation that enables them to survive long-term hypoxia.

Other research with *D. melanogaster* suggests similarities between mammals and insects in how they cope with hypoxic atmospheres. In mammals, nitric oxide stimulates increased blood flow to areas where hypoxia occurs. In insects, haemolymph does not carry O_2, but nitric oxide was still found to assist in the adaptation to hypoxic atmospheres. In *D. melanogaster* embryos, nitric oxide was involved in coordinating the arrest of the cell cycle. Research with fruit fly larvae found that nitric oxide plays a role in behaviour modulation in response to hypoxia. Also, nitric oxide was found to increase the number of terminal branches of the trachea, where, as in the mammalian system, it would increase the amount of O_2 distributed to an area exposed to hypoxia (Wingrove & O'Farrell, 1999). Similarly, an increased tracheal diameter would increase diffusion of O_2 into the tissues. An experiment found that *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae) larvae reared in 15% O_2 and 10.5% O_2 had increases in tracheal cross-section volume by 40% and 120%, respectively (Hoback & Stanley, 2001).

Response to elevated CO_2

Metabolic effects

Hypercarbia generally reduces the rate of respiration. Oxygen consumption by *E. cautella* pupae was significantly reduced by hypercarbia (Navarro, 1975). Zhou *et al*. (2000) found that elevated CO_2 reduced the O_2 consumption rate of *P. stultana* pupae, even with 21% O_2 present. The O_2 consumption rate decreased by 62% in 20% CO_2+21% O_2 and by 73% in 79% CO_2+21% O_2 at 20°C. The rate of respiration of *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) adults, as measured by CO_2 output, was severely depressed during the initial hours of exposure to elevated CO_2 concentrations (Ali Niazee, 1971). However, Edwards & Batten (1973) observed that the O_2 consumption rate of house flies did not decrease in 33% CO_2+21% O_2 compared with that in air. Carpenter *et al*. (2001) subjected *Ctenopseutis* sp., *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae), *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae), *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), *Sitophilus oryzae* (Linnaeus) (Coleoptera: Curculionidae), and *T. confusum* larvae to 60% CO_2 and 1–10% O_2 atmospheres. The authors reported that once exposed to these atmospheres, the metabolic rates, as measured by calorimetry, decreased in each insect with the exception of *S. oryzae* larvae.

Because elevated CO_2 reduces O_2 consumption, it appears that the net effect of elevated CO_2 on the insect respiratory metabolism is similar to that of reduced O_2. Both reduce oxidative phosphorylation even though the target sites of the two types of atmospheres may be different;

reduced O_2 limits a substrate of respiratory metabolism, whereas elevated CO_2 inhibits respiratory enzymes such as succinate dehydrogenase (Edwards, 1968) and malic enzyme (Fleurat-Lessard, 1990). Reduced oxidative phosphorylation leads to reduced ATP generation. Friedlander & Navarro (1979) found that high CO_2 caused a decrease in ATP levels and energy charge in insect tissues.

Carbon dioxide poisoning inhibits O_2 utilization by specific enzymes, such as succinic dehydrogenase, or causes a weak oxidative metabolism resulting in accumulation of toxic products (Bell, 1984) such as lactate, pyruvate, and succinic acid. Upon transfer to air after treatment, the high level of toxic wastes may increase the mortality rate. Alternatively, mortality upon transfer to air may be due to O_2 stress. Oxidases and peroxidases may play a role in detoxification of free radicals derived from CO_2 accumulation or blocked aerobic pathways (Fleurat-Lessard, 1990).

Although the net effect of elevated CO_2 may be similar to that of reduced O_2, several papers note a difference in insect mortality when they were treated with hypoxic versus hypercarbic atmospheres. Complete mortality occurred sooner in *Callosobruchus subinnotatus* Pic (Coleoptera: Bruchidae) pupae and pharate adults treated with hypercarbic than with hypoxic atmospheres (Mbata *et al*., 2000). Even in plants, elevated CO_2 caused greater damage, such as delayed flowering and mortality, when compared to plants treated with 100% N_2 (Held *et al*., 2001). However, opposite results were found with whiteflies. Low O_2 atmospheres, less than 2%, were found to be more effective than 25 or 50% CO_2 in controlling greenhouse whitefly *Trialeurodes vaporarium* (Westwood) and silverleaf whitefly *Bemisia argentifolii* Bellows & Perring (both Hemiptera: Aleyrodidae). Mortality with low O_2 was greater and more rapid (Han & Konieczny, 2000).

