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FINAL REPORT

PROJECT NO. I-303-80

Modified Atmospheres for Controlling Stored-Product Insects

S. Navarro, E. Jay, E. Donahaye

630.72
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1985

1) Grain - storage - Diseases and
injuries - Control

2) Farm produce - storage - Control

3) Insects as carriers of plant
diseases

630.72 : 633 : 631.563 : 632.7
BAR

2nd copy

12289 0

Standard BARD Cover Page for Scientific Reports

Date July 15, 1985

BARD
P.O. Box 6
Bet Dagan, ISRAEL

BARD Project No. I-303-80

Title of Research Project:
Modified Atmospheres for Controlling Stored-Product Insects.

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and Development Laboratory, Savannah, Georgia.

Project's starting date: September, 1981.

Type of Report: 1st Annual _____ 2nd Annual _____ Final X

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מנהל מחקרים
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C. Abstract

Modified atmospheres (MAs) were investigated as a substitute to conventional stored grain insect control methods. Response of the four developmental stages of Sitophilus oryzae, Oryzaephilus surinamensis, and Tribolium castaneum to different MAs at temperatures from 15° to 32°C were studied. The effect of different carbon dioxide (CO₂) concentrations in air on mortality of larvae of three Trogoderma species at 27°, 32°, and 38°C was also studied. Larvae and pupae of S. oryzae, pupae of O. surinamensis and pupae of T. castaneum were least sensitive to the tested MAs. An exposure of 120 h at 27°C was sufficient for complete control of all stages of O. surinamensis when exposed to 60% CO₂ in air, but not for S. oryzae and T. castaneum. Exposure of successive generations of S. oryzae to CO₂ produced induced tolerance. Similarly the genetic potential of T. castaneum to develop resistance to low O₂ or high CO₂ atmospheres was demonstrated.

The influence of temperature and barometric pressure variation on infiltration of air into a MA treated experimental silo was analysed. The role of the sorption process of CO₂ in reducing the concentration of the gas in the treated bin was determined. For bins treated with MAs with the principle gas being CO₂ recirculation rate requirement for an adequate distribution of the gas was investigated. For this purpose a predictive model was developed.

A field trial was conducted to determine the feasibility of the method in a large bin containing wheat, and the effect of the treatment on a natural population of stored product insects was studied.

D. Objectives of the original research proposal

The original research proposal had two main objectives. The first was aimed at determining the effects of different atmospheric gas compositions on all four life stages of storage pests. The originally proposed four storage pests were Rhyzopertha dominica, Sitophilus oryzae, Tribolium castaneum and Oryzaephilus surinamensis. Since data on R. dominica was published after submission of the proposal, by the cooperating investigator (Jay, 1984), and earlier the principal investigator took part in a study on this species (Calderon and Navarro, 1980), this part of the proposal was not documented in this report. Work with T. castaneum was carried out towards the end of the research period and the time allocated to study this species was not sufficient. Therefore, although T. castaneum is currently under investigation, data given in the present report is not full in its content.

In view of the economic importance that Trogoderma spp. have in both countries, studies were conducted with Trogoderma granarium, T. variabile and T. glabrum. These species are considered to be very important stored product pests because of their resistance to conventional chemical pesticides. Their potential to reappear in the grain industries of both countries and especially that of T. granarium has caused renewed interest in these pests.

A study of the development of tolerance to modified atmospheres was undertaken following the recommendation of the review committee of the original proposal. These studies were conducted with T. castaneum and S. oryzae.

The second objective was to study the application techniques of different modified atmospheres in storage facilities. In accordance with the original proposal detailed studies were undertaken for the determination of the degree of leakage in grain silos and its significance in relation to atmospheric conditions. Since at the early stages of the project CO₂ was shown to play an important role in the control of the tested insects, it was decided to study its sorption by the commodity, and its distribution in a grain bin treated by the recirculation principle.

Several field trials were conducted. The first trial consisted of simulating the gas composition obtainable by a gas burner. It remained as a preliminary study and was documented in the first annual report. The second trial consisted of using CO₂ in a large bin containing wheat, it was studied in detail and forms a considerable part of the present report. At the end of the project period a series of tests were undertaken as preliminary studies to assess the application of CO₂ in bulk flour bins. Because lack of time these studies were not completed and are not documented in the present report.

E. Body of the report

1. Response of insects in laboratory studies to modified atmospheres -

Introduction

Since grain in a post-harvest situation is very close to the consumer, great care is taken by regulatory agencies when a new chemical treatment is submitted for approval. Most pesticide companies realize this and do not even attempt to obtain registration for their products for post-harvest use. When this situation is added to the residue problem (Anon., 1978) the development of insect resistance (Champ and Dyte, 1976), the health hazards, the annual losses and the continued surveillance by regulatory agencies of the currently used pesticides, it can be seen that the control strategy for insect pests in post-harvest situations is in serious trouble.

