LOW OXYGEN CONTENT TO CONTROL STORED PRODUCT INSECTS

C. Reichmuth

Biologische Bundesanstalt für Land- und Forstwirtschaft Institut für Vorratsschutz
D — 1000 Berlin 33, F.R.G.

Abstract

Modified atmospheres are being developed more and more into an alternative means of stored-product insect control. Especially in warm climates, they can have a rapid effect. In this study low oxygen contents between 0.5 % and 4 % was chosen as a criterion to judge the feasibility of the method at temperatures of **15** and **20 °C.** A method of preparing various gas mixtures of the pure components was developed by using evacuated gas cylinders and very accurate manometers.

Five important insect species were selected to assemble laboratory data on the efficacy of inert atmospheres in controlling stored-product pest insects. The susceptibility of eggs of Ephestia elutella (Hubner) and Plodia interpunctella (Hübner), of adult Oryzaephilus surinamensis (L.) and Tribolium confusum (J. du Val), as well as five separate breeding stages and adults of Sitophilus granarius (L.) to these fumigants was determined. Up to 3 % O2 for about **10 days** was sufficient to control all insects except S. granarius **at 15 °C. At 20°C time** for lethal exposure was reduced to 6 days.To achieve 95 % mortality with adult S. granarius, a period of **13 days** was necessary at **both temperatures** tested using N2 as substitute gas. With high CO2 content LT-95 was **8 days** at **15 °C** and **6 days** at **20 °C.** Young eggs and especially pupae of S. granarius at 4 % O2 showed the most pronounced tolerance of all breeding stages. The LT-95 was **55 days** at **15 °C** and **41 days** at **20 °C** for the most tolerant pupal stage.

Introduction

For many years scientists have been considering the idea of reintroducing the very old technique of protecting stored products by gastight covering of the goods, removal of oxygen (O2) from the atmosphere of the enclosed space and replacement either by nitrogen (N2) or carbon dioxide (CO2). This has been done in the distant past when O2 was consumed by respiration of microflora.

Proc 4th Int Work Conf Stored-Product Protection, Tel Aviv, Israel, Sept 1986
[Eds E Donahaye and S. Navarro], pp 194-207

In animal pests the target of the procedure is the animal respiratory system with its requirements for O2 and the poisonous effect of high CO2 dioxide concentrations in the atmosphere. The other objective of the method is the suppression of microflora growing on stored products.

In the course of history, chemistry has produced substances which are toxic to animals (i.e. SO2, HCN, halogenated hydrocarbons, organic esters) and these have been used to facilitate the combat against pest as they appeared. The consciousness of people in this century compared to former times has changed in so far as protection some decades ago involved no foresight as to future problems. Chemicals offered quick solutions. This sort of thinking has been supported by the development of phosphine as a fumigant in stored product protection. The formulations are easy to handle, cheap and effective. There is no need for sophisticated machinery to apply phosphine.

Over recent years, however, the situation is rapidly changing. The chemical industry is under political pressure because of vast chemical catastrophies like Bhopal, Chernobyl and Cameroun and the slowly growing consciousness of pollution of the environment and residues of chemicals in foodstuffs. Our awareness is further enhanced by the development of better analytical methods to measure chemicals and their residues.

All these arguments support the search for so called alternative measures. Nevertheless it has to be noted clearly that in certain cases there is still no reasonable alternative to proper fumigation. Apart from the use of the physical treatments of heat and cold, with their biological effects which can be used for animal control, the application of N2 and (or) CO2 lies at the centre of current interest. The first steps of Bailey (1955), Banks and Annis (1977), Jay and Pearman (1971), Navarro (1978) and Oxley (1963) especially concerning desinfestation of warm grain showed promise. The stage of large scale field experiments has been reached and the financial competitiveness with classical fumigants can be evaluated. However, evaluation of the method is not only dependent on cost but also on political interests.

One initial objective is gastightness of the treated premises or enclosed foodstuffs this being the most important prerequisite. Due to the increasing problem of insect resistance against fumigants this aspect has in any case required radical improvement.

Up to present the use of so called controlled atmospheres (CAs) has been considered mainly for regions with warm climatic conditions (average temperature greater than 25 °C). Bell (1984), Fleurat Lessard (1986), Harein and Press (1968), Jay (1980), Marzke et al., (1970) also describe and report on experiments with CAs and stored product pest insects at temperatures below 20 °C.

