A REVIEW OF RECENT STUDIES OF THE EFFECTS OF CONTROLLED ATMOSPHERES ON STORED PRODUCT PESTS

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ABSTRACT

Controlled atmosphere storage of grain involves the alteration of the proportion of the normal atmospheric gases, nitrogen, CO_2 and oxygen, to give an atmosphere in the storage lethal to stored grain pests. Progress in the laboratory study of the action of controlled atmospheres on such pests is reviewed with particular emphasis on recent developments. Lethal and sublethal effects are considered. Sublethal effects include delayed or disturbed development, other deleterious phenotypic effects and behavioural phenomena. The literature is apparently conflicting on the relative susceptibilities of various species, the relative speed of action of nitrogen-oxygen mixtures and those containing CO_2 and the temperature dependence of the action of air-CO_2 mixtures. The effects of controlled atmospheres on stored product mites and predators are reviewed and the possible development of tolerance to such atmospheres is discussed.

INTRODUCTION

Controlled atmosphere storage of cereal grains and grain products is generally understood to consist of the medium or long term storage of such commodities in atmospheres consisting only of the major gaseous components of the earth's atmosphere: that is storage without the introduction of any gases or vapours normally considered to be poisons. In a world that is currently very conscious of pollution by toxic materials, it is this latter fact that has drawn so much attention to the technique. Nevertheless, in view of the great potential of the method in providing long term solutions to storage problems, the number of research workers involved is very small. We urgently need more accurate information on the biological mode of operation of controlled atmosphere storage. The aims of this section of the symposium are to discuss our current knowledge of the biological effects on the arthropod fauna of stored grain and to point out where our greatest deficiencies in our knowledge occur in the hope that they will receive effective study in the near future.

This review is intended to update that of Bailey and Banks (1975), which dealt largely with the acute mortality response of stored product insects to controlled atmospheres. It aims to present a broader outline of the effects of controlled atmospheres including sublethal effects and refers to the earlier literature where necessary to give a complete picture.

The major gaseous components of the earth's atmosphere are nitrogen, oxygen, argon and carbon dioxide. Of these gases argon has been considered to be totally inert biologically and, in the context of controlled atmosphere storage, has received no attention from researchers. Oxygen and carbon dioxide, the obviously biologically active components, have received much attention but, more recently, attention has been focussed on the interaction of water vapour concentration with the other components. Nitrogen, four-fifths of the earth's atmosphere, has been largely looked upon, probably correctly so, as a diluent, since its action has been shown to be similar to that of the inert gas, helium (Lindgren and Vincent, 1970; Aliniazee, 1972).

LETHAL EFFECTS OF CONTROLLED ATMOSPHERES

Mixtures of nitrogen and oxygen

Since the last general review in 1974 (Bailey and Banks, 1975) few investigations of the effects of changing the proportions of nitrogen and oxygen on mortality have been conducted. Shejbal (1979) has tabulated current knowledge on this. Such studies are very relevant to practical use of low oxygen atmospheres, as they help define the commercial target oxygen level. This is an important consideration because, the lower the target level, the more difficult it is to maintain the specified atmosphere in the face of inevitable leakage. It appears that different pest species have different responses to low oxygen atmospheres and that the available data on the subject may even be conflicting. In the most comprehensive study to date Navarro (1978a) found the lethal response of S. oryzae adults was dependent largely on exposure time, being almost independent of oxygen concentration below 3%, with 1% oxygen atmospheres being slightly more rapid in action than 0.2% or 2% atmospheres. The lethal effect on T. castaneum adults or E. cautella pupae was strongly dependent on oxygen content with more rapid kill occurring with lower oxygen levels (see Fig. 1). Person and Sorenson (1973a,b) found 0.5% O_2 to be only slightly more effective than 1% O_2 particularly at lower temperatures against S. oryzae adults and immatures, but considerably more effective against "flat grain beetle" adults. Careri et al. (1972) found no difference between the speed of action of technical (< 0.5% O_2) or pure nitrogen against T. confusum adults but a slightly more rapid action of pure nitrogen against S. granarius adults. Shejbal et al. (1973) found an increase in speed of action for adults of T. confusum, T. castaneum and S. granarius as the oxygen was progressively reduced from 1% to 0.1%. The apparent difference between the action on the two Sitophilus species adults is notable and, as Navarro (1978a) concludes, it may be dangerous to generalise from one species to another. A possible reason for wide variations in the results of different studies may be explained by the findings of Shejbal et al. (1973) who demonstrated the interesting phenomenon that the flow rate of

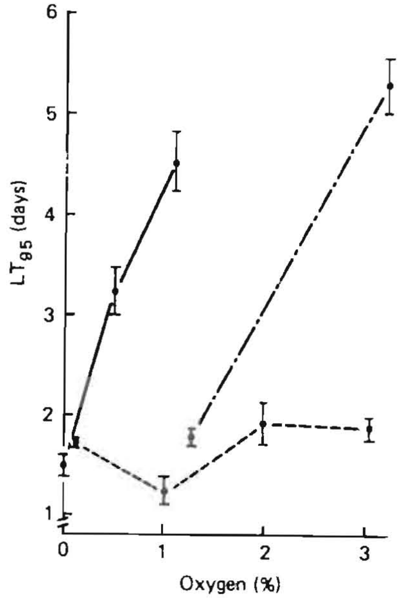

Fig. 1. Variation of LT$_{95}$ on oxygen concentration in an oxygen-nitrogen mixture at 26°, 54% for S. oryzae adults (– – –), T. castaneum adults (——) and E. cautella pupae (–·–·–). Redrawn from Navarro (1978a).

gas mixture through the grain has an effect on insect mortality, a faster rate enabling insects to survive longer. This result suggests that localised regions of still lower oxygen concentration may form at low flow rates resulting in increased mortality under such conditions.

Studies on high CO_2 atmospheres

Several studies of the effects of carbon dioxide-air mixtures have been carried out recently. Stoyanova and Shikrenov (1976) determined the time required for a 50% and 99.9% kill of 4 species at 20°C and 28°C using the relatively low concentrations of 20% and 40% CO_2 in air. The longest time necessary was 19.5 days for 99.9% kill of S. granarius at 20°C and 20% CO_2. They suggested the lower observed tolerance of S. oryzae than S. granarius may be due to a higher respiratory rate.

