

A comparison of the efficacy of CO₂-rich and N₂-rich atmospheres against the granary weevil *Sitophilus granarius* (L.) (Coleoptera: Curculionidae)

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Abstract

For temperatures below 15°C and above 22°C almost no literature data could be found on the efficacy of N₂ or CO₂ fumigations against the granary weevil. Laboratory experiments were therefore carried out at 5, 10, 25 and 30°C, 75% r.h., with the following gas mixtures (% by vol.): 98% N₂, 2% O₂; 32% N₂, 8% O₂, 60% CO₂ and 8% N₂, 2% O₂, 90% CO₂.

The results of these experiments, and literature data, were graphed to show the exposure times needed to achieve 95–100% mortality of different life stages. Compared was the influence of temperature on the efficacy of atmospheres consisting of 97–100% N₂ (rest O₂) or 60–100% CO₂ (rest N₂, O₂), respectively.

The resulting functions of mortality over temperature, seem to indicate that atmospheres with high contents of CO₂, while causing more rapid death below 20°C, do not produce shorter LT values than hypoxic atmospheres at 25°C or above. At high grain temperatures, factors other than minimum fumigation time may therefore be more important determinants of the choice of an atmosphere to be used in a treatment.

Introduction

Studies on the efficacy of controlled atmospheres (CA) on stored-product insects have been carried out intensively since around 1970. Due to the many different factors affecting insect mortality and the various ways to present mortality values (see Table 1) it is very difficult, however, to draw general information from this vast mass of accumulated data. Literature findings were reviewed by Bailey and Banks (1980) and Banks et al. (1990). A first graphical approach to give an overview on dosage schedules to control the main stored-product insect species was the work published by Annis (1987). Banks and Annis (1990) studied the comparative advantages of high CO₂ and low O₂ types of controlled atmospheres. The present paper aims to give a more detailed picture on the effects of temperature on nitrogen or carbon dioxide fumigations. Because stored-product insects vary in their susceptibility to CA treatments, this study concentrates on only one species: the granary weevil *Sitophilus granarius* (L.) (Coleoptera: Curculionidae).

The granary weevil is a very common grain pest in moderate climates and proved to be among the species least sensitive to CA fumigations. This fact has made *S. granarius* the main test species when studies on the efficacy of controlled atmospheres have to be carried out for registration purposes in Germany.

In general, it may be suggested that lethal exposure times calculated from experiments with the granary weevil should be safe for fumigations aimed to control other, less tolerant stored-product pest species. However, this cannot be guaranteed, since too often insect biology has held surprises for generalising entomologists. As Banks and Annis (1990) pointed out, *Trogoderma granarium* Everts may be another species quite tolerant to controlled atmospheres.

Material and Methods

Literature review

Literature data (see Tables 2 and 3 for sources) fulfilling the following requirements were tabulated:

- 1 species: *Sitophilus granarius*,
- 2 fumigation atmosphere: 97–100 % N₂ or 60–100% CO₂/(balance: O₂, CO₂ or N₂),
3. achieved mortality: 95–100 %.

From these data two graphs were drawn showing the efficacy of atmospheres containing high contents of either N₂ or CO₂ on different life stages of the granary weevil at various temperatures.

Laboratory experiments

Because almost no data could be found at temperatures below 15°C or above 22°C, fumigation experiments were carried out in the laboratory at 5 ± 1°C, 10 ± 1°C, 25 ± 1°C and 30 ± 1°C, 75 ± 5 % r.h.

The following gas mixtures were produced manometrically from pure components (Adler and Reichmuth 1988) (in % by volume):

- 98% N₂, 2% O₂;
- 32% N₂, 8% O₂, 60% CO₂;
- 8% N₂, 2% O₂, 90% CO₂.