An alternative technique available for insect control in post-harvest situation is the application of modified atmospheres. This technique basically involves changing the proportions of the normal atmospheric constituents of the storage environment, O_2 , N_2 and CO_2 , to create an atmosphere lethal to insects. The makeup of this modified atmosphere must be maintained within set limits for an adequate length of time to give insect control.

With the apparently great potential of the modified atmosphere method of grain storage, it is remarkable that the exact limits of dosage and mortality for stored product pests has been so inadequately defined. Work has concentrated largely on the adult stage which appears to differ in susceptibility from the larvae, pupae and eggs that so far have largely been ignored.

The time required to obtain a certain level of insect mortality on exposure to a given atmospheric gas composition is dependent on the temperature of the environment. In fact, on a physiological basis development of insects in normal atmospheres can only occur within a fairly narrow range of temperatures. Developmental threshold temperatures have been determined for a number of stored product insects at different stages of development (Birch, 1945; Howe, 1960).

This effect is shown to be similar in tendency for different insect species, which differ in their susceptibility in the given gas concentration. In practice, these data should be considered very carefully. It is clear that at a given gas composition, much longer

exposures will be needed to obtain effective control when low temperatures prevail in the grain bulk. Furthermore, since differences in temperature exist in the various parts of the grain bulk, the lowest temperature recorded should be that which determines the length of the exposure time required for the treatment. In the use of modified atmosphere treatments, the most resistant stage of the most resistant insect species found in the grain bulk to be treated should be considered. However, there is at present insufficient data available to assess accurately the influence of temperature upon the effectiveness of modified atmospheres.

The following report was prepared based on a series of investigations aiming at elucidating the effects of different mixtures of the normal constituents of the atmosphere on different life stages of the major storage grain pests in Israel and the U.S.

1.1. Sitophilus oryzae

1.1.1. Materials and Methods:

Sitophilus oryzae were obtained from stock cultures reared continuously on 12.5% moisture content wheat held at 27°C and 70% relative humidity (RH). The cultures for the tests were established by seeding 255 g of this wheat with ca. 600 newly emerged adults for 48h. At the end of this period, the adults were removed leaving immature insects 0 to 2-d old in the cultures. S. oryzae used in these tests were from cultures that were 0 to 3-d old (eggs), and 21 to 24-d old (larvae and pupae) at the beginning of the exposure. The same cultures were kept until adults emerged, and 7 to 10-d post emergence adults were used in these tests. Wheat internally infested with immature insects was blended and then 25 g of this blend was placed in chambers of 100 ml capacity for exposure to three atmospheres plus a group serving as control that was held at 27°C in normal air. For tests carried out on adults, groups of 50 adults were exposed in the same exposure chambers containing 3 g of wheat.

The gas mixtures were obtained by preparing each mixture in a 3-liter container supplied with gases from pressurized cylinders of O₂, CO₂ and N₂. These mixtures were passed into the test chambers at a

rate of 15 ml/min, after they had been conditioned to 55% RH by purging them through gas washing bottles containing appropriate concentration of H_2SO_4 (Navarro and Donahaye, 1972). The gas washing bottles and the exposure chambers were kept in constant temperature rooms at 15°, 21°, 27°, and 32°C. Gas concentrations of each mixture were monitored daily using a gas chromatograph equipped with a thermal conductivity detector and dual columns packed with porapak Q and molecular sieve 5A.

Insects were exposed to the following gas mixtures: 1% O_2 , 14% CO_2 and 85% N_2 (to simulate the atmosphere obtained from a gas burner); 1% O_2 in N_2 ; and 60% CO_2 in air. Four replicates were tested at each of the four temperatures and five exposure periods of 24, 48, 72, 96, and 120 h for normal air and the three modified atmospheres. At the end of each exposure period, the test chambers containing either immature insects or adults were returned to normal atmosphere and transferred to an incubation chamber at 27°C. For exposure of eggs, and larvae and pupae, emergence counts of adults were made 45 and 30 days respectively after the end of the exposure period. Mortality counts for adults were made 10 d after the termination of each exposure period. Effectiveness of treatment for immature stages was determined by dividing the total number of adult insects that emerged after the end of the treatment by the total number that emerged in the controls and converting this to percent reduction in emergence of adults (RIE). A correction factor for adult mortality was made by comparison with the relevant untreated groups (Abbott, 1925). To determine possible interactions between variables, a factorial design was adopted using analysis of variance (Snedecor and Cochran, 1969). The data were analyzed using arcsin transformation.

