

IMPERFECTIONS IN OUR CURRENT KNOWLEDGE OF INSECT BIOLOGY AS RELATED
TO THEIR RESPONSE TO CONTROLLED ATMOSPHERES

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ABSTRACT

Several recent review papers have commented on the fact that there is little relevance between laboratory studies and the field application of controlled atmospheres (CA) to control stored-product insects. Most of this research used either larvae or adults of one or two species of insects, usually *Tribolium* spp., to demonstrate that there is a difference in mortality when the insects are exposed to different CA compositions. We have developed generalities from this research such as "pupae are harder to control than are the other three life stages of these stored-product insects" and that "a 60% carbon dioxide (CO₂) concentration is more effective against most of these species than is a 98 to 100% CO₂ concentration". Are these generalizations true for all of the major pest species? This paper attempts to develop some order in this area by reviewing recent and chiefly unpublished research by the author. An examination of the responses within and between species and life stages and their relationship to temperature, relative humidity and CA composition of these insects is presented. Shorter exposure times are generally needed to control stored-product pests in field situations than would be expected from laboratory results. Attempts are made to explain the causes of this phenomenon, how it relates to the overall information transfer from the laboratory to actual CA field applications and how the available laboratory data can logically be applied in the field. Future research needs are described on the basis of the information contained in this paper.

INTRODUCTION

Review papers on the effects of controlled atmospheres on stored product pests were published by Bailey and Banks (1975 and 1980). In these papers the effective atmospheric gas compositions to control stored-product insects, regardless of the practical application in a field situation, were mentioned. Some authors have investigated rare atmospheres including helium (Aliniaze, 1972) but the high cost of this gas would not make its use practical. Other authors have investigated either very low relative humidities (r.h.s) such as 7 to 30%, or very high r.h.s such as 72 to 95% (Jay, et al., 1971; Navarro, 1978). These studies are justifiable from a scientific standpoint and contribute to knowledge on the mode of action of controlled atmospheres (CA).

However, it is not a common practice to store grain at extreme humidity levels, so this information is again of little value to a practical storage insect control scheme.

Bailey and Banks (1980) concluded from the available literature that there are still gaps in our knowledge of the biological action of CA, that we need additional work on dose-mortality responses to various gas mixtures with particular emphasis on tolerant stages and species to provide a basis for an exposure schedule dependent on the temperature of the bulk to be treated; and that we need information on the interaction of high carbon dioxide (CO₂) atmospheres and temperatures.

What is wrong with this overall research effort? It can be seen from the above that, after years of study on the effects of CA on stored-product insects, little data has emerged which can be translated to field studies. Several economically important species such as Sitotroga and Cryptolestes have been overlooked, and much of the research has been conducted with gas mixtures which would be economically impossible to produce in field situations.

When CA is used to control stored-product insects many factors enter into the success of the treatment. Some of these factors are shown in Figure 1 and these factors will form the basis of this paper.

The purpose of this paper is to contribute additional information to our current knowledge of storage insects in response to CA. The information reported is generally based on recent research conducted by the author with Tribolium castaneum (Herbst), Oryzaephilus surinamensis (L.), Rhizopertha dominica (F.), Sitophilus oryzae (L.), Sitophilus zeamais Motschulsky, Trogoderma glabrum (Herbst), Trogoderma variable (Ballion), and Esphestia cautella (Walker). The information presented here deals mainly with the effects of CO₂ and to some extent nitrogen (N₂) atmospheres on these insects and does not consider the effects of generated atmospheres on these pest species.

MATERIALS AND METHODS

When actually purging out a storage bin or structure to obtain various CO₂ concentrations, the oxygen (O₂) concentrations usually present are shown in Table 1.

Table 1 - Approximate O_2 concentrations found when purging with CO_2 or when mixing a given percentage of CO_2 with air. 1/

% CO_2	% O_2
45	12
60	8
75	5
90	2
99	0.3

1/ Balance of mixture will be nitrogen (N_2), argon and rare gases.