Press and Flaherty (1973) exposed adult moths of Ephestia khueniella, E. cautella and Plodia interpunctella to a 96% CO_2 atmosphere for 2 hours per day for 6 days. They found a large reduction in oviposition in all 3 species and observed that egg hatch was entirely eliminated in E. khueniella, was less than 2% normal in E. cautella and was 5% normal in P. interpunctella.

Banks and Sharp (1979) reported a trial CO_2 fumigation of wheat and rye using an atmosphere of about 60% CO_2 generated from dry ice under plastic sheeting at 11 to 13°C. The CO_2 level was maintained for 22 days. The pest infestation was predominantly R. dominica with small numbers of T. castaneum, Cryptolestes ferrugineus, E. cautella and S. oryzae. Small numbers of R. dominica only survived the treatment, emerging as adults four weeks or more after the start of incubation: they were presumably early instar larvae at the time of gassing. Cantwell and Neidhardt (1978) working on the disinfestation of hospital food carts, found that 8 hours exposure to > 35% CO_2 at about 32°C gave complete kill of adult Blatella germanica and prevented hatching from oothecae.

The mortality of stored product pests found in field trials using high CO_2 atmospheres has been summarised by Banks (1979). It was noted that while very high mortalities were often attained, complete mortality of added test insects or natural populations seldom occurred. This was attributed to a failure to provide sufficient exposure time to CO_2.

There have been a few studies on the relative toxicities of high CO_2 and low oxygen atmospheres. More are required to provide full details of their relative effects, especially against particular species, and to define the speed of action of the two atmospheres. It is well known that CO_2 acts in the presence of oxygen and that mixtures of CO_2 and air (e.g. 80% CO_2, 4% O_2, 16% N_2) are more speedily lethal to some species (e.g. Sitophilus spp. (Lindgren and Vincent, 1970)) than pure CO_2. However studies on the relative rates of action of CO_2 or N_2-rich atmospheres under conditions where the oxygen concentration is low enough to kill stored product species (> 2% O_2) give a complex picture. Press and Harein (1966) found pure nitrogen to be slightly more effective against T. castaneum adults and P. interpunctella larvae than pure CO_2. Lindgren and Vincent (1970) found no difference between 100% CO_2 and 100% N_2 against different developmental stages of both S. oryzae and S. granarius whereas Zakladnoi (1976) showed pure CO_2 to be more rapidly toxic than pure nitrogen to the adults of S. granarius, S. oryzae, R. dominica and T. castaneum, at all three experimental temperatures (20°, 25° and 35°C). Verma (1977) and Verma and Wahdi (1978) exposed a number of different stored product pests to pure CO_2 and N_2 atmospheres. The action of the CO_2 atmosphere was significantly slower than the nitrogen one against T. castaneum adults, O. mercator eggs, larvae and pupae and E. cautella eggs and larvae; significantly faster against O. mercator adults, E. cautella adults and T. granarium larvae and not significantly different for T. castaneum eggs, larvae and pupae, E. cautella pupae and T. granarium pupae. As may be expected under oxygen levels that are only just insecticidal in pure nitrogen mixtures, the replacement of nitrogen by CO_2 gives a more toxic mixture, presumably combining the effect of the low oxygen level with CO_2's specific toxic effect on the insect. Thus Aliniazee (1971) obtained complete mortality of T. confusum and T. castaneum adults at 27°/38% r.h. in less than 1.5 days with 2% O_2 in CO_2, but it took 4 days using 2% O_2 in nitrogen.

The possible synergistic effects of CO_2 and chemical pesticides feature in two studies. Young and Mcdonald (1970) examined the effect of CO_2 anaesthesia on the toxicity of malathion to adults of T. castaneum, T. confusum, and Lasioderma serricorne and to larvae of Attagenus megatoma. Anaesthesia for 60 minutes increased the toxicity of malathion to all four species. Anaesthesia for as little as five minutes increased the toxicity to all species except T. confusum. The study arose from concern over the use of CO_2 anaesthesia during the handling of experimental insects. Bond and Buckland (1978) tested the effect of adding CO_2 to a number of fumigants at 0^o, 10^o and 25^oC using adult S. granarius and T. castaneum and fourth-instar larvae of Tenebrioides mauritanicus. The fumigants used were acrylonitrile, methyl bromide, a mixture of these two, and phosphine and hydrogen cyanide. Results varied ranging from a seven-fold increase of the toxicity to T. castaneum of acrylonitrile in the presence of 30% CO_2 to no increase at all with hydrogen cyanide. The authors suggest the use of low concentrations of fumigants in controlled atmospheres employing CO_2 as a means of reducing the concentration of CO_2 required, or of reducing the exposure times.

Controlled atmospheres based on mixtures of CO_2 and nitrogen

Lindgren and Vincent (1970) found no difference in the mortality response of S. granarius and S. oryzae to pure N_2, CO_2 or helium. Accordingly, it was assumed (e.g. Bailey and Banks, 1975) that at low oxygen levels the proportion of CO_2 and nitrogen present is unimportant. Recently this has been shown to be untrue and it is now clear that, at low oxygen levels, the presence of a few per cent of CO_2 accelerates the lethal action of the atmosphere compared with pure nitrogen-oxygen mixtures.

Calderon and Navarro (1979) found that at $< 5\%$ O_2 the mortality of adult T. castaneum was dependent on the quantity of CO_2 also present. For instance at 2% oxygen, $> 90\%$ mortality was attained with 5% CO_2 present compared with about 50% mortality in the pure nitrogen-oxygen mixture (30^oC, 57% r.h.). Fig. 2 shows the overall variation of effectiveness of given oxygen levels with different combinations of CO_2 and N_2 at 30^oC. A similar variation was found at 26^oC. Girish (1978) using 1.1 to 1.2% oxygen found the LT_{50} for T. granarium larvae at 35^oC was 27, 30, 32 and 39 hours for atmospheres containing 15, 11, 6 and 0% CO_2 respectively (balance nitrogen).