1.1.2. Results and Discussion

The results of percent RIE of adults and mortality of adults obtained at four temperatures and three atmospheres are shown in Tables 1 to 4. At 15°C the only developmental stage affected by the tested MAs was the adult. Among the tested MAs the 1% O_2 in N_2 was shown to be the least efficient (Table 1). For the adult stage increase in temperature in the test environment resulted in reduction of exposure period required to obtain complete mortality. At 32°C all the tested MAs produced 100% adult mortality in less than 24h (Table

4). However, at all the experimental temperatures none of the tested MAs produced 100% RIE of the treated larvae and pupae after 120h. The tested MAs were found to be only effective in producing 100% RIE of the treated eggs at 27° and 32°C (Tables 3 and 4). From the results shown in Tables 1 to 4 it can be deduced that the most sensitive stage to the tested MAs was the adult followed in decreasing order by egg, larva and pupa.

The influence of an atmosphere created by an exothermic inert atmosphere generator (1% O₂; 8.5-11.5% CO₂; the balance being principally N₂) on S. oryzae adults at four different temperatures was studied by Storey (1975a). He reported that at 15°C, 296.7 h were required to obtain 95% mortality. However in the present work the atmosphere containing 1% O₂, 14% CO₂ and 85% N₂, at the same temperature and relative humidity produced complete mortality within 48 h exposure. This pronounced difference in exposure time required to obtain a high mortality level may be attributed to the CO₂ concentration (which was higher in the present work) and to differences in experimental technique. The results obtained by Storey (1975a) at 27° and 32°C fall within the range of mortality levels obtained in the present study (Tables 3 and 4). Storey (1975b) also investigated the influence of the atmosphere produced by an exothermic inert-atmosphere generator on different developmental stages of S. oryzae when exposed to 21° and 27°C. At both temperatures the earlier and later stages were more susceptible and the middle stages were less susceptible to the tested MAs. At 27°C, the exposure time required to produce 95% mortality during the 4th instar through early pupal development was about 10 days for S. oryzae. In the present study, an attempt was made to increase the influence of the tested MAs by exposing the insects to 32°C. However, from results shown in Table 4 it is evident that 120 h exposure even at 32°C is not sufficient to obtain complete control for the most resistant stages, namely larvae and pupae. Among the tested MAs the mixture containing 60% CO₂ in air was the most promising, especially against the larvae and pupae. Although exposure times longer than 120 h were not tested in the present study, it is evident that to obtain effective control of S. oryzae at 27°C and higher temperatures an exposure of at least 10 days should be allowed with preference for use of a MA produced by an

exothermic inert atmosphere generator or one containing 60% CO₂ in air.

Table 1. Percent reduction in emergence of adults of Sitophilus oryzae eggs, larvae and pupae, and mortality of adults exposed to three modified atmospheres at 15°C.

Modified atmosphere composition (%)			Exposure time (h)	% reduction in emergence of adults of treated -		% mortality of treated adults
O ₂	CO ₂	N ₂		Eggs	Larvae & pupae	
1	0	99	24	0	7.2	27
			48	5.7	12.6	81
			72	0	2.0	87
			96	19.2	6.1	99
			120	29.0	17.6	100
1	14	85	24	5.5	0	24
			48	2.5	7.9	100
			72	0	12.2	100
			96	30.0	16.7	100
			120	62.5	20.3	100
8	60	32	24	7.2	0	74
			48	0	7.3	100
			72	21.3	4.5	100
			96	0	15.5	100
			120	36.2	35.5	100

Table 2. Percent reduction in emergence of adults of Sitophilus oryzae eggs, larvae and pupae, and mortality of adults exposed to three modified atmospheres at 21°C.

Modified atmosphere composition (%)			Exposure time (h)	% reduction in emergence of adults of treated -		% mortality of treated adults
O ₂	CO ₂	N ₂		Eggs	Larvae & Pupae	
1	0	99	24	0	3.2	57
			48	12.5	20.0	100
			72	10.3	9.0	100
			96	75.5	8.5	100
			120	89.8	28.7	100
1	14	85	24	11.1	2.6	99
			48	19.1	31.7	100
			72	54.5	2.6	100
			96	71.4	15.5	100
			120	96.8	34.4	100
8	60	32	24	0	17.9	100
			48	34.5	37.9	100
			72	70.3	41.1	100
			96	96.2	76.4	100
			120	100	91.6	100

Table 3. Percent reduction in emergence of adults of Sitophilus oryzae eggs, larvae and pupae, and mortality of adults exposed to three modified atmospheres at 27°C.