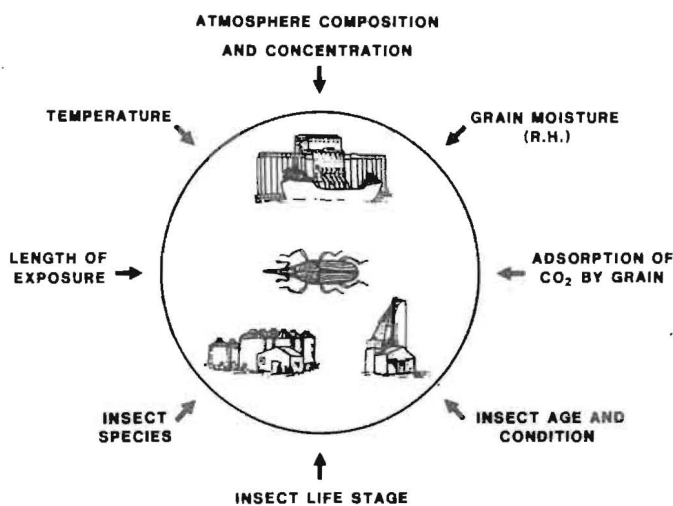


Figure 1 -- Some factors affecting the treatment with controlled atmospheres to control stored-product insects.

This paper will only be concerned with these concentrations of CO_2 at > 50% r.h. unless otherwise noted. The exposure chambers used were similar to those described by Jay and Cuff (1981). They consisted of 2.8-l glass jars

that were partly submerged in laboratory water baths which could be heated or cooled to provide different temperatures for some studies. The jars were closed with metal screw-top lids fitted with 0.6 cm o.d. copper tubing that were used as gas inlet and gas exit tubes. The lids were also fitted with a neoprene stopper so a humidity sensor could be inserted. During the exposure the cages containing the insects were suspended in the chamber from a 5-cm length of steel wire hung from the underside of the neoprene stopper holding the thermometer.

The gas mixtures were released from the cylinders through two-stage regulators and flowed through a micrometering valve and flowmeter into gas washing bottles that contained the glycerol-water mixture to adjust the r.h. of the gas. Flow rates of 200 cc/min were used for the first hour and 30 cc/min for the balance of the exposure periods. The r.h. was monitored with an electric hygrometer (model 15-2001 humidity indicator and narrow range humidity sensors, HygroDynamics, Inc.), and the temperature was recorded daily by using the dial thermometers. A Fisher-Hamilton model 29 gas partitioner equipped with dual columns was used to measure the areas under the peaks.

The effectiveness of the treatment for S. oryzae and R. dominica immature stages was determined by dividing the total number of adult insects that emerged after treatment by the total number that emerged in the control and converting this to percent reduction in emergence (RIE) (Jay, 1980a).

RESULTS AND DISCUSSION

Low temperatures

Temperature is an important factor in the use of CA for insect control, as it is in conventional fumigation. However, little research has been conducted at temperatures below 16°C on the effects of CA. Table 2 shows the effects of 2° and 16°C on mixed age (1,2,3,4 and 5 week old) immature life stages of S. oryzae and R. dominica and shows that at 2°C the cold air alone was more effective than the two CA's tested after a 1-wk exposure against S. Oryzae but not against R. dominica where the 60% CO₂ atmosphere was more effective. A 2-wk exposure at this temperature showed that the cold air and 99% N₂ was more effective than 60% CO₂ against S. oryzae but 100% RIE of R. dominica could be achieved only with the 60% CO₂ atmosphere. At 16°C the effects of the air alone on both species was diminished, whereas R. dominica appears to be the more resistant than S. oryzae at this temperature. At this temperature 60% CO₂ was more effective against both species than was 99% N₂.

High temperatures

Table 3 shows the effects of elevated temperatures on mortality of larvae of T. glabrum and T. variabile when exposed to 61% and 99% CO₂ at 33° and 38°C. Although at 33°C exposure to 99% CO₂ for 48 h produced high mortalities in both species 100% mortality was obtained only for T. glabrum. However, at 38°C exposure to 99% CO₂ for 24 h produced 100% mortality in both species, while 61% CO₂ was only slightly more effective at this temperature than it was at 33°C.

Table 2 - Percent reduction in emergence (RIE) of adults for immature S. oryzae (SO) and R. dominica (RD) exposed for 1 or 2 wk to two low temperatures at 55-60% r.h.