Insect cultures

To study the effects of the gas mixtures on developmental stages of defined age, insects were cultured as follows: At 25°C, 75% r.h., adult granary weevils were placed weekly on uninfested wheat for an oviposition period of 3 days, leaving age groups of developmental stages with a deviation of ±1.5 days. At 25°C, this culture technique produces 5 preadult stages. Details of this procedure are given in Adler (1991). From each preadult group, 70 kernels of infested wheat were placed into a wire mesh cage. Fifty adult weevils, 1–3 weeks old, and some 5 g of wheat, were filled into a slightly larger cage completing the set of developmental stages.

Before fumigation experiments at 5 and 10°C, the test insects were adapted to cold by a storage at 15°C for 24 hours before exposing them to the desired temperature. The lethal effects of cold alone were studied by keeping untreated samples at the experimental temperature and at 25°C.

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Fumigation

A set of developmental stages and adult granary weevils was placed into a 2L Dressel flask and fumigated with one of the three gas mixtures mentioned above. Before use, each gas mixture was heated or cooled to the desired temperature and purged through a saturated sodium chloride solution to adjust to a close-to-optimal moisture content of 75–76% r.h. (see Winston and Bates 1960). Each Dressel flask was purged with

the respective gas mixture until the atmosphere in the vessel was completely replaced by the desired atmosphere. The flask was then closed and stored in a climate chamber for exposure periods between 1 and 12 weeks in experiments at 5 or 10°C and between 3 and 13 days at 25 or 30°C. After this time, grain and insects were transferred into petri dishes. These were stored at 25°C, 75% r.h., and emergence and mortality were recorded for up to 12 weeks.

Table 1. Factors impairing the comparability of data on the efficacy of CAs

Insect biology	Variations in the susceptibility of different species, variations in relative susceptibility on the subspecies level, medium age of stages, age deviations due to variations in developmental speed, general health conditions, behavioural aspects
Experimental set up	Gas composition, moisture conditions, gas flow rates, fumigation technique (e.g. continuous, pulsed, one purge), time span for temperature adaptation, maintenance of atmospherical composition during insect exposure, effects of culture medium or microorganisms on insects or atmosphere, number of individuals and effects of respiratory processes on the CA and its moisture content, reliability of data (number of individuals and number of replicates)
Presentation of data	Exposure time leading to complete kill or 100 % reduction in emergence, LT ₉₉ , LT ₉₅ , LT ₅₀ or other LT-values

Table 2. Literature data on lethal exposure times of *S. granarius* caused by 97-100% N₂^a

Author/year	Temp. °C	r.h. %	N ₂ %	O ₂ %	CO ₂ %	Mort. %	Eggs	Lethal exposure time (days)				
								L1/L2	L3/L4	L4/P	adults	
Reichmuth 1987/1990	15	75	99.5	0.5		95					6.3	
			99	1			30	40	40	45	10.8	
			98	2			30	40	60	52	13.1	
			97	3			30	31	65	53	6.9	
Adler & Reichmuth 1988	15	75	98	2		95	(>42)	35	(>42)	(>42)	21	
Lindgren & Vincent 1970	16	65	100			95					12.5	
Krishnamurthy et al. 1986	20	70	99.5	0.5		100					10	
Reichmuth 1987/1990	20	75	99.5	0.5		95					4.4	
			99	1			20	13	30	30	13.0	
			98	2			12	13	31	25	7.4	
			97	3			17	12	35	30	5.3	
Adler 1991/1993	20	75	99	1		99						
			strain F-L					14.9	7.6	19.1	20.4	9.0
			strain USA-L					14.7	5.1	18.6	22.2	6.6
			strain CN-L1					11.7	8.5	22.5	28.8	6.3
			strain CN-L2					12.3	8.5	21.5	24.1	7.6
			strain AUS-L					17.2	6.0	17.2	21.8	4.2
			strain AUS-f					14.5	7.8	22.5	28.4	4.3
			strain GB-L1					13.0	6.6	14.7	20.5	4.3
			strain GB-L2					14.8	10.6	18.6	19.5	8.0
			strain D-L					15.6	9.9	23.8	22.5	7.3
			strain D-f					13.0	8.2	28.4	26.6	5.5
Adler & Reichmuth 1988	20	75	98	2		95	28	21	(>42)	(>42)	(<14)	
Shejbal et al. 1973	22	70	99.9	0.1		100					2	
			99.7	0.3							3	
			99.5	0.5							4	
			99.2	0.8							9	
			99.0	1.0							10	
Lindgren & Vincent 1970	27	65	100			95	8.5	(4.5) ^b		8	3.5	
Bailey & Banks 1980	30	70	98.7	1.3		100	(<14)	(<14)	(<14)	(<14)	(<14)	