Modified atmosphere composition (%)			Exposure time (h)	% reduction in emergence of adults of treated -		% mortality of treated adults
O ₂	CO ₂	N ₂		Eggs	Larvae & Pupae	
1	0	99	24	53.3	15.3	96.6
			48	93.3	0.9	100
			72	100	58.5	100
			96	100	4.4	100
			120	100	30.9	100
1	14	85	24	32.0	0	100
			48	45.9	0	100
			72	93.8	19.0	100
			96	96.6	24.2	100
			120	100	6.5	100
8	60	32	24	35.4	20.6	100
			48	89.4	58.6	100
			72	100	85.5	100
			96	100	96.6	100
			120	100	98.5	100

Table 4. Percent reduction in emergence of adults of Sitophilus oryzae eggs, larvae and pupae, and mortality of adults exposed to three modified atmospheres at 32°C.

Modified atmosphere composition (%)			Exposure time (h)	% reduction in emergence of adults of treated -		% mortality of treated adults
O ₂	CO ₂	N ₂		Eggs	Larvae & Pupae	
1	0	99	24	43.9	16.6	100
			48	96.6	10.0	100
			72	100	21.0	100
			96	100	44.6	100
			120	100	67.5	100
1	14	85	24	29.8	17.4	100
			48	100	8.4	100
			72	99.0	39.0	100
			96	100	62.3	100
			120	100	64.1	100
8	60	32	24	74.6	30.2	100
			48	91.6	71.2	100
			72	100	80.8	100
			96	100	77.7	100
			120	100	98.5	100

1.2. Oryzaephilus surinamensis

1.2.1. Materials and methods

Oryzaephilus surinamensis were obtained from stock cultures reared continuously on an oatmeal diet and held at 27°C and 60% relative humidity (RH). For each test, three cages each containing 10 insects per cage were exposed to different modified atmospheres (MA). Larvae were 12-15 days old, pupae 0-2 days after pupation, and adults 1-8 days old after emergence. The exposure chambers and the test procedure were similar to those described by Jay (1984).

Cages containing insects and oatmeal diet at four temperatures were exposed to gases supplied from cylinders containing air, or CO₂ concentrations of 60%, 75%, 90% and 99% in air. Exposure periods were 16, 24, 48, 96, 120, 168 and 240 h. The RH of the tested atmospheres in all the experiments was maintained between 50.6% and 52.5%. Three replicates (three cages per replicate) were tested at each of the four temperatures and the exposure periods for normal air and the four modified atmospheres.

The effect of MAs on eggs was investigated by a method different from that referred to above: Eggs 0-1 days old were obtained from O. surinamensis reared on wheat feed and exposed in individual incubation cells, devised to enable observation to be made under the microscope for egg hatch (Navarro and Gonen, 1970). For these experiments one group of 50 eggs exposed to each gas mixture was removed every 24 h until 120 h exposure. Egg mortality was determined by failure of an egg to hatch 7 days after the hatch of the last egg of the same group. For each experiment a control group of 50 eggs was exposed to normal atmospheric air at the same temperature and humidity conditions as those used for exposure to CO₂ concentrations. The experiments were replicated four times. A correction for egg mortality was made by comparison with the relevant untreated groups (Abbott, 1925). The data were analyzed, after arcsin transformation using an IBM 360 computer handling the programs of the SAS Institute (Barr et al. 1976).

1.2.2. Results and Discussion

Mortality of the four developmental stages of O. surinamensis exposed to CO₂ concentrations at four different temperatures are shown in Tables 1 to 4. At 15°C complete mortality was obtained for the larval and the adult stages after 72 h, whereas the egg and pupae showed a lower sensitivity to the tested CO₂ concentrations (Table 1). Although 60% CO₂ was not sufficient to produce complete mortality of pupae even after 240 h exposure, an increase in the CO₂ concentration to 99% was effective after 72 h. At 21°C although pupae were again relatively insensitive, a 120 h exposure was sufficient for complete mortality of eggs (Table 2). Exposure to 60% CO₂ at 27°C showed a slightly greater tolerance of the larvae in comparison with the pupae. At this temperature 96 h exposure to 60% CO₂ was sufficient to obtain complete mortality of all developmental stages of the insect (Table 3). At 32°C, whereas the adults showed a relative insensitivity when exposed to 60% CO₂, when the CO₂ concentration was 75% all developmental stages including pupae were effectively controlled after 48 h.