Atmosphere	SO*		RD	
	1 wk	2 wk	1 wk	2 wk
<u>2°C</u>				
Air	99	100	73	99
60% CO ₂	96	99	89	100
99% N ₂	95	100	63	96
<u>16°C</u>				
Air	67	89	25	54
60% CO ₂	81	99	91	100
99% N ₂	42	86	60	91

* From Jay, 1980a.

Table 3 Mortality (%) of T. glabrum (TG) and T. variabile (TV) when exposed to two concentrations of CO₂ at two temperatures and 50% r.h.

Species	Exposure time (h)	61% CO ₂	99% CO ₂
<u>33°C</u>			
TG	24	22	90
TV		15	73
TG	48	25	100
TV		15	97
<u>38°C</u>			
TG	24	21	100
TV		16	100
TG	48	32	100
TV		31	100

Table 4 presents results of similar exposures of the pupae of the two Trogoderma species. Again it can be seen that at 33°C 99% CO₂ was more effective than 63% CO₂ and produced nearly 100% mortality in both species after a 48-h exposure. An increase in temperature to 38°C reduced the exposure time for 100% mortality to 24 h for both species when the insects were exposed to 99% CO₂. Increasing the temperature from 33 to 38°C had little effect on the effectiveness of the atmosphere containing 63% CO₂.

Table 4 - Mortality (%) of pupae of T. glabrum (TG) and T. variabile (TV) when exposed to two concentrations of CO₂ at two temperatures and 50% r.h.

Species	Exposure time (h)	63% CO ₂	99% CO ₂
		<u>33°C</u>	
TG	24	30	92
TV		22	87
TG	48	96	100
TV		64	100
		<u>38°C</u>	
TG	16	13	100
TV		9	93
TG	24	44	100
TV		31	100

Effects of temperature on three life stages of the same species

Table 5 presents results of exposures of larvae, pupae and adults of O. surinamensis to 77% CO₂ at four temperatures and exposure periods of up to 10 days. One-hundred % mortality of larvae of this species was obtained after an exposure of 3 days at 16°C, after 2 days at 21° and 27°C, and after 1 day at 32°C. Adults of this species displayed similar mortalities when exposed to this CO₂ concentration. However, it took 10 days to obtain 100% mortality of pupae of O. surinamensis at 16° and 21°C but only 3 days at 27° and 32°C.

Table 5 - Time in days required to obtain 100% mortality of O. surinamensis when exposed to 77% CO₂ at different temperatures and 50% r.h.

Temperature °C	Larvae	Pupae	Adults
16	3	10	3
21	2	10	3
27	2	3	2
32	1	3	1

Species and life stage of stored-product insects

When the results from Tables 3 and 4 are compared it can be seen that pupae of T. glabrum and T. variabile are more susceptible to the effects of 61% (or 63%) and 99% CO₂ than the larvae, except for 16 h exposure at 38°C whereas Table 5 shows that pupae of O. surinamensis are much more resistant to a CO₂ treatment than are larvae and adults. These differences are found between closely related species (Trogoderma spp) and within the different life stages of the same species (O. surinamensis). However, a few comparisons are available from the existing literature on comparative studies between the different life stages of 2 species that were conducted under identical conditions.

A study directly comparing the effects of two CA's on the 4 life stages of T. castaneum and E. cautella was conducted in steel towers containing shelled peanuts (groundnuts). These tests were conducted at ca 27°C and the rh in the bulk was ca 66%. Table 6 presents results of these studies. Eggs of both species were controlled (100% mortality) in 2 days in an atmosphere containing 63% CO₂ and in 3 days in a 99% N₂ atmosphere. This table shows that it took more than 6 days to attain complete mortality of T. castaneum larvae in the CO₂ atmosphere while this level of mortality was obtained in only 2 days when larvae of this species was exposed to the N₂ atmosphere. Larvae of E. cautella exhibited a reverse reaction to these two atmospheres and were controlled in 5 days in the CO₂ atmosphere and in more than 6 days in the N₂ atmosphere. Pupae of both species were controlled more rapidly in the N₂ atmosphere than in the CO₂ atmosphere while E. cautella pupae was more sensitive than T. castaneum pupae.

Table 6 - Time in days required to obtain 100% mortality of all life stages of T. castaneum (TCA) and E. cautella (EC) when exposed to two atmospheres at 27°C and 66% r.h.