^a Literature data mentioned only if 95–100% mortality achieved.

^b Larval age not discriminated.

Results

Literature data are given in Tables 2 and 3. The times needed to achieve complete kill of a certain developmental stage with a given gas mixture in the laboratory experiments are presented in Table 4. These data were added into the compilation of literature values which are displayed graphically in Figures 1 and 2.

Because developmental speed varies strongly in the granary weevil and because larval age is seldom accurately defined in the literature, only young, more sensitive 1st and 2nd instar larvae and old, more tolerant 3rd and 4th instar larvae were discriminated. For better readability, single values were omitted. Mean values and standard deviations of all data at a certain temperature are listed in Table 5.

Discussion

Due to great differences in developmental speed in *Sitophilus granarius*, 4th instar larvae and even some hatching weevils may have influenced the results obtained by fumigating pupal stages in the experimental results and cited literature data. Nevertheless, this stage proved to be most tolerant to both nitrogen and carbon dioxide treatments. For two reasons this fact is not surprising:

- pupae are known to have a very low oxygen uptake (Bailey 1969); and

- the larval stages have accumulated reserves of carbohydrates that the pupal stage can use as a substrate for anoxic energy production through glycolysis (Adler 1993).

Only in the experiments at 5°C did eggs survive fumigations with 60% CO₂ and 90% CO₂ longer than did pupae. Because no values could be derived from literature for this temperature, further studies are needed to verify these findings.

As presented in Table 4, 60% CO₂ was not less effective in the laboratory experiments than was 90% CO₂. This is supported by Fleurat-Lessard and Le Torc'h (1991) who found that atmospheres of only 50% CO₂ and 4% or 20% oxygen (rest N₂) produced mortality rates in *S. granarius* larvae similar to those produced by an atmosphere of 100% CO₂.

Figure 1 shows that in N₂-fumigations the longest LT-values were found around 10°C. Below this temperature the cold may have had an additional effect causing mortality of pupal and larval stages in shorter periods. However, this effect was not detected in CO₂-fumigated developmental stages where lethal times increased with decreasing temperature (Fig. 2). On the contrary, old larvae and eggs in particular seem to be protected from the toxic effects of CO₂ at 5°C better than at 10°C.

Comparing untreated samples exposed to 5°C with those kept at 25°C, it was noticed that the cold alone caused complete kill of eggs and young larvae within 4 to 5 weeks and high mortality rates among late larvae and pupae (while the mortality of adults remained low). This leads to the conclusion that low temperatures not only slow down the effect of controlled atmospheres but also that the effects of cold and CA treatment are antagonistic at temperatures around 5°C.

Table 3. Literature data on the lethal exposure times of *S. granarius* caused by 60-100% CO₂^a.

