SOME RESPONSES OF ARTHROPODS TO GAS EXPOSURES

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ABSTRACT

For centuries various ways of limiting or modifying the atmosphere in an enclosure have been employed as a means of controlling insect infestation. This has evolved from hermetic storage of crops in underground pits and practices such as the burning of sulphur candles in mills to modern applications of fumigant gases and operation of sophisticated controlled atmosphere systems. Insects and mites still cause problems, however, and this paper examines some of the defences they operate through the range of responses available to them. These may be divided into behavioural and metabolic responses. Behavioural responses include avoidance by responding to concentration gradients, retreating into refuges and/or a shutdown of general activity. Metabolic responses include aspects of increased activity such as the active exclusion of gas or enhanced detoxification pathways, and aspects of reduced activity such as an induced delay in development prolonging a tolerant stage, or a switch to alternative biochemical pathways such as anaerobiosis. The link of these aspects with increased tolerance or resistance to control measures is discussed.

Key words: Stored-product insects, mites, phosphine, controlled atmospheres, stored grain, flour mills, infestation control, resistance.

INTRODUCTION

All living organisms have survived by adopting strategies for life in their ecosystem. For mankind the primary strategy has been one of seeking means of controlling the environment of the ecosystem; for insects and mites the primary strategy has been one of opportunist adaptation. To this end the evolutionary trend has been towards small size, a rapid breeding cycle and close links between environmental cues and behavioural responses. Economy in size leads to economy in the number of cells available to comprise vital organ systems and sensors. The insect nervous system is a wonder of creation in its simplicity and efficiency, enabling the most subtle of environmental stimuli received by a sensor system to elicit precise metabolic or behavioural responses that are of advantage to the individual.

Control intervention by any procedure attempts to render the environment unsuitable for survival of insects and mites that then respond by activating various defence mechanisms. Purging the atmosphere of an enclosure with gases offers a major challenge to the pests present as avoiding exposure by simply moving off a treated surface or rejecting a bait is not an option. However, as reflected by the need of ongoing research into the use of gases and indeed the very *raison d'etre* of these conferences, many individuals have, and still do,

survive these procedures. In this paper the behavioural and metabolic responses employed by insects that can increase the potential for survival in fumigant or controlled atmosphere are explored. Behavioural responses include movement in response to concentration or temperature gradients, seeking static locations such as crevices or food residue layers, shut-down of activity, activity responses linked to diurnal rhythms and aggregation responses. Metabolic responses include active exclusion of toxicant, switching to anaerobiosis, increased capacity of detoxification or elimination of toxicant, desensitisation of active sites and developmental aspects such as the prolonging of a tolerant stage or entry into diapause.

BEHAVIOURAL RESPONSES

Response to gradients
In each of the different storage or food processing environments that are encountered in practice, local microclimates exist and give rise to gradients, gradients of temperature, moisture or humidity, light intensity and even gradients of atmospheric gases where respiration of stored-products or pest populations is evident. When a building or silo is sealed, or a bag stack or cereal bulk sheeted, prior to a fumigation or controlled atmosphere treatment, some of these gradients may be buffered or modified but they will still be present.

The optimum requirements of insects and mites for active development are well known and much information has been gathered on their capacity to locate environments supplying the right conditions for breeding. In grain bulks insects have been shown to respond to gradients of temperature (Surtees, 1964; Jian et al., 2003), moisture content (Yinon and Shulov, 1969; Parde et al., 2004), oxygen (Navarro et al., 1981; Adler, 1992), carbon dioxide (Navarro et al., 1981; Parde et al., 2004) and light (Smereka and Hodson, 1959). In chamber tests they have also been shown to respond to moving gas fronts of the fumigants methyl bromide and phosphine (Bell, 1987). Table 1 lists some of the species for which some strains have been shown to respond to different gradients.