Life Stage	Species	63% CO ₂	99% N ₂
		Days	Days
Eggs	TCA	2	3
	EC	2	3
Larvae	TCA	greater than 6	2
	EC	5	greater than 6
Pupae	TCA	5	4
	EC	3	2
Adults	TCA	4	3
	EC	2	2

Adults of T. castaneum were completely controlled in 4 days in the CO₂ atmosphere and in 3 days in the N₂ atmosphere while adults of E. cautella were controlled in 2 days in both atmospheres.

Relative humidity

The response of 3 life stages of T. castaneum to 66% r.h. (from the data on peanuts reported above) and to 50% r.h. (Jay and Cuff 1981) are compared in Table 7. These r.h.'s are in the range of conventional grain storage practice. Table 7 shows that after 48 h exposure mortality of larvae and pupae is much higher at the lower r.h. while there is no significant difference between adult mortalities recorded at two r.h.'s. After 72 h, mortality of larvae and pupae remained markedly higher at 50% than at 66% r.h. but mortality of adults at 50% are close to those recorded at 66% r.h.

Table 7 - Mortality (%) of 3 life stages of T. castaneum when exposed to ca 60% CO₂ at 50% and 66% r.h. for 48 and 72 h.

Life Stage	48 h		72 h	
	50%*	66%	50%	66%
Larvae	48	20	80	53
Pupae	89	46	99	81
Adult	96	94	99	98

* From Jay and Cuff (1981).

Effect of CA composition

Jay (1971) stated that a 60% CO₂ concentration at or above 27°C was adequate for controlling most stored product insects in a 4-day exposure period. This statement was based on unpublished laboratory studies described below. However, from data already presented (ie Tables 3 and 4) it can be seen that some species and life stages respond much faster to higher CO₂ concentrations than they do to those around 60%.

Table 8 shows the time required to obtain 100% mortality of larvae, pupae, and adults of O. surinamensis exposed to four CO₂ concentrations at four temperatures. This table shows that there is a dramatic difference in the time required to attain 100% control of these life stages at 16° and 21°C when the CO₂ concentration is raised from 91 to 98%. At 91% CO₂ at these two temperatures, it takes 10 days to obtain 100% mortality; at 98% CO₂ it only takes 3 days to attain this level of control. This difference is not so

pronounced at 27° and 32°C; however, time required to obtain 100% mortality drops from 2 days to 0.7 days at 32°C when the CO₂ concentration is increased from 91 to 98%.

Table 8 - Time in days to obtain 100% mortality of larvae, pupae and adults of O. surinamensis when exposed to four CO₂ concentrations at four temperatures and 50% r.h.

% CO ₂	Temperature - °C			
	16	21	27	32
62	10	10	4	4
77	10	10	3	3
91	10	10	3	2
98	3	3	2	0.7

Table 9 presents data on the effects of four high CO₂ concentration atmospheres on T. glabrum and T. variabile larvae and pupae at 36°C. In most cases there is a gradual increase in mortality as the CO₂ concentration is increased from 61 to 89%. However, there is again a large increase in mortality when the CO₂ concentration is increased from 89 to 99%.

Table 9 - Mortality (%) T. glabrum and T. variabile larvae and pupae when exposed for 24 h to four CO₂ concentrations at 36°C and 50% r.h.

% CO ₂	% Mortality			
	<u>T. glabrum</u>		<u>T. variabile</u>	
	Larvae	Pupae	Larvae	Pupae
61*	21	44	16	31
76	37	49	23	45
89	42	61	20	67
99	100	100	100	100

* Pupae were exposed to an atmosphere containing 63% CO₂.

Jay and Cuff (1981) also presented data on the effects of 58 and 97% CO₂ atmospheres on three life stages of T. castaneum. This data is summarized in Table 10 and shows that there was less marked difference between these two atmospheres on larvae and pupae when compared to the response of the adults.

Table 10 - Mortality of three life stages of T. castaneum when exposed to two CO₂ atmospheres at 27°C and 50% r.h.

Life Stage	Exposure time (hr)	% Mortality	
		58% CO ₂	97% CO ₂
Larvae	72	80	99
Pupae	72	99	100
Adults	24	28	100

* From Jay and Cuff (1981).