Author/ year	Temp. °C	r.h. %	N ₂ %	O ₂ %	CO ₂ %	Mort. %	Eggs	Lethal exposure time (days)				
								L1/L2	L3/L4	L4/P	adults	
Reichmuth 1987/1990	15	75	16	4	80	95	45	15	39	40	6.2	
			12	3	85	21	11	24	34	3.9		
			8	2	90	13	12	15	27	6.7		
			4	1	95	30	9	22	35	8.0		
			2	0.5	97.5	-	-	-	-	5.2		
Lindgren & Vincent 1970	16	65			100	95					11.5	
Reichmuth 1987/1990	20	75	16	4	80	95	(23?)	(18?)	(40?)	(38?)	2.2	
			12	3	85	10	2	16	26	2.3		
			8	2	90	10	5	7	19	2.9		
			4	1	95	16	2	20	24	4.7		
			2	0.5	97.5	-	-	-	-	3.2		
Adler 1991/1993	20	75	4	1	95	99						
			strain F-L					14.3	2.6	15.5	15.5	4.4
			strain USA-L					13.6	3.0	14.0	20.2	4.4
			strain CN-L1					12.1	3.8	17.9	22.5	5.5
			strain CN-L2					11.9	4.4	15.6	19.5	5.8
			strain AUS-L					14.7	4.7	13.0	19.3	4.0
			strain AUS-f					15.4	3.2	15.0	23.2	3.8
			strain GB-L1					15.0	2.7	9.2	11.3	3.4
			strain GB-L2					14.1	4.0	15.0	18.3	8.1
			strain D-L					15.7	2.5	19.9	18.5	4.5
			strain D-f					13.2	3.3	18.6	19.7	5.5
Fleurat-Lessard & Le Torc'h 1991	25	75			100	95			9			
Lindgren & Vincent 1970	27	65			100	95	8.5	(4.3) ^b		8	3.5	

^aliterature data mentioned only if 95–100% mortality achieved

^bLarval age not discriminated

Generally, at lower temperatures CO₂-atmospheres are significantly faster than other CAs in lethal action. Clearly, this is due to the toxic properties of CO₂ being less affected by low temperatures. CO₂ has a high solubility in water and the solubility of gases increases with decreasing temperature. Therefore, at temperatures decreasing from 15°C to 5°C the higher passive uptake of CO₂ may to some extent compensate for the temperature-triggered slow down in insect metabolism. In eggs this effect may not be detectable because this stage has a smaller ratio of body and respiratory surface to body volume than the later stages.

In contrast to the findings on *S. oryzae* adults (Banks and Annis 1990), the type of controlled atmosphere seems to cause

only minor differences in the lethal exposure period of adult *S. granarius*.

At 25°C and above, the toxic effects of CO₂ seem to cause no faster mortality of granary weevil stages than the reduction of O₂ by a treatment with N₂.

As Banks and Annis (1990) demonstrated, many factors influence the question whether atmospheres of high CO₂ or low O₂ are advantageous under certain practical conditions. For example, one of the great advantages of CO₂ is that it may be diluted by air down to 40% while still being efficient against insects. If, however, N₂- and CO₂-treatments of *S. granarius* are compared solely from the point of view of achieving disinfestation in the shortest possible time, the

Table 4. Lethal exposure times, results of the experimental study

	Temp. °C	r.h. %	N ₂ %	O ₂ %	CO ₂ %	Mort. %	Lethal exposure time (days)				
							eggs	L1/L2	L3/L4	L4/P	adults
High N ₂ - contents	5	75	98	2	-	100	42	28	63	70	21
	10						42	56	70	77	14
	25						5	5	13	(>13)	7
	30						3	3	10	10	7
High CO ₂ - contents	5	75	32	8	60	100	56	21	42	49	18
			8	2	90		56	21	38.5	49	18
	10		32	8	60		31.5	(<21)	24.5	45.5	14
			8	2	90		35	(<21)	28	45.5	14
	25		32	8	60		5	5	10	13	3
			8	2	90		7	7	5	13	3
	30		32	8	60		3	3	10	7	3
			8	2	90		3	3	7	7	3

Table 5. Lethal exposure times of *S. granarius* caused by 97–100 % N₂-(rest O₂) and 60–100 % CO₂-(rest N₂/O₂) atmospheres, literature and experimental data^a