Banks and Annis (1977) suggested that at 15 °C at least 24 weeks will be necessary to control all stages of Sitophilus. Bailey and Banks (1980) and Bell (1984) indicated the very limited existence of data on low temperature and mortality of stored product insects under CAs. The question still remains therefore as to how far CAs can replace fumigants like phosphine in moderate (i. e. central european) climates. For such methods to be competitive, complete mortality has to be achieved within a reasonable time.

As an outcome of this situation a research program has been initiated to build-up a catalogue of mortality data on important species and stages of stored product pest insects at 15 °C and 20 °C with gas mixtures of 0.5, 1.0, 2.0, 3.0 and 4.0 % by volume of O2 respectively - with N2 and (or) CO2 being balance components. A method has been elaborated to amass data without being dependent on expensive calibrated gas mixtures or continuous adjusting of gas flows. This paper describes the new method and presents results obtained at this stage of the program.

Materials and Methods

Experiments were performed on young eggs (1 - 2 days old at 25 °C) of:

- Ephestia elutella (Hübner) and
- Plodia interpunctella (Hubner),
- 2 - 3 weeks old adults and 5 breeding stages of Sitophilus granarius (Linnaeus)
- adults of Tribolium confusum (J. du Val) and
- Oryzaephilus surinamensis (Linnaeus).

The moth cultures were reared at 25 °C and 70 % relative humidity (r.h.). For experimentation batches of 30 eggs were kept singly in a perspex frame (25 X 60 X 3 mm) containing 30 holes which were covered with fine stainless steel gauze (mesh 0.135 mm) on both sides.

Developing stages of S. granarius were established by placing adults weekly on fresh wheat (soft summer, kept at -20 °C for 14 days in advance to kill all possible infestation) for three days at 25 °C and 70 % r.h. Reichmuth (1986) . Some 2500 adults including about 40 % females were exposed on 3000 grain kernels (142 g) in 2 l glas jars. From 70 - 90 % of the kernals there was development to weevils. At 25 °C test insects could be used for experiments according to the following time intervals :

age	1	:	0 - 3 days	old	eggs
age	2	:	7 - 10 days	old young	larvae
age	3	:	14 - 17 days	old	larvae
age	4	:	21 - 24 days	old larvae and	pupae
age	5	:	28 - 31 days	old	pupae
age	6	:	2 - 3 weeks	old adult	weevils

Fifty grain kernels of each stage or 50 adults on non-infested grain were introduced into separate stainless steel mesh wire cages (6 cm long , 1.5 cm diam., closed with foam rubber stoppers). Adult O. surinamensis and T. confusum taken from cultures at 25 °C were placed in cages on culture media. Up to 5 cages each containing one species or age respectively, were introduced into a gas-washing bottle (dressel flask).

- Gas mixtures

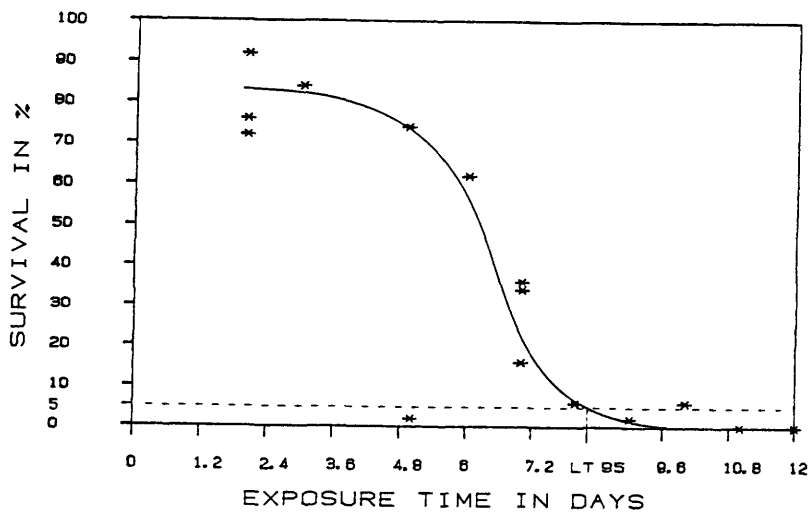
The gas mixtures were prepared from pure O2, CO2 and N2. The components were released from pressurized cylinders into evacuated cylinders using very accurate manometers (SETARAM/ France, rampe a gaz) with an accuracy of about 0.1 vol.% absolute. This was monitored using a paramagnetic oxygenmeter (SERVOMEX/ England). In many cases the concentration of all components was determined additionally by GC (INTERSMAT/ France, minigrator SPECTRA PHYSICS, spherocarb 60/80; N2/O2: 30 °C; CO2: 150 °C; inj. 200 °C; det. 225 °C; He 2 bar). To improve distribution of the components the cylinders with gas mixtures were kept at room temperature for two days before starting the experiment.