Storey (1975a, 1975b, 1975c, 1977, 1978) subjected a number of species of stored product pests to a gas mixture produced by burning natural gas and containing about 9% CO_2, $< 1\%$ O_2 and with balance N_2 and some carbon monoxide and other gases. Zakladnoi et al. (1974) and Zakladnoi (1976) report similar work using a burner producing an atmosphere containing from 0.4 to 2% oxygen and up to 13% CO_2. It is not possible to compare the results directly with

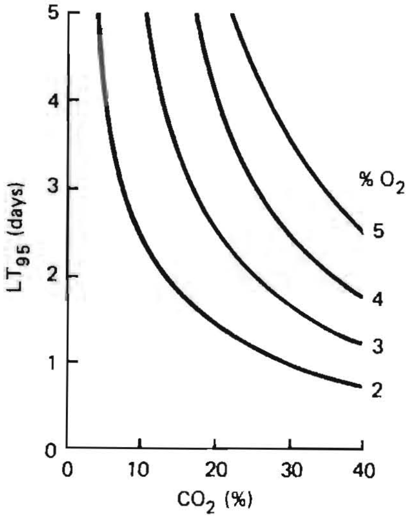

Fig. 2. Variation of LT_{95} with % CO_2 in atmosphere at various oxygen levels (balance nitrogen) at 30°, 57% r.h. for T. castaneum adults. Redrawn from Calderon and Navarro (1979).

those obtained using pure gases because the influence of the minor components, carbon monoxide, oxides of nitrogen and other combustion products, is not known and the oxygen level was not precisely regulated. The general trends are similar to that found by other workers using low oxygen atmospheres (see Bailey and Banks, 1975), with S. oryzae being the most tolerant species both as adult and immature forms. S. oryzae adults gave an LT_{95} of 12.4 days at 15°C, falling to 0.8 days at 32°C (50% r.h.) (Storey, 1975a). It is notable that Zakladnoi (1976) found S. granarius to be much more tolerant than S. oryzae. Moth species (Storey, 1975b) were relatively susceptible to the gas mixture with all stages of Sitotroga cerealella killed after five days, of E. cautella, after two days and of P. interpunctella within one day at 27°C, 50% r.h. Some immature stages of S. oryzae were more tolerant than the adults to the gas mixture, with the greatest tolerance being shown by the fourth-instar larvae and pupal stage. The LT_{95} of prepupae was found to be 27 and 10 days at 21° and 27°C (50% r.h.) respectively. There was no survival of immature S. oryzae exposed for 14 days in maize, wheat or sorghum at 27°C, 50% r.h. This exposure period is similar to that needed to obtain complete mortality using 1% oxygen in nitrogen under the same conditions (Banks and Annis, 1977). Thus, although the results of Storey and those given above suggest that under commercial conditions there may be some advantage in using a proportion of CO_2 in low oxygen atmospheres, the reduction in exposure time allowable will not be substantial if it is necessary to ensure that Sitophilus spp. are eliminated.

Relative susceptibility of various species to controlled atmospheres

It is important to know what is the most tolerant stored product pest in a given atmosphere in order to be able to set the exposure limit to control it and thus all others too. It is usually assumed for both high-CO_2 and low oxygen atmospheres that immature stages of S. oryzae are the most tolerant form of the most tolerant pest likely to be encountered but that stages of S. granarius may also be as tolerant. However there are no comparative studies on immature stages of other species and the evidence based on the adult forms is conflicting. Table 1 summarises the order of susceptibility to various controlled atmospheres found by various workers for the main stored grain pests. There is general agreement that T. confusum is similar in susceptibility or slightly more tolerant than T. castaneum both as adults and immature stages to all types of controlled atmosphere (Jay and Pearman, 1971; Storey, 1977; Aliniazee, 1971; Jay et al., 1971; Shejbal et al., 1973). Bearing this information in mind, it can be seen from Table 1 that Sitophilus granarius is consistently rated as a tolerant species, but authorities are divided as to the degree of susceptibility of S. oryzae. Whether the apparent inconsistencies result from strain differences, difference in technique or a true change in relative susceptibility in different gas mixtures is yet to be determined.

The susceptibility of Trogoderma granarium larvae to pure nitrogen and CO_2 atmospheres, reported by Verma and Wadhi (1978), is similar to that of Sitophilus larvae (Lindgren and Vincent, 1970; Storey, 1975a). A detailed comparison should be made to determine which is the more tolerant species to various modified atmospheres.

Influence of relative humidity on action of controlled atmospheres

A component of controlled atmospheres that has been largely neglected so far as its biological action is concerned is water vapour. Pearman and Jay (1970) reported a marked increased in the mortality of T. castaneum exposed for 46% CO_2 where the relative humidity was decreased to 33% or lower. Jay, Arbogast and Pearman (1971) exposed adult T. castaneum, T. confusum and O. surinamensis to atmospheres containing less than 1% oxygen with the balance as nitrogen at relative humidities ranging from 9% to 68%. All three species showed a marked increase in mortality as the relative humidity decreased. Also with essentially similar relative humidities used in conjunction with atmospheres containing about 38% carbon dioxide they showed that the mortality of all three species increased with decreasing humidity. The authors suggested that desiccation at low humidities was an essential factor in mortality in both instances. Zakladnoi (1976) demonstrated a more rapid mortality at low relative humidity in assessments employing "dry grain, moderately dry grain and wet grain".

Subsequently, Navarro and Calderon (1973) examined the inter-relationship of CO_2 and relative humidity as it affects pupae of E. cautella. They confirmed the finding of high mortality at lower relative humidity and found that for this species death appeared to be associated with a critical water loss representing about 30% of weight of the pupae. The higher the concentration of CO_2 and the lower the humidity the more pronounced was water loss. In a further study using pupae of E. cautella, Navarro and Calderon (1974) showed that there was little difference in rate of action of CO_2 concentration between 21-88% at 21 and 55% r.h. but a difference became apparent at high r.h. (95%) with lower CO_2 levels being slower in action. At 21 and 55% r.h. mortality appeared to be correlated with weight loss and an inverse relationship between CO_2 concentration and relative humidity was found for a given mortality level. At the highest relative humidity used, 95%, weight loss was small and a direct toxic effect of CO_2 acting as a fumigant appeared to be involved.