The developmental pattern and mortality response of *P. stultana* pupae under various elevated CO_2 and low O_2 atmospheres indicated that the pupae had a greater energy shortage under elevated CO_2 than under reduced O_2, despite a similar decrease in metabolic rate (Zhou *et al*., 2001). This greater energy shortage could result from lower energy supply and/or higher energy demand. The lower energy supply could be caused by an inefficient production of ATP under elevated CO_2. Carbon dioxide has been considered an uncoupler of phosphorylation, similar to 2, 4-dinitrophenol (Fanestil *et al*., 1963). Therefore, even with similar metabolic heat dissipation, pupae under elevated CO_2 may generate less ATP than under reduced O_2. Friedlander & Navarro (1979) found that the ATP content and energy charge in the tissues of *E. cautella* pupae decreased more with 80% CO_2 than with 1% O_2 after 1 day of treatment. The higher energy demand could result from an increase in membrane permeability. Zhou *et al*. (2000) observed that high CO_2 concentrations caused body fluid of *P. stultana* pupae to leak out, suggesting that the membrane systems of the pupae were affected by high CO_2.

Metabolic arrest was found to be a major response of *P. stultana* pupae to elevated CO_2 concentrations with 21% O_2 present (Zhou *et al*., 2000). The metabolism of the pupae decreased at elevated CO_2 concentrations, and the percent decrease of metabolism, as indicated by the percent decrease of metabolic heat rate, was comparable to the percent decrease in O_2 consumption rate at various CO_2 levels. It has also been shown that insects initiate anaerobic metabolism under elevated CO_2 concentrations. Kerr *et al*. (1993) suggested that, in crickets, high CO_2 atmospheres induced anaerobiosis even with 20% O_2 present. Navarro & Friedlander (1975) observed that lactate rose in *E. cautella* pupae exposed to 80% CO_2+20% O_2.

Metabolic arrest, when coupled with higher membrane permeability, has been thought to be the cause of hypoxic/anoxic toxicity (Hochachka, 1986). This decoupling between metabolic arrest and membrane function could also be the major cause of hypercarbia toxicity. However, Zhou *et al*. (2001) suggested that such decoupling occurred more quickly under hypercarbia than under hypoxia, contributing to the higher susceptibility of *P. stultana* pupae to hypercarbia. The failure of membrane function under hypoxia is mainly caused by an insufficient energy supply to maintain membrane gradients (Hochachka, 1986). However, Zhou *et al*. (2001) suggested that elevated CO_2 could increase the permeability of membranes. Therefore, the failure of membrane function under hypercarbia could result from both energy insufficiency and increased membrane permeability. It is more likely that the decreased energy supply under metabolic arrest cannot meet the need of maintaining a more permeable membrane due to elevated CO_2.

Carbon dioxide has also been shown to increase intercellular Ca^{2+} concentration by decreasing pH (Lea & Ashley, 1978). According to Hochachka (1986), a high concentration of Ca^{2+} in the cytosol can cause the cell and mitochondrial membranes to become more permeable, again suggesting that high CO_2 can increase membrane permeability.

Under anoxia, pyruvate and lactate levels increase in the same proportion such that the ratio between the two is constant (Price & Walter, 1987). Under hypercarbia, the ratio of pyruvate to lactate is reduced to 25% of normal, indicating a change in the redox potential and a lesion in the electron transport chain, presumably caused by a modification in the permeability of mitochondrial membranes (Friedlander, 1983). In studies with *T. castaneum*, redox potential was not greatly affected by controlled atmosphere during the initial part of treatment, but after 4 days of exposure, the NAD+/NADH ratio in the mitochondria decreased in relation to inhibition of mitochondrial electron transport (Donahaye, 1985).