- Exposure procedure

Insects in gastight connected dressel flasks were held in constant temperature rooms at 15 °C and 20 °C and were exposed to the gas mixtures by leading gas out of the gas mixture cylinder through copper tubes and a humidifying unit at 70 % r.h. - consisting of saturated NaCl/H2O solution- into the flasks.

At the outlet of the flasks O2 content was determined continuously. After about 15 min (time for about 10 replacements of total flask volume by gas mixture) outlet concentration was identical with inlet concentration. Then gas was flushed at a low rate for one day to overcome sorption effects. The gastight sealed flasks were then separated from the gas mixture cylinder to permit further experiments with other flasks. After different exposure periods ranging from days to months, single flasks were aerated and insects transferred to a culture room and examined for mortality at 25 °C. Each sample was accompanied by an untreated one which was exposed to the same temperature change. Atmospheric compositions were as follows: O2 contents by volume were 0.5, 1.0, 2.0, 3.0 and 4.0 %. N2 and (or) CO2 were balance gases to simulate replacement of air, with N2 (99.5 to 96.0 %), CO2 (97.5 to 80.0 % with rest N2 and O2; ratio 4:1) and burner gas or catalytic combustion (80 % N2 and 19.5 to 16.0 % CO2). Experiments were repeated three times. In general probit analysis was used to determine LT values (p = 0.05). Some values were taken from graphs. In Fig. 1 a step in the preparation of the data is demonstrated. For each insect stage a dose - response function was determined at two temperatures for 5 different O2 contents and three gas mixtures per O2 content.

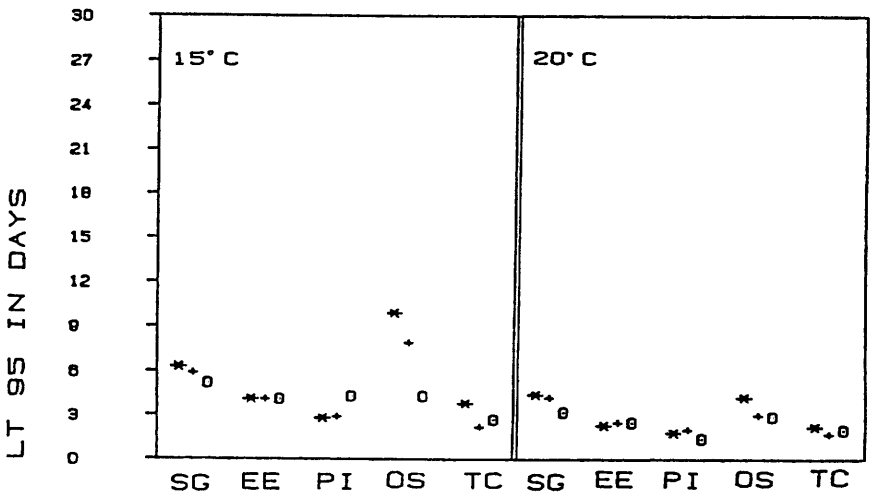
Figure 1: Example for determination of LT 95 value using the dose - response graph : surviving adults of Sitophilus granarius as a function of exposure time in days at 15 °C, and 3 % O2 and 97% N2.



Results

LT 95 values (in days) of adult S. granarius, O. surinamensis and T. confusum, and eggs of E. elutella and P. interpunctella are given for : 0.5 % O2 (Fig.2), 1.0 % O2 (Fig.3), 2.0 % O2 (Fig.4), 3.0 % O2(Fig. 5), and 4.0 % O2 (Fig.6).