Navarro (1978a) reported that in the absence of CO_2 a relationship existed between oxygen tension and relative humidity for a set level of mortality. With pupae of E. cautella and adults of T. castaneum the higher the relative humidity the lower the oxygen concentration necessary to produce 95% mortality.

Action of controlled atmospheres on mites

There is little published work on the influence of controlled atmospheres on stored product mites. Stepien (1979, 1975) investigating the action of 99.5% CO_2 on Tyrophagus putrescentiae Schrank found the 0-24 hour old eggs to be the most tolerant stage of development with complete mortality attained only after six days (25°C, 85% r.h.) but with other stages controlled in less than one day. CO_2 under high pressure is rapidly toxic to T. putrescentiae (? adults only) (Mitsura et al., 1973) requiring 30 minutes exposure at 16 atm pressure at 30°C. Nitrogen even at 100 atm was ineffective at 30°C even after one hour. The lethal effect was dependent on CO_2 pressure and exposure time requiring 15, 30 and 55 minutes exposure at 20°C at 26, 21 and 16 atm respectively for complete mortality. Hughes (1943) found Acarus siro (? adults only) was killed by an exposure of four days at 20°C (high r.h.) to either pure N_2 or CO_2 but could survive four days at 0.4% O_2 in N_2. These observations suggest that controlled atmospheres may be potential control agents for stored product mites, but additional data, notably on A. siro, is required to confirm this and extend the range of atmospheres tested.

Action of controlled atmospheres on beneficial insects associated with grain storage

Banks and Sharp (1979) found that significant numbers of the hymenopteran parasitoids Anisopteromalus calandrae and Choetospila elegans survived conditions (11-13°C, 25-90% CO_2 over 22 days) under which a high level of mortality was attained for their host, Rhyzopertha dominica. There is a possibility that this differential susceptibility could be exploited for suppression of R. dominica under conditions where an incomplete kill was achieved in the presence of these parasitoids. A similar situation does not appear likely to occur with the predator Xylocoris flavipes under low oxygen atmospheres as it is very susceptible : 97% CO_2, 1% O_2, 2% N_2 at 30°C, 60% r.h. gave complete mortality of eggs and adults, the most tolerant stages, within 24 hours (Press and Flaherty, 1978).

Temperature dependence

There is general agreement in the literature (see Bailey and Banks, 1975) that the action of low oxygen atmospheres is strongly dependent on temperature, with action at low temperatures being slow. This generalisation includes those low oxygen atmospheres containing CO_2 produced by burning hydrocarbons (Storey 1975c, 1977). However there is a general lack of information on the temperature dependence of high CO_2 atmospheres containing substantial quantities of oxygen (e.g. 60% CO_2 in air) and little information on the action of low oxygen at between 20 and 15°C, a range in which death from cold does not occur, but in which low oxygen systems are likely to be very slow acting. Banks and Annis (1977) by extrapolating the available data for temperatures at > 20°C, predicted that about 15 weeks exposure to 1% O_2 in nitrogen at 18°C was required for complete mortality of all species and developmental stages of stored product insect pests.

As an extreme case of temperature dependence under oxygen conditions only just adequate for insect control, the response of mixed cultures of S. granarius containing approximately equal numbers of various developmental ages was studied (Bailey and Banks, unpublished data) at three temperatures at 1.3% O_2 in nitrogen under an r.h. not likely to cause additional stress (70%). The cumulative emergence curves for this experiment are given in Fig. 3 for various periods of exposure to the low oxygen gas. Control cultures yielded 5000 to 7000 insects each before the start of emergence of the next generation. Almost complete mortality (> 99.5%) was attained in 2 weeks at 29.4°, in 3 weeks at 23.9°, but at 18.3°C only 78% mortality was attained after 12 weeks exposure. Numbers emerging at various times after various exposures are given in Table 2 for the latter experiment (see below).

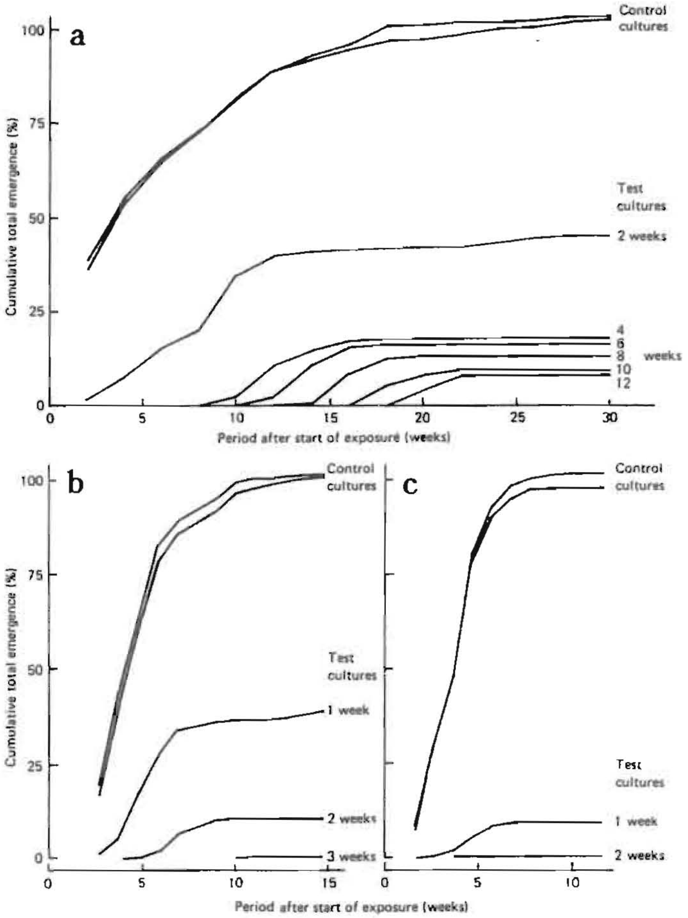

Fig. 3. Cumulative total emergence of *Sitophilus granarius* cultures containing approximately equal numbers of each developmental age expressed as a percentage of the average total observed numbers in the control cultures. Emerged adults removed after each inspection. Cultures continuously exposed to 1.3% O_2 in nitrogen at 70% r.h. at (a) 18.3^{o}, (b) 23.9^{o} and (c) $29.4^{o}C$ for various periods and then incubated at the same temperature.