The metabolism of *P. stultana* pupae decreased rapidly by 60% as the CO_2 concentration was elevated to 20% at 20°C (Zhou *et al*., 2000). Further decrease in metabolism was slight when the CO_2 concentration was elevated from 20 to 79% (all with 21% O_2 present). Since respiratory enzymes are inhibited by CO_2 (Edwards, 1968), this quantitative response seemed to indicate that the capacity of respiratory enzymes was increasingly inhibited by increasing concentrations of CO_2, but after a point more CO_2 did not further inhibit the capacity. Although the percent decrease of metabolism showed no difference from 20 to 79% CO_2 at 10°C, the efficacy of CO_2 for mortality of *P. stultana* pupae increased greatly in this concentration range (Zhou *et al*., 2000). This further suggests that mechanisms other than the decrease of metabolism, such as an increase in membrane permeability, are contributing to the toxicity of CO_2.

The greater response to high CO_2 than low O_2 may also be due to the greater permeability constant for CO_2, which is 36-fold higher than for O_2, and the fact that respiratory regulation mechanisms are largely dependent on brain receptors which are sensitive to CO_2 but not to O_2. Carbon dioxide detoxification or exclusion from the receptors seems to occur in tolerant species or life stages (Kashi, 1981;

Desmarchelier, 1984). These mechanisms may be important in the tolerance of arthropods to CO_2 (Donahaye, 1985).

Other direct effects

Empirical mortality data have shown that levels of CO_2 toxic to insects are generally above 20% (Banks & Annis, 1990; Carpenter & Potter, 1994; Mitcham *et al.*, 1997; Zhou *et al.*, 2001). Carbon dioxide can initially have a narcotic effect leading to knockdown (Edwards & Batten, 1973). Most insects are more easily killed with higher CO_2 concentrations up to 100% (Desmarchelier, 1984; Jay, 1984). In elevated CO_2 atmospheres, as with reduced O_2 atmospheres, RH is assumed to play no role at high humidity levels, while at lower levels RH plays an important role in affecting treatment efficacy (Jay *et al.*, 1971). CO_2 is lethal by causing rapid water loss through opened spiracles when humidity levels are low (Navarro, 1978; Jay & Cuff, 1981; Ofuya & Reichmuth, 2002). However, mortality from high CO_2 at high humidity has been observed to be independent of water loss (Jay & Cuff, 1981). In contrast, Donahaye & Navarro (2000) suggested that the mode of action of elevated CO_2 was desiccation even at high RH levels. Using a laboratory strain of *T. castaneum* resistant to hypercarbic conditions, they determined that the greater quantities of triacylglycerols enabled survival in elevated CO_2 atmospheres (Donahaye & Navarro, 2000). Triacylglycerols are an important source of energy and they are also important for water regulation. Depletion of triacylglycerol levels could influence water regulation and lead to desiccation, even if RH levels are high (Wang & Zhao, 2003).

Research combining the effects of fumigants and CO_2 show a similar pattern of increased mortality at the mid-ranges of CO_2. At low CO_2 concentrations, mortality occurs due to spiracular opening and increased fumigant uptake. However, at higher CO_2 concentrations, metabolic effects become a more important determinant of mortality (Janmaat *et al.*, 2001).

Coping mechanisms

In contrast to low O_2 atmospheres, *T. castaneum* adults exposed to elevated CO_2 atmospheres died when triglyceride reserves were depleted (Ofuya & Reichmuth, 2002). Hydrolysed triglycerides form fatty acids that upon oxidation are an energy source for insects. A strain of *T. castaneum* resistant to hypercarbic atmospheres was found to have greater levels of triglycerides. Interestingly, under hypoxia, triglyceride levels stay relatively stable, suggesting that their depletion is not critical to the mode of action of low O_2 atmospheres (Donahaye & Navarro, 2000).

Despite these differences, researchers believe 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 & Wegener, 1996).

Interactions between controlled atmospheres and temperature

The temperature during exposure to controlled atmospheres has a great effect on arthropod mortality. In general, susceptibility to controlled atmospheres is greater at higher temperatures due to enhanced respiratory demand; however, when the temperature is outside the optimum range for the arthropod, low or high temperature can be an added stress in addition to controlled atmospheres treatment (Mbata & Phillips, 2001). Hoback & Stanley (2001) suggest that there may be a link between cold-hardening in preparation for over-wintering and physiological changes that enable insect survival in anoxic conditions, although they were not able to find direct evidence to support this theory.