One of the problems associated with the assessment of insect responses to a gas concentration gradient is how to differentiate between what is simply an excitory response, whereby activity is increased causing non-directional random movement, and what is a true movement away or towards the stimulus. Where activity is increased, insects moving towards the gas front may become immobilised and create the impression of attractancy as numbers build up. Movements descending a concentration gradient are thus more reliably identified than movements in the opposite direction. Hence caution is required in identifying attractancy and that which has been ascribed to gases such as phosphine and high carbon dioxide concentrations (>30% in air) known to have a rapid knock-down effect may simply have been the result of an initial general stimulus of activity.

The response to a particular gradient in different insects may differ widely. For example in grain, *Trogoderma granarium* (Everts) will descend a moisture gradient while *Sitophilus granarius* (L.) will move towards zones of higher moisture (Yinon and Shulov, 1969; Smereka and Hodson, 1959), and whereas most insects are repelled by high carbon dioxide (CO_2) concentrations some beetles are attracted at least by concentrations up to 10-15% (Willis and Roth, 1954; Parde et al., 2004), *Tribolium confusum* Du Val is also attracted by much higher concentrations and *T. castaneum* (Herbst) even shows an increased level of productivity at a concentration of 10% CO_2 in air containing 5-10% oxygen (Spratt, 1984).

Many insects show a preference for moving up or down in grain bulks and the response to gradients might be overridden by the response to gravity. The positively geotropic movement of *Cryptolestes ferrugineus* (Stephens) in wheat required the combined effect of

high moisture and low CO_2 concentration gradients to be completely overcome (Parde et al., 2004). In contrast other factors may enhance the effect of movement in response to a gradient. The release of semiochemicals is an obvious example. When several insects responding to a stimulus arrive in one locality, sex or aggregation pheromones may be released that cause an increased level of aggregation, very possibly drawing in individuals that were not responding to the original gradient. A temperature gradient may then be set up by the local activity which will act as a further attractant or arrestant for other pests.

Table 1. Some storage insects that have been found to respond directionally to particular physical and chemical (other than pheromone) gradients

Species	Gradient or feature	Response
Cryptolestes ferrugineus	Low temperature gradient	Towards warmth (20°C+)
	High moisture/humidity	Towards high moisture
	Carbon dioxide	Attracted by up to 9% in air
	Gravity	Moved down in grain
	Light	Moves away
Oryzaephilus surinamensis	Methyl bromide	Attracted by a low (2 g m^{-3}) concentration at 25°C
	High carbon dioxide	Repelled
	Low oxygen	Repelled
Sitophilus granarius	Temperature	Towards warmth (20°C+)
	Humidity	Towards higher humidity
	Carbon dioxide gradients	Repelled by 19 or 95% CO_2
	Low oxygen	Attracted towards low O_2
	Methyl bromide	Repelled by a low (2 g m^{-3}) concentration (at 25°C)
	Phosphine	Repelled by high (>0.6 g m^{-3}) concentrations
Tribolium castaneum	Carbon dioxide gradients	Attracted by concentrations up to 15%, repelled by concentrations over 50%
Tribolium confusum	Carbon dioxide	Attracted by concentrations up to 90% in air
Trogoderma granarium	Humidity	Attracted to less than 40% r.h. Repelled by r.h. over 60%

From a practical viewpoint the question is whether the ability of pests within a treatment enclosure to move away from toxic gas fronts or low oxygen can enhance survival. If the seal on the enclosure is complete, the only benefit to the individual would be that the

time survived would be extended by the time taken for an even concentration of gas to be achieved throughout the enclosure. While this situation may apply to chamber or sealed bag stack treatments, it certainly will not apply to fumigation of a cereal bulk or food processing facility where it is exceedingly difficult to achieve an absolute seal. Here the potential will always be for insects to aggregate at leakage points where a local ingress of the external atmosphere will reduce the chances of a lethal atmosphere being maintained. The prospects for a successful fumigation treatment are reliant on calm weather, windy conditions necessitating addition of further gas and a prolongation of the fumigation period if adequate control is to be achieved. However, leakage points may also feature gradients of reduced temperature or increased moisture which may or may not counteract the effect a concentration gradient may have on the movement of insects. The survival of individual insects or mites in such situations will depend on the ability to respond to the life-threatening gradient amid these other stimuli.