There are no published studies on the temperature dependence of mortality to atmospheres containing mixtures with 60-90% CO_2 in air. However, Harein and Press (1968) found little difference in speed of action of 47-49% CO_2 in air against *Tribolium castaneum* larvae exposed at 16, 27 or $39^{o}C$ (61-64% r.h.). With adults, exposure at $27^{o}C$ appeared slightly less effective than at the other two temperatures. It appears that, in contrast to that of low oxygen atmospheres, there may be only a small temperature dependence of atmospheres containing high CO_2 with air. With pure gases, Zakladnoi (1976) demonstrated little temperature dependence in 100% CO_2 but a marked temperature dependence in 100% nitrogen. By contrast, Aliniazee (1971) found a substantial positive temperature effect in the lethal action of 100% CO_2 on both *T. confusum* and *T. castaneum*.

TABLE 1

Relative susceptibility of common stored product pests (beetles) to various controlled atmospheres as reported by various authorities.

Order of susceptibility	Stage	Gas Mixture	Temperature (°C)	Approximate r.h. (%)	Reference
S.g. < S.o.[a] < T.co. < R.d.	adult	100% N_2	20,25,35	75	Zakladnoi, 1976
T.ca. = S.o.	adult	100% N_2	26	54	Navarro, 1978a
S.o. < S.g.	all stages	100% CO_2 or N_2	27	65	Lindgren & Vincent, 1970
S.g. < S.o. < T.ca.	adult	100% CO_2	20	?	Busvine, 1942
S.g. < T.co. < S.o. = R.d.	adult	100% CO_2	20,25,35	75	Zakladnoi, 1976
T.ca. < S.o.	adult	1% O_2/N_2	26	54	Navarro, 1978a
R.d. = T.ca.	adult	2% O_2/N_2	32	70	Bailey, 1965
S.g. < T.co. = T.ca.	adult	low oxygen	22	70	Shejbal et al., 1973
S.o. = or < S.g.	all stages	b	27	50	Storey, 1975a
S.o. < S.g. < R.d. = T.ca.	adult	b	21,27,32	50	Storey, 1975c
S.o. < S.g. < R.d. < T.ca.	adult	b	15	50	Storey, 1975c
T.ca. < R.d.	adult	50% CO_2/ 21% O_2/N_2	32	70	Bailey, 1965
S.g. < S.o.	adult	high CO_2 CO_2/O_2 mixtures	27	65	Lindgren & Vincent, 1970
S.g. < T.co. < R.d. < S.o.	adult	20,40% CO_2/ air	20,28	?	Stoyanova & Shikrenov, 1976

[a] A < B signifies species A is less susceptible than B.

[b] low oxygen atmosphere ($< 1\%$ O_2) produced by burning natural gas

S.g. = *Sitophilus granarius*; S.o. = *S. oryzae*; T.ca. = *Tribolium castaneum*; T.co. = *T. confusum*; R.d. = *Rhyzopertha dominica*.

SUBLETHAL EFFECTS OF CONTROLLED ATMOSPHERES

Behavioural effects associated with controlled atmospheres

There have been two reports (Shejbal et al., 1973; Navarro et al., 1976) of stored product Coleoptera, presumably within the grain bulk, congregating around leaks in stores under low-oxygen atmospheres. Bailey (unpublished data) observed a similar phenomenon in an hermetic storage. The high concentration of insects observed suggests that movement had occurred and thus that the insects can sense and move up the oxygen concentration gradient towards the leak. Navarro (1978b) was unsuccessful in his attempts to demonstrate this aggregation under laboratory conditions with various species using gradients of either oxygen or carbon dioxide.

Barrer and Jay (1980) demonstrated that E. cautella females found a source of wheat odour more attractive if containing 30% CO_2 than if mixed with pure air. A source of odour with 60% CO_2 was as attractive as pure air while that from a low oxygen source (1% O_2) was significantly repellant. Oviposition close to the source was increased in the presence of 30% CO_2 relative to air. Willis and Roth (1954) found 60% CO_2 to be repellant to T. castaneum.

Aggregation at leaks both from within and outside a storage are relevant to the controlled atmosphere technique as such abilities could allow localised survival in a treated grain bulk or infestation of a region around a leak from external sources.

The 'anaesthetic' effect of carbon dioxide or nitrogen on insects is well-known. For stored product insects, immobilisation in response to low oxygen atmospheres is rapid after a brief period of hyperactivity (Aliniazee, 1972; Storey, 1975c). At about 40% CO_2 in air, immobilisation may take some hours for some species (Stoyanova and Shikrenov, 1976) but be rapid for others (Oosthuizen and Schmidt, 1942). Individual S. granarius remain active to varying degrees (Bond and Buckland, 1979). The latter effect appears to be related to the ability to develop tolerance to 42% CO_2.

Influence of controlled atmospheres on development

An important and neglected aspect of the action of both low oxygen and high-CO_2 atmospheres concerns the influence of these gas mixtures on development rate. With both types of atmosphere, a significant retardation of development occurs. The effect is more easily observed under slower rates of development. Table 2 gives the observed emergence during successive 2-week periods of S. granarius cultures set up as described above and exposed to 1.3% oxygen in nitrogen for various periods. In this case the time at which peak numbers emerge increases directly with the period of exposure to the low oxygen atmosphere. The pattern is consistent with the survival of one developmental age group by some form of slowing or suspension of development, thus presumably lessening its oxygen consumption and partially avoiding the effects of anoxia. The number emerging between 8-10 weeks after 2 weeks of exposure to low oxygen is significantly higher than expected from the numbers observed emerging each fortnight from the controls between 4 and 12 weeks ($\bar{x}$ = 399, s.d. = 75, n = 8, $P < 0.01$) suggesting that some development occurred into the tolerant stage, increasing the total numbers surviving for that emergence period. It is clear from the declining overall numbers that even the tolerant stage was slowly killed by the low oxygen atmosphere under these conditions.