In a review on the effects of modified atmospheres on stored product insects, Bailey and Banks (1980) concluded that additional work is needed on dose-mortality response to various gas mixtures, with particular emphasis on tolerant stages and species. This in order to provide a basis for an exposure schedule dependent on the temperature of the bulk to be treated. Since in the present study the pupal stage was shown to be the most tolerant stage, an attempt was made to demonstrate the relationship between various CO₂ concentrations and temperature at this stage. In Fig. 1 this relationship was expressed in terms of CO₂ concentration and the exposure time required to obtain 99% mortality of pupae. For this purpose a probit analysis was performed for each temperature and CO₂ concentration, in order to enable calculation of exposure times needed to obtain different mortality levels. This data was further analyzed using a multiple regression procedure. Fig. 1 shows that the calculated exposure times to produce 99% mortality are slightly prolonged as the CO₂ concentration is reduced. Temperature had a marked effect on exposure time, the higher the temperature the shorter

the time needed to obtain a 99% mortality. Although to the best of our knowledge this type of presentation is not common for most biological work, it enables us to demonstrate the expected effect of CO_2 within the range of test conditions. However, any extrapolation beyond the limits of the above described test may lead to erroneous conclusions.

Figure 1 clearly shows that the response of O. surinamensis pupae at 15° and 21°C is distinctly different from their response at 27° and 32°C. For the application of CO_2 in a field situation the family of curves shown in Fig. 1 have an importance in enabling decisions to be made as to the duration of treatment required for the maintenance of a given CO_2 concentration over a given temperature range of the commodity. The shallow slope of the curves at 21° and 27°C also shows that for CO_2 concentrations higher than 60% only a limited reduction in exposure time could be achieved.

Little information on the effect of MAs on O. surinamensis is available. Storey (1980) has shown that adults of this species when exposed to 1% O_2 and 9.0-9.5% CO_2 with the balance N_2 suffer 95% mortality after 47 h exposure at 15°C. The high sensitivity of this species to atmospheres containing less than 1% O_2 with the balance as N_2 at relative humidities ranging from 9% to 98% was investigated by Jay et al. (1971). However, the results obtained in the present work clearly demonstrate the low sensitivity of O. surinamensis pupae, and the relationship between CO_2 concentration and temperature.

Table 1. Mortality of four developmental stages of Oryzaephilus
surinamensis exposed to different CO₂ concentrations at 15°C

%CO ₂	Exposure time (h)	% mortality			
		Eggs	Larvae	Pupae	Adults
60	24	56	42.6	30.7	16.1
	48	53	98.8	45.8	98.9
	72	80	100	54.3	100
	96	82	-	-	-
	120	84	-	47.8	-
	168	-	-	71.0	-
	240	-	-	92.2	-
75	24	41	37.0	31.3	13.2
	48	53	94.3	58.2	98.9
	72	58	100	57.6	100
	96	76	-	-	-
	120	90	-	61.5	-
	168	-	-	85.0	-
	240	-	-	100	-
90	24	58	29.0	39.3	23.6
	48	64	80.6	59.8	100
	72	82	100	61.8	100
	96	91	-	-	-
	120	99	-	60.1	-
	168	-	-	96.2	-
	240	-	-	100	-
99	24	-	21.9	76.9	64.1
	48	-	77.7	99.0	100
	72	-	100	100	100

Table 2. Mortality of four developmental stages of Oryzaephilus surinamensis exposed to different CO₂ concentrations at 21°C

%CO ₂	Exposure time (h)	% mortality			
		Eggs	Larvae	Pupae	Adults
60	16	-	70.8	-	26.4
	24	55	92.1	36.9	76.2
	48	75	100	52.2	100
	72	93	-	67.3	-
	96	99	-	77.0	-
	120	100	-	95.4	-
	168	-	-	89.6	-
	240	-	-	100	-
75	16	-	-	-	38.0
	24	57	96.6	23.5	76.0
	48	89	100	62.7	97.8
	72	94	100	71.7	100
	96	100	-	69.5	-
	120	100	-	88.0	-
	168	-	-	95.4	-
	240	-	-	100	-
90	16	-	46.5	-	32.9
	24	63	85.1	41.2	88.2
	48	84	100	58.0	100
	72	99	-	85.3	-
	96	100	-	83.3	-
	120	100	-	89.8	-
	168	-	-	95.3	-
	240	-	-	100	-
99	16	-	32.8	-	75.8
	24	-	95.2	76.0	92.7
	48	-	100	100	100
	72	-	-	100	-