Refuge seeking behaviour
Although the retreat of insects into refuges offers an obvious advantage for survival when surfaces are sprayed with insecticide, the advantage to insects within a fumigation enclosure may seem to be minimal unless the harbourage is at a site of leakage or, more importantly, ingress of the external atmosphere. Nevertheless some survival value must exist because bounce back of pest populations in flour mills and other food processing facilities is strongly linked with pockets of survival in the fabric of the building often well away from obvious leak sources. Very seldom is the residual population completely eradicated.

Refuge seeking behaviour is evident in many stored-product insects. Most prefer dark conditions for activity and seek a refuge in light, particularly bright light. In *C. ferrugineus* the response is enhanced by lowered temperature and the presence of food in the refuge (Cox et al., 1989; Cox and Parish, 1991). In *Oryzaephilus surinamensis* (L.) there is a diurnal rhythm of movement in and out of refuges that is entrained by the daily light cycle and dampened by the presence of food in the refuge (Bell and Kerslake, 1986). Occupancy of the refuge is naturally accompanied by a reduction or cessation of activity, particularly if food is absent, resulting in reduced respiration and hence reduced susceptibility to low oxygen or fumigant gases.

One aspect that is difficult to assess is the effect of microclimate on the rate of gas diffusion into a crack or crevice. The presence of insects and food residues in such harbourages create microclimates that are radically different to the external airspace and gradients may be set up that hamper entrance of gas into the recess. Pressures of as little as 10 mm water gauge have been demonstrated to greatly influence the movement of fumigant gases along an 8 mm diameter tube (Bell, 1987) and such positive pressures can be created by very small increases of temperature. Developing or feeding insects can produce marked temperature rises in commodities resulting in the creation of 'hot spots' in grain. The Mediterranean flour moth, *Ephestia kuehniella* Zeller, developing in 325 g cultures can raise the temperature from $25^{o}C$ to $32^{o}C$ at the peak of larval growth (Bell, 1976). A group of insects feeding on residues in a crevice may thus be protected from exposure to the treatment atmosphere for a considerable period.

With many fumigation applications in cool climates the addition of heat is necessary to increase the chance of a successful treatment. While this enhances gas diffusion and toxicity, for insects hiding and feeding in food residues a short term protective effect may be afforded by evaporative cooling, moisture being produced by the metabolism of carbohydrates as insects feed. In this situation the evaporation of water vapour from the food surface may

counteract the diffusion of gas and also delay the rate of heating in the vicinity of the pests (Bartlett et al., 2005).

Diurnal rhythms
Besides the diurnal rhythm associated with foraging behaviour mentioned above, there are many other instances of the response of insects to the daily light – dark cycle. Activities such as mating, oviposition, hatching of eggs, larval developmental rate, pupation and adult eclosion from the pupal case have all been found to run on entrained cycles in stored-product moths (Table 2), the most prominent trigger or zeitgeber being the onset of darkness. Photoperiodicity may also be an agent for the induction of resting stages in the life cycle. The receipt of a number of lengthening scotophases at the time of the last inter-instar larval moult gives rise to an overwintering diapause after completion of feeding in the warehouse moth, *Ephestia elutella* (Hübner) (Bell, 1977). Though this delayed response does not confer any advantage to the developing larvae at the time of receiving the photoperiodic signal, subsequently the susceptibility to a wide range of control measures is much reduced on progression to the inactive diapausing stage. Furthermore the challenge of a toxic gas on diapausing stages can result in an increased synchronisation of diapause termination after treatment, resulting in a flush of emergence shortly afterwards, thus improving prospects for the rapid establishment of fresh infestation.