Exposure (weeks)	Assay date (weeks after start)												
	2	4	6	8	10	12	14	16	18 $F_2 \rightarrow$	20	22	24	26
0	1709	816	501	332	424	338	169	125	165	54	50	55	37
2	73	290	353	219	**672**	231	58	20	11	10	11	57	56
4		0	0	1	119	**378**	186	117	14	2	1	3	2
6			0	0	1	118	**388**	212	19	1	0	1	3
8				0	0	0	23	**373**	182	24	4	0	0
10					0	0	0	0	**246**	134	52	1	4
12						0	0	0	6	**184**	165	15	2

Table 2. Numbers of adult *S. granarius* emerging and removed at fortnightly intervals from cultures held at 18.3^{o}C/70% r.h. under 1.3% O_2 in nitrogen for various periods and subsequently in air under the same conditions. Bold figures denote maximum emergence for a given period of exposure to low oxygen conditions.

A delay in developmental period corresponding to that spent under high-CO_2 (46-53%) was observed by Oosthuizen and Schmidt (1942) for *Callosobruchus chinensis*. Intermittent exposure of *Blatella germanica* to high CO_2 levels even for only three minutes a week can substantially slow development (Brooks, 1957). Unspecified delays in development have been noted for *T. castaneum* pupae and larvae under pure nitrogen and CO_2 (Aliniazee, 1971, 1972) and for their eggs in 20% CO_2 in air (Aliniazee and Lindgren, 1920), for *S. granarius* and *oryzae* immature stages under pure nitrogen and CO_2 (Lindgren and Vincent, 1970), of *T. granarium* larvae under high CO_2 and low oxygen gas mixtures (Bailey, 1965) and *T. castaneum*, *T. confusum*, *S. oryzae*, *S. granarius* and *C. maculatus* in low oxygen atmospheres from hydrocarbon burning (Storey, 1975a, 1977, 1978). Spratt (1979a) found continuous exposure of *S. zeamais* to 10% O_2, 10% CO_2, 80% N_2 (30^{o}C, 71% r.h.) resulted in a delay in development of about 11 days compared with development in air. The intrinsic rate of increase, r, fell from 0.32 in air to 0.12 in the gas mixture (Spratt, 1979b) with the fall in rate caused by a combination of delay in development and other factors. The general effect on development rate merits further study as it is an important component of the effect of controlled atmospheres. Enhancement of the ability to delay development could lead to a form of tolerance to the treatment. If the phenomena is not recognised, it may also lead to errors in laboratory or field assessment of controlled atmosphere applications.

There have been a number of observations of sublethal effects of controlled atmospheres apparently common to both high CO_2 and low oxygen atmospheres which influence the overall fitness of the exposed population and thus reduce the rate of increase. These are perturbed metamorphosis (Storey, 1977, 1978; Aliniazee, 1971, 1972; Stepien, 1979), decrease in fecundity (Press and Flaherty, 1973; Spratt, 1979a; Stepien 1979), decrease in longevity (Spratt, 1979b; Stepien, 1979) and partial paralysis (Storey, 1975c, 1977). A general review of sublethal effects of CO_2 on insects was given by Brooks (1957).

RESISTANCE OR INCREASED TOLERANCE TO CONTROLLED ATMOSPHERES

It has been stated (e.g. Shejbal, 1978) that resistance to controlled atmospheres, specifically low oxygen systems, is unlikely to develop. It is indeed, difficult to envisage any stored product insect pest rapidly becoming capable of continuing to develop under low oxygen conditions ($< 0.5\%$ O_2) as this would entail a substantial change in its biochemistry of metabolism. However many insects possess anerobic metabolic pathways (Gilmour, 1965) and some are capable of continued development under very low oxygen conditions, although none are known which will complete its life cycle in such a situation. In practical terms, resistance does not entail continued development under a control measure but only survival until it no longer operates. Thus an accentuation of the delay in development observed when stored product insects are treated with low oxygen atmospheres together with an increased overall tolerance would result in a form of resistance. There appears no reason why such a pattern could not be selected for and why it could not eventually become significant in commercial practice, particularly when short exposures (e.g. 1 month) are used.

A similar form of tolerance could arise to high CO_2 atmospheres. Additionally, because such atmospheres can contain sufficient oxygen ($> 5\%$) for development under otherwise normal conditons, a further form of resistance analogous to that found for fumigants (Champ and Dyte, 1976) can be expected. Bond and Buckland (1979) have demonstrated such a tolerance to CO_2 atmospheres in a strain of S. granarius selected in the laboratory. After four selections with 75% CO_2 the LT_{50} to 75% CO_2 increased from 1.7 to 4.6 days and for 7 selections with 42% CO_2 the LT_{50} to 42% increased from 5.1 to 17.5 days (25°, 100% r.h.). A similar level of tolerance was also found at 60% r.h. to 75% CO_2. For relatively short exposures to nitrogen or CO_2, precedent already exists for a low level of resistance. Strains of Drosophila melanogaster show a range of susceptibility (about 1.5x) to these gases, with increased tolerance associated genetically with resistance to desiccation (Matheson and Parsons, 1973, 1975).

The possibility of the eventual rise of resistance cannot be taken to invalidate the controlled atmosphere technique but must be borne in mind, as in fumigation, so that the technique is not used in situations where it is likely to be incompletely effective thus providing a population of insects selected for tolerance to the control measure. An increase in tolerance may eventually require longer exposure periods to low oxygen atmospheres to achieve complete insect kill and in the case of CO_2 where a 'one-shot' system is used, a system of maintenance of atmosphere may have to be provided. Both eventualities will increase the overall cost of the technique.

CONCLUSION

It is clear from this review that there are several inconsistencies and gaps in our knowledge of the biological actions of various controlled atmospheres. During the 1970's a large quantity of information on the general subject has been gathered but only a few generalisations are apparent: low humidities accelerate the action of controlled atmospheres; increase in temperature or CO_2 content assists the action of low oxygen mixtures and exposures producing incomplete mortality cause a number of deleterious effects, including delayed development. We still require further work on the basic dose-mortality response to the various gas mixtures, with particular emphasis on tolerant developmental stages and species in order to provide a soundly based exposure schedule for particular conditions and atmospheres. Clarification of the delayed emergence phenomenon, the temperature sensitivity of the action of high CO_2 atmospheres and the influence of gas flow rate on mortality to low oxygen atmospheres is important to the practical use of controlled atmospheres.