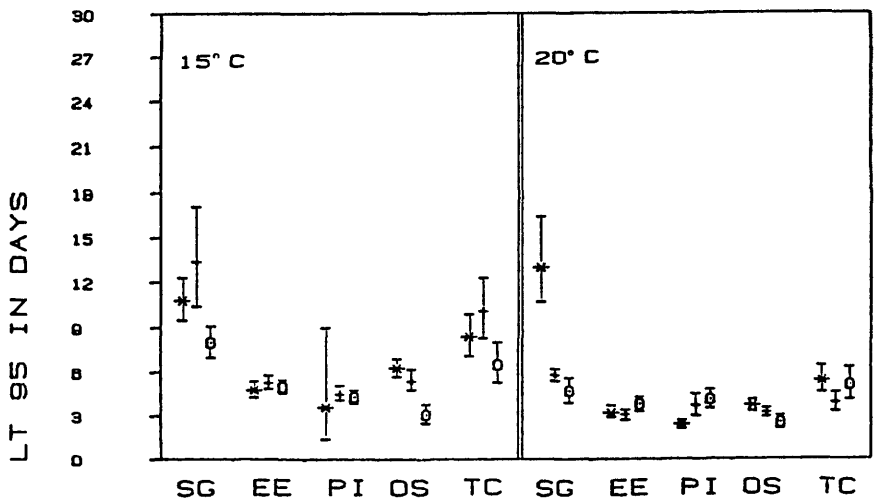
Figure 2: Lethal exposure time in days (LT 95) for Sitophilus granarius (SG), Ephestia elutella (EE), Plodia interpunctella (PI), Oryzaephilus surinamensis (OS) and Tribolium confusum (TC) at 15 °C and 20 °C; 0.5 % O2 and * = 99.5 % N2, += 80 % N2 and 19.5 % CO2 (burner gas), 0 = 2 % N2 and 97.5 % CO2.



The experimental atmosphere was either replacement of air with N2 or CO2 or a gas mixture simulating combustion of O2 into CO2. Some experiments are not yet completed.

At **0.5 % O2** (Fig. 2) O. surinamensis was most tolerant to N2 and burner gas at 15 °C. Differences between all other species, temperatures and kinds of gas mixture were not very pronounced with S. granarius being slightly more tolerant. Within 10 days all tested insects were dead. Clear differences could be found at **1 %** and **2 % O2** (Figs. 3 and 4). The vertical lines indicate the fiducial limits. At both temperatures longest exposure periods were required for control of S. granarius. At 20 °C exposure of S. granarius to N2 was rather ineffective in contrast to 3 % O2 (Fig. 5). High CO2 contents were relatively more toxic for

Figure 3: Lethal exposure time in days (LT 95) for Sitophilus granarius (SG), Ephestia elutella (EE), Plodia interpunctella (PI), Oryzaephilus surinamensis (OS) and Tribolium confusum (TC) at 15 °C and 20 °C; 1 % O2 and : * =99 % N2, + = 80 % N2 and 19 % CO2 (burner gas), 0 : 4 % N2 and 95 % CO2.



S. granarius. At 1 % O2 T. confusum was generally slightly more tolerant at both temperatures than the other tested insects. Efficacy was reduced at low temperature for all species and gas mixtures.

At **3 % O2**, CO2 was again most effective (Fig. 5), 10 days being sufficient to kill all tested insects and stages with any mixture.

With **4 % O2** (Fig. 6) differences in mortality between the mixtures were rather marked high CO2 dosages still being lethal in the shortest time. P. interpunctella data are not yet ready for presentation. With N2 as replacement gas - except for E. elutella - LT 95 was markedly longer for 4 % O2 in comparison with 3 % O2, S. granarius was still the most tolerant of all tested species. At 20 °C all mixtures were generally more toxic than at 15 ° C even though this

Figure 4: Lethal exposure time in days (LT 95) for Sitophilus granarius (SG), Ephestia elutella (EE), Plodia interpunctella (PI), Oryzaephilus surinamensis (OS) and Tribolium confusum (TC) at 15 °C and 20 °C; 2 % O2 and: * = 98 % N2, + = 80 % N2 and 18 % CO2 (burner gas), O = 8 % N2 and 90 % CO2.