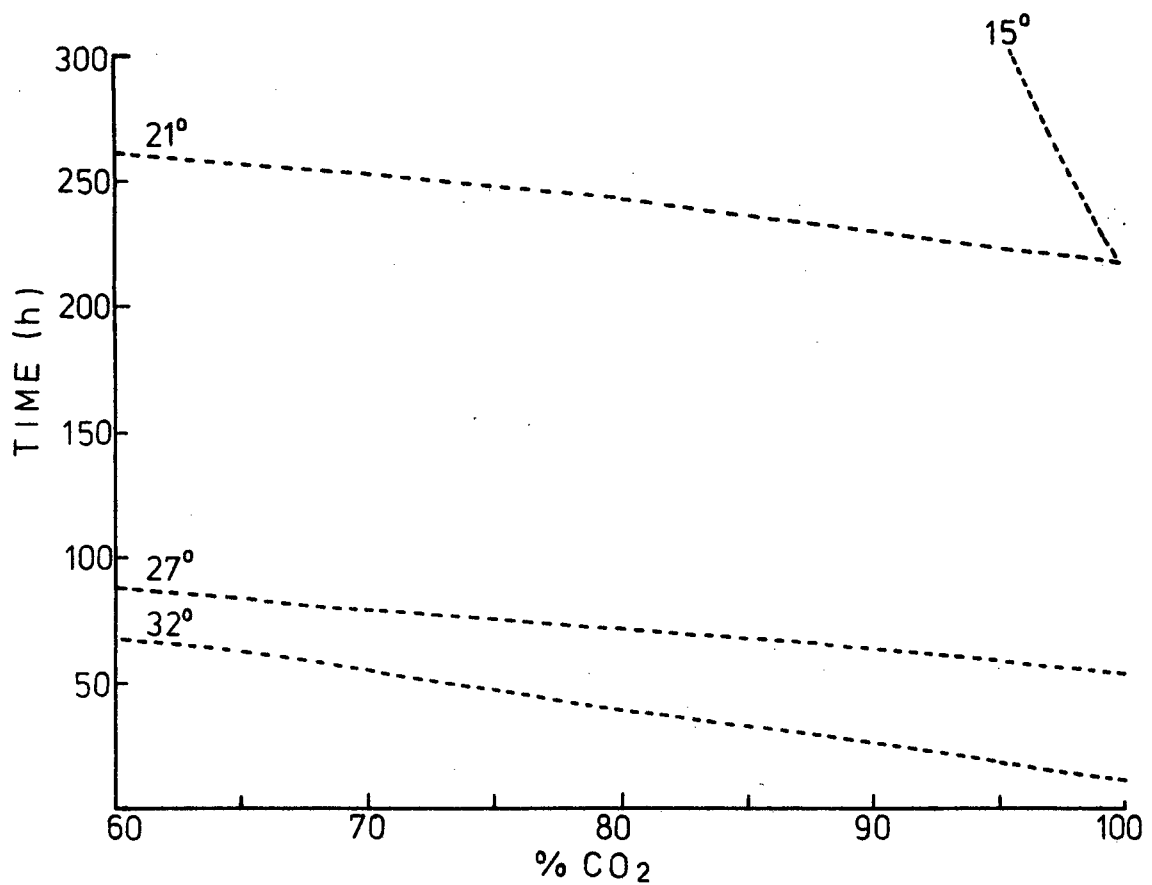
Table 3. Mortality of four developmental stages of Oryzaephilus
surinamensis exposed to different CO₂ concentrations at 27°C

%CO ₂	Exposure time (h)	% mortality			
		Eggs	Larvae	Pupae	Adults
60	16	-	51.1	5.3	24.7
	24	81	51.8	19.8	69.5
	48	100	93.4	69.5	98.9
	72	100	98.9	100	100
	96	100	100	-	-
75	16	-	90	18.4	80.7
	24	90	95.6	33.4	94.2
	48	100	100	73.0	100
	72	100	-	100	-
	96	100	-	-	-
90	16	-	100	25.7	94.5
	24	78	100	23.7	100
	48	100	100	78.7	100
	72	100	-	100	-
	96	100	-	-	-
99	16	-	100	46.2	100
	24	-	99.0	95.4	100
	48	-	100	100	100

Table 4. Mortality of four developmental stages of Oryzaephilus
surinamensis exposed to different CO₂ concentrations at 32°C

%CO ₂	Exposure time (h)	% mortality			
		Eggs	Larvae	Pupae	Adults
60	16	-	52.4	36.1	57.3
	24	100	56.8	56.5	26.7
	48	100	88.5	81.3	79.2
	72	100	96.7	100	92.5
	96	-	-	-	99.0
75	16	-	89.9	43.4	94.1
	24	100	100	86.1	100
	48	100	100	98.9	100
	72	100	100	-	-
90	16	-	100	70.8	100
	24	100	100	98.7	100
	48	100	100	100	100
	72	100	100	-	-
99	16	-	100	100	100
	24	-	100	100	100
	48	-	100	100	100
	72	-	100	-	-

Fig.1 -Regression lines to demonstrate the relationship between CO_2 concentration and the time needed to obtain 99% mortality of Oryzaephilus surinamensis pupae exposed at four temperatures.