It is hoped that this Symposium will provide some of the answers to these questions and give the stimulus necessary to ensure that those problems not yet elucidated will be given the attention they deserve, thus providing a sounder basis for the practical application of the important technique of controlled atmosphere grain storage.

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REFERENCES

Aliniazee, M.T., 1971. The effect of carbon dioxide gas alone or in combinations on the mortality of *Tribolium castaneum* (Herbst) and *T. confusum* du Val (Coleoptera, Tenebrionidae). J. stored Prod. Res., 7: 243-252.

Aliniazee, M.T., 1972. Susceptibility of the confused and red flour beetles to anoxia produced by helium and nitrogen at various temperatures. J. econ. Entomol., 65: 60-64.

Aliniazee, M.T. and Lindgren, D.L., 1970. Egg hatch of *Tribolium confusum* and *T. castaneum* (Coleoptera, Tenebrionidae) in different carbon dioxide and nitrogen atmospheres. Ann. Entomol. Soc. Amer., 63: 1010-1012.

Bailey, S.W., 1965. Air-tight storage of grain; its effect on insect pests - IV *Rhyzopertha dominica* (F.) and some other Coleoptera that infest stored grain. J. stored Prod. Res., 1: 25-33.

Bailey, S.W. and Banks, H.J., 1975. The use of controlled atmospheres for the storage of grain. Proc. 1st Internat. Working Conf. Stored-prod. Entomol., Savannah, USA (1974), pp. 362-374.

Banks, H.J., 1979. Recent advances in the use of modified atmospheres for stored product pest control. Prod. 2nd Internat. Working Conf. Stored-prod. Entomol., Ibadan, Nigeria (1978), pp. 198-217.

Banks, H.J. and Sharp, A.K., 1979. Insect control with CO_2 in a small stack of bagged grain in a plastic film enclosure. Aust. J. exp. Agric. Anim. Husb., 19: 102-107.

Banks, H.J. and Annis, P.C., 1977. Suggested procedures for controlled atmosphere storage of dry grain. CSIRO Aust. Div. Entomol. Tech. Pap. No. 13, 23 pp.

Barrer, P.M. and Jay, E.G., 1980. Laboratory observations on the ability of *Ephestia cautella* (Walker) (Lepidoptera, Phycitidae) to locate, and to oviposit in response to a source of grain odour. J. stored Prod Res., 16: 1-7.

Bond, E.J. and Buckland, C.T., 1979. Control of insects with fumigants at low temperatures: toxicity of fumigants in atmospheres of carbon dioxide. J. Econ. Entomol., 71: 307-309.

Brooks, M.A., 1957. Growth retarding effect of carbon dioxide anaesthesia on the German cockroach. J. Insect Physiol., 1: 76-84.

Busvine, J.R., 1942. Relative toxicity of insecticides. Nature (Lond.), 150: 208-209.

Calderon, M. and Navarro, S., 1979. Increased toxicity of low oxygen atmospheres supplemented with carbon dioxide on *Tribolium castaneum* adults. Ent. exp. Appl., 25: 39-44.

Cantwell, G. and Neidhardt, K., 1978. Fumigation of hospital food carts to control German cockroaches. Pest Control., 46(Dec): 14-16.

Careri, G., Shejbal, J. and Tonolo, A., 1972. Conservazione di cereali e di altri prodotti in atmosfero di azoto tecnico. Proc. 1st Symp. sulla Difesa Antiparassitaria nelle Industrie Alimentari e sulla Protezione degli Alimenti, Piacenza, Italy, pp. 280-286.

Champ, B.R. and Dyte, C.E., 1976. Report of the FAO global survey of pesticide susceptibility of stored grain pests. FAO Plant Production and Protection Series No. 5, Rome, 297 pp.

Gilmour, D., 1965. The metabolism of insects. Oliver and Boyd, Edinburgh. 195 pp.

Girish, G.K., 1978. Susceptibility of full grown larvae of *Trogoderma granarium* Everts to varying concentrations of carbon dioxide at low oxygen tension. Bull. Grain Technol., 16: 199-202.

Harein, P.K. and Press, A.F.Jr., 1968. Mortality of stored-peanut insects exposed to mixtures of atmospheric gases at various temperatures. J. stored Prod. Res., 4: 77-82.

Hughes, T.E., 1943. The respiration of *Tyrophagus farinae*. J. exp. Biol., 20: 1-5.

Irwin, D.G., Smith, L.W. Jr. and Pratt, J.J. Jr., 1972. Effects of carbon dioxide and nitrogen on the secretion of parabenzoquinones by *Tribolium castaneum* (Herbst). J. stored Prod. Res., 8: 213-219.

Jay, E.G. and Pearman, G.C. Jr., 1971. Susceptibility of two species of Tribolium (Coleoptera, Tenebrionidae) to alterations of atmospheric gas concentrations. J. stored Prod. Res., 7: 181-186.

Jay, E.G., Arbogast, R.T. and Pearman, G.C. Jr., 1971. Relative humidity: its importance in the control of stored-product insects with modified atmospheric gas concentrations. J. stored Prod. Res., 6: 325-329.

Lindgren, D.L. and Vincent, L.E., 1970. Effect of atmospheric gases alone or in combination on the mortality of granary and rice weevils. J. econ. Entomol., 63: 1926-1929.

Matheson, A.C. and Parsons, P.A., 1973. The genetics of resistance to long-term exposure to CO_2 in Drosophila melanogaster; an environmental stress leading to anoxia. Theor. Appl. Genet., 43: 261-268.

Matheson, A.C. and Parsons, P.A., 1975. Long-term exposure to CO_2 in Drosophila melanogaster and Drosophila simulans, using isofemale strains from natural populations. Amer. Nat., 109: 593-595.

Mitsura, A., Amano, R., Tanabe, H., 1973. The acaricidal effects of compressed gas treatments on the grain mite, Tyrophagus putrescentiae. Fd hyg. Soc. Japan., 14: 511-516. (in Japanese)

Navarro, S., 1978a. The effects of low oxygen tensions on three stored-product insect pests. Phytoparasitica, 6: 51-58.

Navarro, S., 1978b. Report of the visiting scientist at the Plant Research Institute Burnley Victoria, Australia. Stored Products Division, Agricultural Research Organization, Israel, 81 pp.