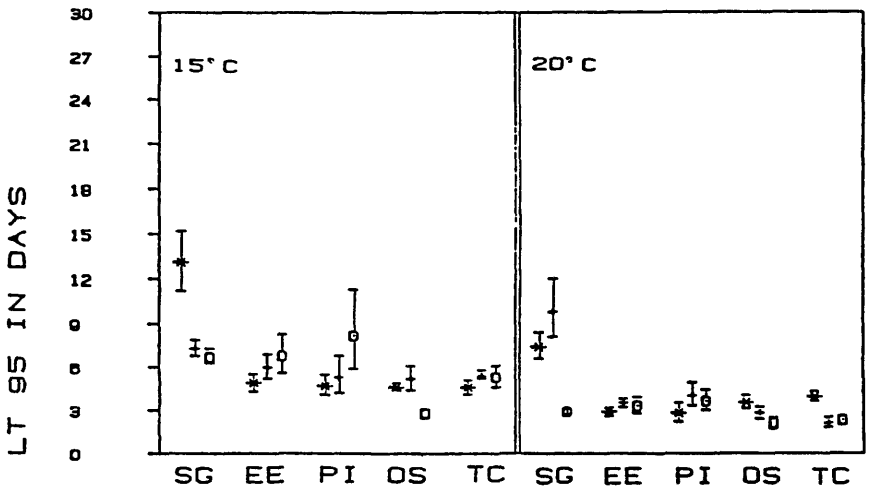


Figure 5: Lethal exposure time in days (LT 95) for Sitophilus granarius (SG), Ephestia elutella (EE), Plodia interpunctella (PI), Oryzaephilus surinamensis (OS) and Tribolium confusum (TC) at 15 °C and 20 °C; 3% O2 and: * = 97 % N2, + = 80 % N2 and 17 % CO2 (burner gas), O : 12 % N2 and 85 % CO2.

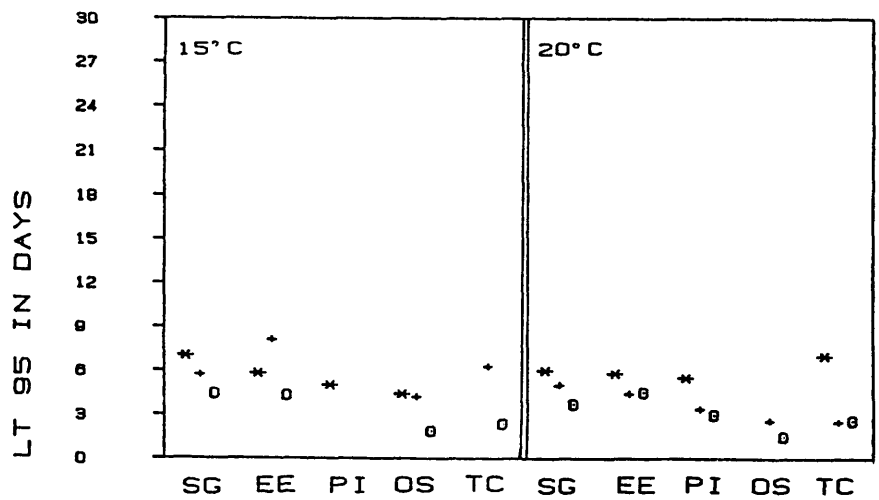


Figure 6: Lethal exposure time in days (LT 95) for Sitophilus granarius (SG), Ephestia elutella (EE), Oryzaephilus surinamensis (OS) and Tribolium confusum (TC) at 15 °C and 20 °C; 4 % O2 and: * : 96 % N2, + : 80 % N2 and 16 % CO2 (burner gas), 0 : 16 % N2 and 80 % CO2.