The data from Tables 8, 9, and 10 are all based on the effect of different CO₂ concentrations on species which feed externally while Table 11 presents data on R. dominica and S. zeamais, which are internal feeders. These insects were exposed at 27°C and 43% r.h. as 1, 2, 3, 4 or 5 week-old immatures for 1, 2, 3 or 4 days to the CO₂ concentrations indicated in Table 11. The data in this table are from a statistical analysis of the individual degree of freedom of counts of adult emergence (as compared to controls exposed in air) resulting from these tests.

Table 11 - Mean number of adults that emerged from immatures exposed for 1, 2, 3 or 4 days to five atmospheres at 27°C and 43% r.h.

Atmosphere %	<u>R. dominica</u>	<u>S. zeamais</u>
Air	70.7	22.6
39 CO ₂	30.4	14.9
48 CO ₂	17.1	9.4
99 CO ₂	14.5	8.9
60 CO ₂	9.7	5.8

The data in Table 11 shows that as the CO₂ concentration is increased from 39% to 48% the number of emerging adults decreases. However, when the number of adults emerging from 99% CO₂ is compared to those emerging from those exposed to the 60% CO₂ the number emerging in the later atmosphere are less than those emerging from the former atmosphere.

These data on R. dominica and S. zeamais were used as a background for the 60% CO₂ control regime recommended by Jay (1971). This regime is inadequate for species such as T. glabrum and T. variabile which cannot be successfully controlled with 60% CO₂. Also, O. surinamensis and some life stages of T. castaneum are controlled more rapidly by CO₂ concentrations approaching 100% than they are at CO₂ concentrations of ca 60%.

Translating laboratory studies to field use

Generally, when a field test using CO₂ is conducted on infested grain higher mortality is obtained in the natural population than would be expected from laboratory studies. This can be seen in the field test reported by Jay and Pearman (1973) where a 4-day treatment at a CO₂ concentration of ca 60% of 958 kl of maize resulted in 99.9% control of a natural population of Sitophilus spp (probably most were S. zeamais). This infested maize had a higher m.c. (11 to 16% m.c. or c.a. 55 to 80% r.h.) than did the infested maize used in the laboratory tests with S. zeamais (Table 11) and a lower mean temperature of 20.7° vs 27°C for that used in the laboratory tests. This 99.9% control can be compared to 74.4% control obtained in the laboratory studies (% taken from the data used to prepare Table 11) and would not be expected based on the higher r.h.'s and lower temperature encountered in the field study.

The possible reasons for the difference between the degree of control obtained in the laboratory and that obtained in the field could be due to the technique used in the laboratory where, at the end of the exposure, the insects are immediately removed from the CA exposure container and transferred to another container for post-exposure observations in a normal atmosphere. This practice probably causes a rapid desorption of the CO₂ absorbed during the exposure. In field situations the grain may not be moved or aerated for several days or weeks after the treatment is concluded, and there would be a gradual desorption of the CO₂ from the grain into the surrounding atmosphere. The speed of this desorption would be, of course, dependent on the tightness of the storage vessel that had been treated.

Concentrations of CO₂ below 35% can be effective in providing continued control of stored-product insects. In laboratory studies on the effects of 5% to 25% CO₂ concentrations, 25 S. oryzae adults were confined on 100g of wheat

for 24 h before they were exposed for periods of 15 or 30 days. These tests were conducted at 27°C and 60% rh and post-exposure readings were made for 4-weeks after the insects were removed from the tested atmospheres.

Table 12 shows that when insects were exposed for 15 days to 5% CO₂ there was a 39% RIE but only a 1% RIE was obtained with the insects exposed for 30 days to this atmosphere. Increasing the CO₂ concentration to 15% resulted in a 89% RIE after a 15-day exposure and 73% for the 30-day exposure. Exposures to the atmospheres containing 20 and 25% CO₂ resulted in almost 100% RIE for both exposure periods.

Table 12 - Mean number of adults that emerged and % reduction in emergence (RIE) of *S. oryzae* after a 15 or a 30 day exposure to indicated CO₂ concentrations at 27°C and 60% r.h.