When *Pseudococcus affinis* (Maskell) (Hemiptera: Pseudococcidae) were exposed to various O_2 concentrations (0.4 to 20.9%) at high temperatures (35 to 45°C), the time required for 99% mortality decreased with increasing temperature and decreasing O_2 concentration at 35 and 40°C. At 45°C, the effect of reducing O_2 concentration was diminished (Whiting & Hoy, 1997). Metabolic heat changes in *E. kuehniella* were observed before and after a 1 h treatment with 1 and 5% O_2 (Carpenter *et al.*, 2001). With 5% O_2, metabolic heat was reduced to 85%, 79% and 82% of the initial metabolic heat at 0, 20 and 40°C, respectively. Metabolism was reduced further with 1% O_2, with metabolic heat rates after treatment at 66%, 79% and 38% of the initial metabolic rate at 0, 20 and 40°C, respectively. However, although metabolism was in some cases greatly reduced, no mortality was observed (Carpenter *et al.*, 2001).

Mortality of New Zealand flower thrips *Thrips obscuratus* (Crawford) (Thysanoptera: Thripidae) was greatest at high temperatures and elevated CO_2 levels (Potter *et al.*, 1994). For long-tailed mealybug *Pseudococcus longispinus* (Targioni-Tozzetti) (Hemiptera: Pseudococcidae), mortality was greatest at 0°C and 18% CO_2, and there was a sharp increase in mealybug survival between 0 and 7°C (Carpenter, 1997). Based on the results of large factorial experiments, Carpenter (1997) concluded that increases in time and temperature during controlled atmosphere exposure had a greater effect on mortality of thrips and aphids than increases in CO_2 or reductions in O_2.

The normal metabolic rate of *P. 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 arthropod metabolism. *P. stultana* pupae were more susceptible to elevated CO_2 at higher temperatures, and this susceptibility seemed to correlate with higher metabolic rates (Zhou *et al.*, 2000). However, the metabolic response to elevated CO_2, as indicated by the percent decrease of metabolism, was only slightly different at 10, 20 and 30°C. It appeared that it is not the relative percent decrease of metabolism, but the absolute decrease of metabolism that was related to susceptibility. Because the absolute decrease of metabolism was 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.

Recent reviews have concluded that there was no enhancement of arthropod mortality above 40–60% CO_2 (Banks & Annis, 1990; Carpenter & Potter, 1994). The data of Zhou *et al.* (2000) show that this conclusion is mostly applicable to temperatures such as 20 and 30°C. At 10°C, increasing the CO_2 concentration from 40 to 79% increased mortality of *P. stultana* pupae. Increased efficacy of CO_2 at concentrations above 40–60% on Pacific spider mites, *Tetranychus pacificus* McGregor (Acari: Tetranychidae), was also observed at 0°C as compared with 5°C (Zhou & Mitcham, 1998). The greater efficacy at higher concentrations of CO_2 and low temperatures could be related to the higher solubility of CO_2 in tissues at low temperatures (Yacoe, 1986).

Temperature had a significant effect on the metabolic response of *P. stultana* to reduced O_2 concentrations. The percent decrease in metabolism by a given low O_2 concentration was higher at higher temperatures when compared to elevated CO_2 concentrations (Zhou *et al*., 2000). Carpenter *et al*. (2001) observed a similar trend when adult and larval confused flour beetles, *T. confusum*, and adult rice weevils, *S. oryzae*, were treated with 60% CO_2 and 5% O_2 over a temperature range of 15–45°C.

Interactions between reduced O_2 and elevated CO_2

Empirical studies on the additive effects of combinations of elevated CO_2 and reduced O_2 on arthropod mortality have yielded mixed results. When CO_2 is added to low O_2 environments, there can be a synergistic effect (Calderon & Navarro, 1980) or an antagonistic effect (Ali-Niazee, 1971; Mitcham *et al*., 1997). The synergistic effect may be similar to the enhancement of effectiveness of fumigants by CO_2 (Bond & Buckland, 1979; Calderon & Leesch, 1983). In some cases, when only a small amount of CO_2 is present in an O_2 deficient atmosphere, it can enhance mortality by up to 10-fold. This increase in mortality is temperature dependent (Calderon & Navarro, 1979). Some arthropods are more tolerant of 100% CO_2 than an atmosphere containing a small amount of O_2 with CO_2 (Lindgren & Vincent, 1970). Mitcham *et al*. (1997) found that, at 0°C, mortality of *P. stultana* was greater with 45% $CO_2 + 11.5\%$ O_2 as compared with 45% $CO_2 + 0.5\%$ O_2.