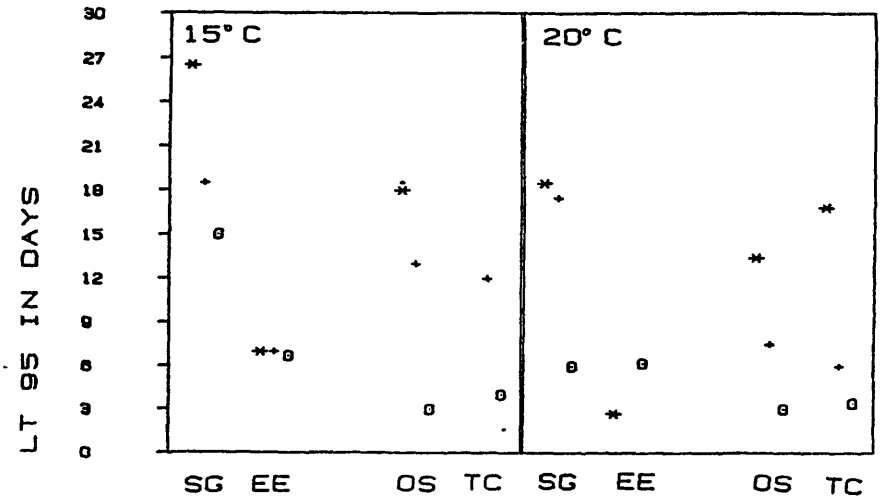
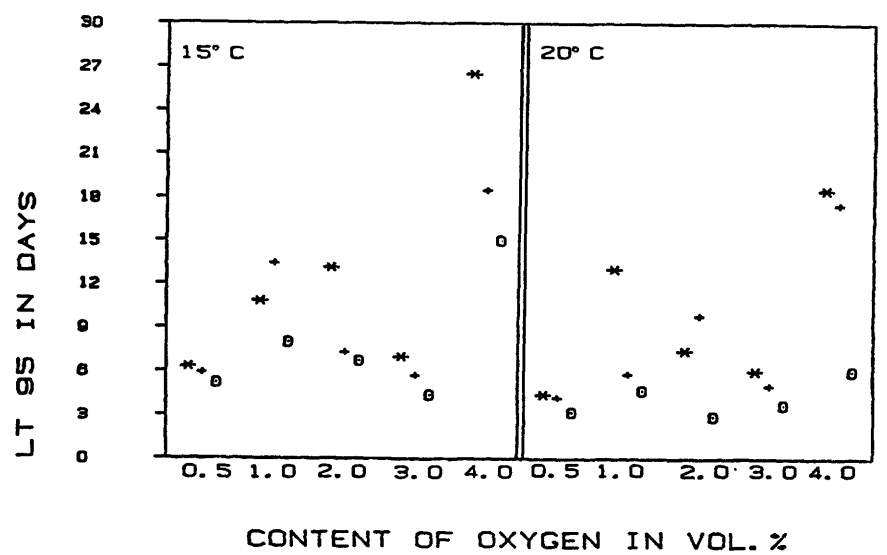


Figure 7: Lethal exposure time in days (LT 95) for Sitophilus granarius adults at 0.5, 1.0, 2.0, 3.0 and 4.0 % O2 at 15 °C and 20 °C replacement gas being: * = N2, + = 80 % N2; rest CO2 (burner gas), 0 = CO2.

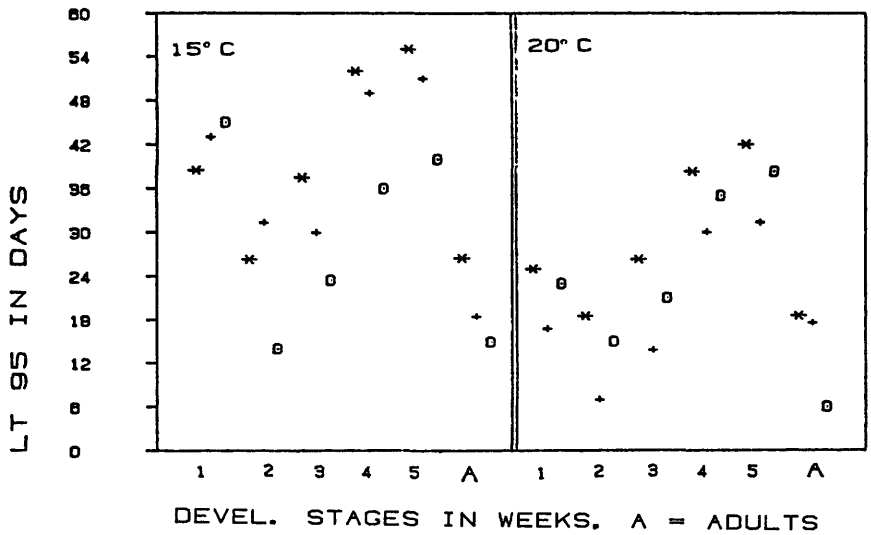


tendency was not very pronounced (S. granarius: burner gas; E. elutella, O. surinamensis and T. confusum: high CO2). With burner gas LT 95 was not achieved for O. surinamensis until 18 days.