Flight activity in pyralid moths is triggered at dusk with another response at dawn and here light intensity and ambient temperature are important additional stimuli. Mating and oviposition are closely linked with flight activity and generally follow a similar pattern, as in *Plodia interpunctella* (Hübner) (Lum and Flaherty, 1970; Lovitt and Soderstrom, 1973), *Corcyra cephalonica* (Stainton), *Ephestia elutella* (Bell, 1981) and *Ephestia cautella* (Walker) (Steele, 1970; Hagstrum and Tomblin, 1973).

Most of these entrained essential biological responses are associated with an increased level of activity and hence increased potential vulnerability to control measures based on respiratory action. As with other activity linked responses such as retreating to refuges or zones of reduced exposure to toxicant, any ability to modify behaviour patterns in response to the detection of a toxic atmosphere will enhance the prospect of survival. Such responses may be termed behavioural resistance. From the viewpoint of control, knowledge of the factors influencing activity can help to decide the time of starting a fumigation, particular when using fast acting fumigants such as methyl bromide or sulfuryl fluoride, to ensure that pests are most likely to be active soon after the application of gas.

METABOLIC RESPONSES

Developmental aspects
It is well known that large differences occur between the susceptibility levels of different stages of arthropods to fumigants and controlled atmospheres. The egg stage is more tolerant of a wide range of fumigants and modified atmospheres than other stages in mites, while in stored-product insects the most tolerant stage varies with species and fumigant. Treatment dosages and exposures are designed to kill all stages and often rely on tolerant stages to carry on development under gas so that the period of highest tolerance can be bridged. This has long been the strategy for the control of *Sitophilus* spp. with phosphine where tolerance peaks around the time of pupation within the grain and then declines (Howe, 1973). Further tests on this species revealed that a concentration of 280 ppm needed to be maintained for 16 days to achieve complete control in laboratory tests at $15^{o}C$ (Hole et al., 1976).

Clearly the ability to survive a fumigant exposure in one insect stage can potentially be acquired in another, this being an obvious route to the development of resistance. Indeed an insight into the potential for resistance can be gained from looking at the natural tolerance spectrum which in the case of phosphine is very wide. Phosphine has been in widespread use since the 1960s and today resistant strains of many species are known. In Australia a strongly resistant strain of *C. ferrugineus* requires a 30-day exposure at 360 ppm for control at 20°C, displaying a resistance factor of 875 compared to non-resistant strains (Nayak et al., 2010).

Exposure to a toxic gas may slow the rate of development in an insect and an effective survival mechanism would be for an individual to remain at a stage of relative tolerance to the hostile atmosphere. Such a response could be described as 'developmental' resistance and has been observed in stored-product mite species exposed to high CO_2 levels (60-99% in air) or

Table 2. Responses of some storage insects to daily cycles of photoperiod or temperature

Species	Stimulus	Response
Corcyra cephalonica	The daily onset of darkness	Oviposition
Ephestia cautella	The daily onset of darkness	Adult emergence Flight, mating Oviposition
	Night-time falling temperature	Oviposition
	Lengthening scotophases	Diapause in mature larvae
	Lengthening photophases	Termination of diapause
Ephestia elutella	The daily onset of darkness	Flight Oviposition
	Lengthening scotophases	Diapause in mature larva
Ephestia kuehniella	The daily onset of darkness	Adult emergence Oviposition
Oryzaephilus surinamensis	The daily onset of darkness	An increase in foraging behaviour
Plodia interpunctella	The daily onset of darkness	Flight, mating Oviposition Pupation
	Daily temperature peak	Oviposition
	Lengthening scotophases	Diapause in mature larva
	Lengthening photophases	Termination of diapause

low oxygen atmospheres (0.5-2%) at 15°C where delays of hatch in excess of 20 days were recorded after exposure in *Tyrophagus longior* (Gervais), *Acarus siro* L., *A. farris*

(Oudemans) and *Lepidoglyphus destructor* (Schrank) (Conyers and Bell, 2003). Similar delays in the hatch of the first two species have also been observed following exposure to phosphine (Bowley and Bell, 1981).