1.3. Tribolium castaneum

1.3.1. Materials and Methods

Tribolium castaneum was reared on wheat feed mixed with 5% brewer's yeast (by weight) in a controlled temperature (26°C) and relative humidity (70%) room. Eggs 0-24 h old, were collected and confined in individual incubation cells, devised to enable observation under the microscope for egg hatch (Navarro and Gonen, 1970). During exposure to modified atmospheres (MA) the individual cells (2 mm diameter, 1 mm depth) were uncovered to permit gas exchange through the top. At the end of each exposure they were covered by a microscope slide. Larvae 7-14 d old (after egg hatch), were collected and exposed in groups of 30 individuals in a chamber containing 1 g of rearing media. Pupae 1-3 d old (after pupation) were exposed in groups of 50 individuals without food. Groups of 100 adults, 7-10 d old, were exposed on 3 g culture media. Chambers of 100 ml capacity were used to expose the insects to the tested MAs.

Insects were exposed to the same gas mixtures described in section 1.1.1. of this report. The test temperatures consisted of 15°, 21° and 32°C. Four replicates were tested at each of the three temperatures and five exposure periods of 24, 48, 72, 96 and 120 h were used. After treatment insects were transferred to an incubator at normal atmosphere and 27°C.

Egg mortality was determined as failure of an egg to hatch 14 days after the hatch of the last egg of the same group. Larval and pupal mortality was determined by failure of an adult to emerge 14 days after adult emergence in the control group. Mortality counts for adults were made 14 days after the end of each exposure.

1.3.2. Results and Discussion

Mortality of four developmental stages of T. castaneum exposed to three MAs at different temperatures are shown in Tables 1, 2 and 3. Tests with this species were run towards the end of the termination of the project period and have not been completed. Therefore, results shown in these tables contain limited information and make comparison

difficult between the different development stages. At 15°C complete mortality was not achieved with the tested developmental stages except for the 60% CO₂ atmosphere. Although mortality data on the effect of 60% CO₂ on eggs and adults is not complete, the most resistant stages appear to be the larvae and pupae. At 21°C pupae were more resistant than eggs and larvae to 1% O₂ in N₂ and to the GB atmosphere (namely 1% O₂, 14% CO₂ and 85% N₂). For 60% CO₂ comparison was possible only between larvae and pupae, the latter being more resistant. Exposure at 32°C showed that the most resistant stage for the 60% CO₂ atmosphere was the egg stage. For the other two atmospheres tested, the most resistant stage (adults excepted) was again the egg stage. At this temperature complete mortality was obtained within 120 h for eggs exposed to 1% O₂, while for the other atmospheric compositions shorter exposure times were needed.

In tests carried out with an atmosphere of 2% O₂ and 15% CO₂ in N₂ at 26°C and 57% RH, complete mortality of T. castaneum adults was obtained within 96 h (Calderon and Navarro, 1980). The same authors report also that similar conditions of temperature and humidity but with an atmosphere of 2% O₂ and 10% CO₂ in N₂ are sufficient for complete control of T. castaneum eggs. In a study to test the response of different stages of T. castaneum to an atmosphere of 1% O₂ and 9-9.5% CO₂ in N₂, Storey (1977) found that at 27°C larvae and adults were more sensitive than eggs and pupae. In his work a range of 25-40 h for eggs and a range of 17-47 h for pupae were required to achieve a 95% mortality depending on the age of each stage. For the GB atmosphere tested in the present work it was shown that at 32°C 96 h was required for complete mortality of eggs and 48 h for pupae. The longer exposure time required in the present work, in spite of exposure at a higher temperature (32°C) than the 27°C used by Storey (1977), may be attributed to differences in experimental procedures.

Exposure at 26.7°C and 38% RH, to a 100% CO₂ atmosphere produced complete mortality of T. castaneum eggs, larvae, pupae and adults within 60, 48 and 18 h, respectively (AliNiazee, 1971). However, in the same work AliNiazee (1971) reported that at 15.6°C 100% mortality was obtained for the larval stage exposed to 100% CO₂ within 84 h. This result may be compared with the results shown in Table 1, for

which at 15°C a 60% atmosphere caused complete mortality within 120 h. At the same temperature AliNiazee (1971) reports that 168 h was required when pupae were exposed to 100% CO₂.