Fig. 7 contains a combination of all results obtained for adult S. granarius. These show:

- very little differences between mortality data with gas mixtures containing 0.5 % O2;
- increase in tolerance at 1 % O2;
- slight decrease in tolerance at 2 and 3 % O2;
- pronounced increase in tolerance at 4 % O2;
- high CO2 content was most effective;
- temperature influence was not strong (except at 4 % O2 with the two mixtures containing CO2.

Figure 8: Lethal exposure time in days (LT 95) for 1 to 5 weeks old developing stages and two weeks old adult Sitophilus granarius at 4.0 % O2 at 15 °C and 20 °C replacement gas being: * = 96 % N2, + = 80 % N2/16 % CO2, θ = 16 % N2; 80 % CO2.



Five developing stages of S.granarius were investigated for susceptibility in atmospheres with low O2 contents. Fig. 8 contains mortality results with 4 % O2 including those for adult S. granarius. At **15 °C** 8 weeks were required to obtain 95 % mortality of all stages with mixtures of 4 % O2 in 96 % N2, or 4 .% O2 in 80 % N2/16 % CO2 respectively. Except for the one week old stage, CO2 as replacement gas was most toxic to all other stages. The first stage was relatively tolerant to high CO2 contents at this temperature. After 6 weeks

exposure at 20 °C all stages were dead, the order of tolerance being the same for all stages as at 15 °C. The developmental stages were more susceptible to 16 % CO2 than to 80 % CO2.

Discussion

Scientific aspects

Bailey and Banks (1980) have already stated that some insects are more tolerant to very low O2 content than to 2 or 3 % O2. This tendency is confirmed here with O. surinamensis at 15 °C and low CO2 content. The absence of O2 combined with low temperature may induce a very deep anesthesia which protects some insects from being killed. However, this did not happen to the other test insects.

At all O2 contents less than 4 % -with some exceptions like S. granarius- susceptibility to anoxia is rather similar for all insects independent of the mixture at both temperatures used - mostly high CO2 contents having the strongest effect. Essential is the lack of O2 which leads to increase in acidity by formation of lactic acid in the insect (Navarro and Friedlander, 1975), so that increased CO2 content in the atmosphere does not necessarily increase mortality.

With adult S. granarius it is interesting to note that O2 contents of 1 and 2 % are lethal in shorter periods than O2 contents of 0.5 and 3 %. Very high CO2 content reduces this effect. A strong increase in tolerance occurs at O2 contents greater than 3 %, especially at 15 °C. At higher temperatures the metabolism of the insects requires more O2 and thus the toxic effect of anoxia is more pronounced. On the other hand low temperature is a lethal factor itself which became obvious during the experiments with developmental stages of S. granarius. Sometimes offspring from treated samples was larger than from untreated controls at the same low temperature. These two effects may explain that sometimes differences in tolerance at the two temperatures are relatively small.

O2 consumption of insect eggs is low so that O2 deficiency may not harm this stage (Fig. 8) in the same short time as the larvae. The reduced need for O2 may also explain the high tolerance of the pupal stage. Fig. 8 shows a change in susceptibility of immature S. granarius to high and low CO2 content in the atmosphere between 15 °C and 20 °C. Presumably at 20 °C the anesthetic effect of high CO2 is similar to 15 °C. With 16 % CO2 this effect may be reduced at 20 °C. It seems that 4 % O2 is sufficient to allow some metabolism

which accelerates the onset of death (Bell, 1984).