	Temp. °C	Exposure period (days) causing 95–100 % mortality									
		eggs		L1/L2		L3/L4		L4/P		adults	
		av.	s.d.	av.	s.d.	av.	s.d.	av.	s.d.	av.	s.d.
High N ₂ - contents	5	42		49	±7	63	±7	70		21	
	10	42		56	±7	70	±14	77	±7	14	
	15	30		36.5	±3.8	55	±10.8	50	±3.6	9.3	±2.8
	16									12.5	
	20	15.6	±4.1	9.8	±3.9	23.3	±5.9	24.6	±3.7	7.1	±2.4
	22									5.6	
	25	5		5		13		(>13)		7	
	27	8.5		4.5 ^b				8		3.5	
High CO ₂ -contents	30	3		3		10		10		7	
	5	56		21		40.3	±5.8	49		18	
	10	33	±3.0	(<21)		26.3	±3	45.5	±3.5	14	
	15	27.3	±20.4	11.8	±2.5	25	±8.8	34	±4.6	6	±1.4
	16									11.5	
	20	14.2	±3.1	3.3	±1	15.1	±3.7	19.8	±3.6	4.7	±1.4
	25	6	±1	6	±1	8.3	±1.3	13		4.8	±2.5
	27	8.5		4.3 ^a				8		3.5	
30	3		3		8.5	±1.5	7		3		

^aaverage values (av.) and standard deviations (s.d.), s.d.-values only in case of contradictory data; - see figures 1 and 2.

^bLarval age not discriminated

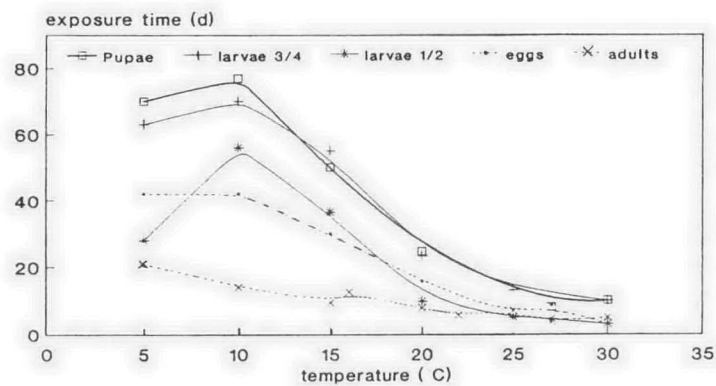


Fig. 1. Lethal exposure times of *S. granarius* produced by 97–100% N₂ (rest O₂)

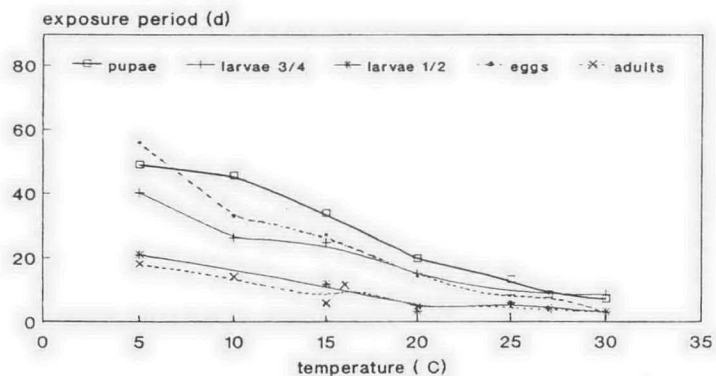


Fig. 2. Lethal exposure times of *S. granarius* produced by 60–100% CO₂ (rest N₂/O₂)

present study seems to support the assertion that CO₂-fumigations will give a more rapid disinfestation at temperatures below 20°C but not at 25°C or above. Therefore, at high grain temperatures, factors other than minimum fumigation time may determine the choice of an atmosphere to be used in a treatment.

Acknowledgments

The author wishes to thank Dr Christoph Reichmuth for his advice and help and Mrs Sylvia Krause for her technical assistance.

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