Exposure:		15 Days		:	30 Days		
Time	Number	:	% RIE	:	Number	:	% RIE
% CO ₂	: of adults	:		:	: of adults	:	
0.1 (Air)	720	:	--	:	1,004	:	--
5	436	:	39	:	998	:	1
10	305	:	50	:	559	:	44
15	79	:	89	:	274	:	73
20	19	:	97	:	34	:	97
25	10	:	99	:	4	:	99

The design of these tests, which only included adults at the beginning of the exposures indicates that these low CO₂ atmospheres may be controlling only eggs and/or young larvae and possibly some of the original adults at the 20 and 25% CO₂ concentrations.

These studies show that atmospheres above 10 or 15% CO₂ give a high degree of control. Certainly, these are the type atmospheres encountered in the gradual desorption of CO₂ by the grain and this gradual desorption continues to give control after the treatment has been terminated.

Condition of the insect

We have shown that insect life stage is an important factor in the length of time required for a given CA composition to produce adequate control of a species. Other factors that also play an important role is the condition of the insect and the availability of food. Table 13 presents data on the simultaneous exposure of normal and quiescent larvae of T. glabrum to four CO₂ atmospheres. The quiescent larvae were obtained by crowding them without food for 30 days prior to exposure. During a 48h exposure at 32°C the quiescent larvae were not as affected by atmospheres containing 60, 75, or 90% CO₂ as were the normal larvae but when they were exposed to 99% CO₂ they exhibited the same response (100% mortality) as the normal larvae.

Table 13 - Mortality (%) of normal and quiescent T. glabrum larvae when exposed to four CO₂ atmospheres for 48-h at 32°C and 49% r.h.

%CO ₂	% Mortality	
	Normal	Quiescent
60	39	16
75	47	28
90	94	52
99	100	100

CONCLUSIONS

Table 11, if presented in its entirety, would show the effects of 8 CAs (and air) on 2 species of stored product insects and could be expanded to show the individual effects on 1, 2, 3, 4 and 5 week old immatures of these insects. These studies as they now stand have statistically 479 total degrees of freedom for each species and are probably two of the most complete laboratory studies ever conducted in this area. However, this research was conducted at only one temperature and one r.h. and the effects of the atmospheres on eggs and adults was not considered. Also, the exposure times tested were not long enough to achieve data on 100% mortality. A complete laboratory study on these or any other species should include exposure at several temperatures and relative humidities and the length of exposure should be expanded until complete mortality is obtained. There is a need for data such as this for each of the economically important stored-product

insects. More research is needed on the mode of action of CA on the insect at the several concentrations recommended for use in actual field situations. Adsorption and desorption of CO₂ by grain should be studied further, particularly as it relates to the immature insects feeding inside the kernel. Additional research is also needed on the effects of CA on diapausing or quiescent stages of stored-product insects. When this information is obtained it should be translated into a format to be used by industry in a practical manner to achieve effective control in the field.

The knowledge available at the present time provides some guidelines for treating grain with CO₂ in grain or oilseed storage bins or silos in a grain marketing system like the U.S.A. employs. If the grain to be treated is at or below 14% m.c. and at or above 27°C and if it does not contain Trogoderma spp. then a concentration of ca. 60% should be attained and maintained for 5 to 6 days if it is infested only with internal feeders. If external feeders are present a concentration as near to 100% CO₂ should be maintained for this period. Grain below 27°C and/or having a high m.c. or infested with Trogoderma should be treated for longer periods to obtain satisfactory control. Grain infested with species which have little or no research conducted on them, such as Sitotroga and Cryptolystes spp, should be treated in a similar manner and carefully sampled after treatment to determine the degree of control obtained.

The research necessary to eliminate the current inconsistencies and to provide a complete overview for the use of CA for stored-product insect control may have been brought up to date by this data. However, this research should be completed so that this control technique can become a viable alternative to conventional, residue-producing chemical fumigation.

ACKNOWLEDGEMENTS

The author wishes to acknowledge the assistance of G C Pearman, Jr. of ARS, USDA, Stored-Product Insects Research and Development Laboratory, Savannah, Georgia, U.S.A., in the actual conduct of the laboratory phase of this research and Dr S. Navarro, Agricultural Research Organization, Bet-Degan, Israel for his constructive criticism of the manuscript.

This research was supported in part by BARD Grant I-303-80 and by funds from the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Hoboken, N.J., U.S.A.

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