Experiments with developmental stages of S. granarius are not yet completed. It can be expected from the available data that LT 95 will be shorter with lower O2 contents than 4 %. Egg stages and old larvae and (or) pupae are most tolerant - much more than the adults. This corresponds to data of Desmarchelier (1984) and Reichmuth (1986) who found these stages to be very tolerant to inert atmospheres and (or) phosphine. Adults are unsuitable for evaluating the tolerance of this species to CAs due to their rather high susceptibility.

Technical aspects

The possibility to control stored product insects at low temperatures with CAs including low O2 contents has been proven. At very low contents of O2 (less than 3 %) depending on species (except for S. granarius), temperature, and gas mixture, 4 to 10 days are required to achieve 95 % mortality. A high standard of gastightness is required to make warehouses, silos and covered bag stacks suitable for application of these mixtures. A constant flushing of gas -to overcome O2 ingress from the surroundings- should be installed at a pressure of some 10 Pascal. Otherwise the risk of development of resistant strains due to incomplete control may be expected.

Grain disinfestation with controlled atmospheres can also be the objective when time is not a limiting factor. Six to 8 weeks are necessary to control the most common insect pests in grain. Experiments are in progress to investigate pulsed treatments with aeration of about 3 weeks between two periods of some weeks depending on temperature, to force the tolerant stage to develop into susceptible adults and larvae before the second treatment is started. Investigation is required to verify how these basic data can be transferred into practice. It should be noted that generally temperatures of infested areas in grain are higher than 20 °C (hot spot).

At the moment very little granaries in Germany meet the necessary standard of gastightness. This could eventually be solved by using gastight laminates and foils inside the premises to install a gastight enclosure around the stored grain. Often enough grain is stored for years so that the measure would pay also for keeping insects away from the stored commodity.

Acknowledgements

I am very grateful to the DFG (German Research Society) for funding this research and to Mrs. B. Haile-Raub and Mrs. R.Schneider for technical assistance. Mr. C. Adler together with Mrs. I. Papke developed the method of rearing the Sitophilus granarius.

References

Bailey S.W. (1955) Airtight storage of grain : its effect on insect pests - I : Calandra granaria. Aust. J. agric. Res. 6, 33 - 51

Bailey S.W. and Banks H.J. (1980) A review of recent studies of the effects of controlled atmospheres on stored product pests. In J. Shejbal (ed.) Controlled atmosphere storage of grains pp. 101 - 108 Elsevier, Amsterdam

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.

Bell C.H. (1984) Effects of oxygen on the toxicity of carbon dioxide to storage insects. In Ripp, B.E. et al. (eds.) Controlled atmosphere and fumigation in grain storages pp. 67 - 74 Elsevier, Amsterdam

Desmarchelier J.M. (1984) Effect of carbon dioxide on the efficacy of phosphine against different stored product insects. Mitteilungen aus der Biologischen Bundesanstalt fur Land - und Forst - wirtschaft, Berlin - Dahlem Heft 220

Fleurat-Lessard F. (1986) Controlled atmosphere fumi - gations for insect control. GASGA seminar on fumigation technology in developing countries, pp. 164 - 172 / TDRI, Storage Dept./ London Road, Slough, Berks., SL3 7HL England

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

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

Jay E.G. (1980) Low temperatures : effects on control of Sitophilus oryzae (L.) with modified atmospheres. In J. Shejbal (ed.) Controlled atmosphere storage of grains pp. 65 - 71 Elsevier, Amsterdam

Marzke F.O., Press A.F. and Pearman G.C. (1970) Mor - tality of rice weevil, Indian meal moth and Trogoderma glabrum exposed to mixtures of atmospheric gases at various temperatures. J. econ. Ent. 63, 570 - 574

Navarro S. and Friedlander A. (1975) The effect of carbon dioxide anesthesia on the lactate and pyruvate levels in the hemolymph of Ephestia cautella (Wlk.) pupae. Comp. Biochem. Physiol. 50 B, 187 - 189

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

Oxley T.A. (1963) The effect of restricted air supply on some insects which infest grain. Ann. appl. Biol. 51, 313 - 324

Reichmuth Ch. (1986) The significance of changing concentrations in toxicity of phosphine. GASGA seminar on fumigation technology in developing countries, pp. 88 - 98 / TDRI, Storage Dept./ London Road, Slough, Berks., SL3 7HL England