Navarro, S. and Calderon, M., 1973. Carbon dioxide and relative humidity: inter-related factors affecting the loss of water and mortality of Ephestia cautella (Walker) (Lepidoptera, Phyticidae). Israel J. Entomol., 8: 143-151.

Navarro, S. and Calderon, M., 1974. Exposure of Ephestia cautella (Walker) pupae to carbon dioxide concentrations at different relative humidities: the effect on adult emergence and loss in weight. J. stored Prod. Res., 10: 237-241.

Navarro, S., Gonen, M., Kashanchi, Y. and Frandji, H., 1976. The use of a controlled atmosphere for the control of stored product insects: I. Experiments in a flat metal bin. Prog. Rep. Israel Agric. Res. Org., Stored Prod. Div. 1975/76, pp. 85-103.

Oosthuizen, M.J. and Schmidt, U.W., 1942. The toxicity of carbon dioxide to the cowpea weevil. J. Entomol. Soc. S. Afr., 5: 99-110.

Pearman, G.C. and Jay, E.G., 1970. The effect of relative humidity on the toxicity of CO_2 to Tribolium castaneum in peanuts. J. Ga. Entomol. Soc., 5: 61-64.

Person, N.K. Jr. and Sorenson, J.W. Jr., 1973a. Use of gaseous nitrogen for controlling stored-product insects in cereal grains. Cereal Chem., 47: 679-686.

Person, N.K. Jr. and Sorenson, J.W.Jr., 1973b. The effects of gaseous nitrogen on the emergence of immature stages of rice weevils (Sitophilus oryzae L.) in cereal grains. Ann. Technol. agric., 22: 541-549.

Press, A.F. Jr. and Harein, P.K., 1966. Mortality of red flour beetle adults and Indian-meal moth larvae in nitrogen and carbon dioxide. J. Ga. Entomol. Soc., 1: 15-17.

Press, J.W. and Flaherty, B.R., 1973. Reduction of fecundity and egg hatch in three stored product Phycitid moths after repetative sublethal carbon dioxide exposures. Environ. Entomol., 2: 147-148.

Press, J.W. and Flaherty, B.R., 1978. Effect of carbon dioxide on the adults and eggs of the predacious bug Xylocoris flavipes (Hemiptera, Anthocoridae). J. Ga Entomol. Soc., 13: 91-93.

Shejbal, J., 1978. Preservation of cereal grains and oil seeds in nitrogen. Meded. Fac. Landbouww. Rijksuniv. Gent., 43: 493-504.

Shejbal, J., 1979. La tecnologia Snamprogetti per la conservazione di cereali in azoto. Tecn. Molitoria, 30: 512-520.

Shejbal, J., Tonolo, A. and Careri, G., 1973. Conservation of cereals under nitrogen. Meded. Fac. Landbouww. Gent., 38: 1133-1144.

Spratt, E.C., 1979a. Some effects of a mixture of oxygen, carbon dioxide and nitrogen in the ratio 1:1:8 on the oviposition and development of *Sitophilus zeamais* Mots. (Coleoptera, Curculionidae). J. stored Prod. Res., 15: 73-80.

Spratt, E.C., 1979b. The effects of a mixture of oxygen, carbon dioxide and nitrogen in the ratio 1:1:8 on the longevity and the rate of increase of populations of *Sitophilus zeamais* Mots. J. stored Prod. Res., 15: 81-85.

Stepien, Z.A., 1975. Mozliwuosci zwalczania szkodnikow magazynowych za pomaca kontrolowarej atmosfery. Ocrh. Rosl., 19: 9-10.

Stepien, Z.A., 1979. Effect of carbon dioxide on *Tryophagus putrescentiae* (Schr.) (Acarina: Acaridae). Proc. 4th Internat. Congr. Acarology, Budapest (1974), pp. 249-255.

Storey, C.L., 1975a. Mortality of *Sitophilus oryzae* (L.) and *S. granarius* (L.) in atmospheres produced by an exothermic inert gas generator. J. stored Prod. Res., 11: 217-221.

Storey, C.L., 1975b. Mortality of three stored product moths in atmospheres produced by an exothermic inert atmosphere generator. J. econ. Entomol., 68: 736-738.

Storey, C.L., 1975c. Mortality of adult stored-product insects in an atmosphere produced by an exothermic inert atmosphere generator. J. econ. Entomol., 68: 316-318.

Storey, C.L., 1977. Effect of low oxygen atmospheres on mortality of red and confused flour beetles. J. econ. Entomol., 70: 253-255.

Storey, C.L., 1978. Mortality of cowpea weevil in a low-oxygen atmosphere. J. econ. Entomol., 71: 833-834.

Stoyanova, S. and Shikrenov, D. 1976. Storage of cereals in an atmosphere with a high CO_2 concentration. 1. Effect of 20% and 40% CO_2 on insect pests. Nauch. Trud. Vissh. Inst. po Khranitelna i Vkusova Prom., Plovdiv., 23: 143-149. (in Bulgarian)

Verma, A.K., 1977. Effect of atmospheric gases on pest infestation during storage and on keeping quality of walnuts. Entomol. Newsl. 7(April): 13-14.

Verma, A.K., and Wahdi, S.R., 1978. Susceptibility of walnut pests to carbon dioxide and nitrogen and effect of gas storage on keeping quality of walnut kernels. Indian. J. Entomol., 40: 290-298.

Willis, E.R. and Roth, L.M., 1954. Reactions of flour beetles of the genus *Tribolium* to carbon dioxide and dry air. J. Exp. Zool., 127: 127-152.

Young, S.Y. and McDonald, L.L., 1970. Effect of CO_2 anaestheaia on malathion toxicity to four species of stored product insects. Ann. Entomol. Soc. Amer., 63: 381-382.

Zakladnoi, G.A., 1976. Regulation of the gas composition of the atmosphere for eliminating insects in grain. USSR Ministry of Procurement, Moscow, 3 pp. (in Russian)

Zakladnoi, G.A., Chekanov, A.A. and Yunskhodzhaeva, S., 1974. Neutral gas media as a means of controlling granary pests. Zash. Rast., 9: 58. (in Russian)