Some researchers have observed additive effects of elevated CO_2 and reduced O_2 atmospheres (Calderon & Navarro, 1979; Krishnamurthy *et al*., 1986), while others have not (Soderstrom *et al*., 1991; Mitcham *et al*., 1997). 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 *P. stultana* pupae were almost fully realized at combinations with $\leq 5\%$ CO_2 and $\geq 4\%$ O_2 (Zhou *et al*., 2000). However, the combined effects became increasingly overlapped as O_2 concentration decreased and CO_2 concentration increased. The percent decreases in metabolism were comparable between 2% O_2 and 20 or 40% CO_2 (with 21% O_2 present) and between 1% O_2 and 79% $CO_2 + 21\%$ O_2 (Zhou *et al*., 2000). Each species and life stage may have different critical concentrations for CO_2 and O_2 working in synergism or antagonism (Person & Sorenson, 1973; Tunc, 1983).

Adaptation and tolerance to insecticidal controlled atmospheres

Chervin *et al*. (1996) suggested that plants could be adapted to extreme controlled atmospheres by exposure to moderate O_2 or CO_2 concentrations, thereby increasing their tolerance of these stress atmospheres. Plant cells clearly sense and respond to O_2 levels that are well in excess of those that limit the terminal oxidase.

For disinfestation, it seems better to use immediate stress conditions as arthropods are also able to adapt to controlled atmosphere conditions and therefore become more resistant to disinfestation treatments (Chervin *et al*., 1996). Tolerance to controlled atmospheres has been induced in granary weevils and selected for in laboratory colonies of stored product beetles (Bond & Buckland, 1979; Navarro *et al*., 1985; Donahaye, 1990a,b). Donahaye (1985) observed in laboratory selected strains of *T. castaneum* tolerant to either hypercarbia or anoxia, a physiological adaptation via two different mechanisms; a general lowering of metabolic intensity and prolonged development periods. Using these selected strains, Donahaye (1992) suggested the mechanisms of resistance were the reduced energy used and ability to maintain water balance to survive in elevated CO_2 atmospheres and the ability to maintain aerobic metabolism in reduced O_2 atmospheres. Strains unselected for resistance died because of water loss (desiccation even at high RH, in this case 95%) and a rapid mobilization of reserves, leading to a rapid loss of energy as reserves are used up and the insects resort to anaerobic metabolism (Donahaye, 1992). Using these selected strains, Donahaye & Navarro (2000) determined energy reserve differences in resistance between elevated CO_2-treated and reduced O_2-treated *T. castaneum* adults. Using a strain resistant to elevated CO_2, reduced O_2, and an unselected strain, the authors determined resistance to elevated CO_2 may be due to greater amounts of triacylglycerol and decreased metabolism. Resistance to reduced O_2 was less clear; potentially resistance was due to the ability to maintain aerobic metabolism, since survival was not dependent on triacylglycerol levels (Donahaye & Navarro, 2000). In the grasshopper, *S. americana*, increases in tracheolar conductance was suggested to be the mechanism for tolerance to hypoxia. An increase in tracheolar conductance occurred during a progressive decrease in atmospheric O_2 (21 to 5%). At concentrations of less than 5% O_2, tracheolar conductance cannot be increased sufficiently to compensate for the lack of O_2 and the effects of O_2 deprivation are observed (Greenlee & Harrison, 1998).

Research needs

Additional research into the mode of action of controlled atmospheres on arthropods is needed to reduce the need for empirical testing in the development of arthropod control treatments. The effect of elevated CO_2 on arthropod membrane systems should be studied. A role or lack thereof for spiracular opening in arthropod mortality under controlled atmospheres and the high humidity conditions present during treatment of fresh commodities must be confirmed, including the role of water loss due to spiracle opening during treatment. The response of representative and economically important pests to various O_2 and CO_2 concentrations, and combinations of O_2 and CO_2 must be determined. Also, comparative studies of the response to temperature with controlled atmosphere for low temperature sensitive and high temperature sensitive species are needed. The potential for arthropod pests to develop resistance to controlled atmospheres must be explored further so that mechanisms can be developed as safeguards against such resistance development.

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(Accepted 27 December 2005)