Resistance mechanisms

Apart from the reduced susceptibility arising from a shutdown of activity or development, there are many other mechanisms available to organisms to help survival. The uptake of the toxicant can be reduced or even actively excluded, the sensitivity of active sites can be reduced, the rate of excretion of toxicant can be increased and the toxicant itself can be metabolised to non-toxic derivatives. Insects and mites show a capacity to tolerate far lower oxygen levels than mammals, being able to breed down to oxygen levels of 4% in air (Conyers and Bell, 2007), their small size enhancing oxygen uptake. There may also be a capacity to survive anaerobically for long periods, particularly during periods of the egg stage, and this can enable extended periods under low oxygen levels to be tolerated. This ability may be the reason why eggs of many species are so tolerant of the fumigant phosphine when compared to other stages, the presence of oxygen being required for phosphine to act.

Resistance to phosphine by insects has become a world-wide problem. In some species an active exclusion mechanism seems to operate in resistant strains. Price (1984) observed that in *Rhyzopertha dominica* (F.) the rate of uptake of phosphine by a phosphine resistant strain was very low, and greatly increased when insects died. In *T. castaneum* concentrations between 0.5 and 1 g/m^3 have been found to induce a kind of narcosis whereby some insects become inactive and reduce their uptake of gas, actually surviving longer than at higher or lower concentration levels (Winks, 1984). When removed from the exposure chamber while in this narcotised state the insects recovered, though prolonged exposure resulted in death (Winks, 1985). A narcotic response has since been observed in several other stored-product insects, the concentration threshold stimulating the response varying widely according to the species (Zhang, 1999; Cao and Wang, 2001). The phenomenon was first observed in scale insects exposed to HCN when it was termed protective stupefaction (Pratt et al., 1931).

The genetics of phosphine resistance has long been a matter of study to shed further light on how the gas interacts with the oxidative metabolic cycle to produce lethality. In several pests resistance has been shown to be the result of two incompletely recessive genes or gene complexes that when expressed fully produce the very high resistance levels seen in *R. dominica*, *Sitophilus oryzae* (L.) and *C. ferrugineus* (Schlipalius et al., 2002; Collins et al., 2005; Thorne et al., 2010; Wang et al., 2010; Nayak et al., 2010). The resistance includes mechanisms for reducing the uptake of phosphine as well as reduced sensitivity of the active sites and an increased capacity for metabolism. Nevertheless it is still possible to control all resistant populations by increases of concentration and exposure time though of course there are limits on the range of circumstances this can be achieved in practice and the possibility remains for still further increases in the level of resistance. Compounds do exist, however, that are specifically active against resistant strains. One such compound is methyl phosphine (Chaudhry et al., 1997).

Phosphine is not the only fumigant or gas that has been implicated with the development of resistance. Reports of resistance in the field towards hydrogen cyanide date back to early last century and laboratory selection studies have produced strains resistant at least to some extent to fumigants such as methyl bromide, and even carbon dioxide (Navarro et al., 1985), though whether resistance is the right term for the increases in tolerance produced is a matter of debate. With such more generally-acting compounds, the levels of resistance or increased tolerance do not approach those obtained with phosphine and so far no

cases of control failure attending their use in practice have been reliably attributed to the resistance of pests.

CONCLUSION

Resistance is a potential problem for any control process and can arise in many different ways. Plants have produced many defences against attack by insects and insects have responded by specialising to be able to utilise the resources provided by specific plants. Hence many bruchid beetle species have specialised to be able to develop on host plants that are poisonous to other animals. In the stored product field the most successful species are generalist feeders with life cycles that can be prolonged to bridge periods where food supplies are not readily available, as for example between harvests, and which can develop rapidly when conditions are favourable. Hence most stored-product beetles have long-lived adult stages and a diapausing larval stage is common among the major moth pests. As this paper has briefly shown, there are many other ways in which insects and mites can respond to changing circumstances and as man intervenes to protect food stocks the measures taken produce changes in the behaviour and biochemistry of pests in response to the challenge.

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