From the results shown in Tables 1,2 and 3 a full comparison of the efficiencies of the atmospheric compositions was not possible. Although the pupae tend to be more resistant than eggs and larvae for all the tested atmospheres at 15° and 21°C, this tendency did not remain constant at 32°C. At 32°C the eggs showed a lower sensitivity in comparison with larvae and pupae.

Table 1 Mortality of four developmental stages of Tribolium castaneum exposed to three modified atmospheres at 15° C

Modified atmosphere composition (%)			Exposure time (h)	% mortality			
O2	CO2	N2		Eggs	Larvae	Pupae	Adults
1	0	99	24	20.8	1.5	1.1	1.0
			48	23.4	5.5	2.6	0.0
			72	73.4	2.3	9.6	5.1
			96	92.9	9.3	8.9	13.0
			120	97.7	42.3		22.4
1	14	85	24	12.8	1.5	5.2	2.6
			48	12.8	16.1	1.9	99.0
			72	35.4	40.6	18.7	99.5
			96	64.6	60.0	25.3	99.5
			120	84.7	83.5	60.3	100.0
8	60	32	24		1.3	0.9	23.8
			48		14.8	4.6	95.9
			72		20.7	12.5	100.0
			96		96.2	26.1	100.0
			120		100.0	28.1	

Table 2 Mortality of four developmental stages of Tribolium castaneum exposed to three modified atmospheres at 21° C

Modified atmosphere composition (%)			Exposure time (h)	% mortality			
O2	CO2	N2		Eggs	Larvae	Pupae	Adults
1	0	99	24	28.7	11.4		0.0
			48	87.0	15.0		6.6
			72	95.3	38.3		
			96	99.5	72.4	30.8	
			120	100.0	99.0	39.5	69.1
1	14	85	24	41.8	4.1	2.3	45.7
			48	70.2	9.8	4.7	100.0
			72	74.5	45.3	29.6	100.0
			96	87.8	89.5	70.1	100.0
			120	82.8	97.0	95.5	100.0
8	60	32	24		6.3	11.6	100.0
			48		16.0	10.8	100.0
			72		38.0	63.5	100.0
			96		93.0	96.7	
			120		100.0	99.5	

מספר הקדמון
למחלקת המחקר
המזון והמגורים

Table 3 Mortality of four developmental stages of Tribolium castaneum exposed to three modified atmospheres at 32° C

Modified atmosphere composition (%)			Exposure time (h)	% mortality			
02	CO2	N2		Eggs	Larvae	Pupae	Adults
1	0	99	24	88.8	55.1	40.5	100.0
			48	99.5	100.0	99.5	100.0
			72	99.5	100.0	100.0	100.0
			96	98.9	100.0		
			120	100.0			
1	14	85	24	79.1	42.0	65.0	100.0
			48	95.1	100.0	100.0	100.0
			72	98.0	100.0	100.0	100.0
			96	100.0	100.0		
			120		100.0		
8	60	32	24	60.2	29.8	72.8	99.5
			48	95.7	78.5	94.3	100.0
			72	99.5	100.0	100.0	100.0
			96	100.0	100.0		
			120				

1.4. Trogoderma granarium

1.4.1. Materials and Methods

Laboratory stocks of Trogoderma granarium reared on a mixture of wheat, broken wheat and brewer's yeast (87;10;3, W/W/W) at 30°C and 70% relative humidity were used for the experiments. Under adverse conditions such as crowding, lack of food or the presence of contaminated and depleted food, T. granarium larvae may enter a resting phase, pupation will be delayed and they remain in a quiescent state (Lindgren and Vincent, 1960). Burges (1959) defined as quiescence this arrest of development which ends as soon as favourable conditions return. Quiescent larvae were obtained according to the method described by Lindgren and Vincent (1960) by removing active larvae from cultures and placing them in groups of several hundred without adding fresh food. Active larvae were those kept for 3 to 4 weeks reared from the egg stage at 30°C. For each test 30 test active or quiescent larvae with 10 g of wheat were placed in a chamber of 100 ml capacity for exposure to CO₂ concentrations in air with a group which served as control held at the three tested temperatures in normal air.

The gas mixtures were obtained by preparing each mixture in a 3-liter container supplied with gases from pressurized cylinders of CO₂ and air. These mixtures were passed into the test chambers at a rate of 15ml/min, after they had been conditioned to 55% RH by bubbling them through gas washing bottles containing appropriate concentrations of H₂SO₄ (Navarro & Donahaye, 1972). The gas washing bottles and the exposure chambers were kept in constant temperature rooms at 26°, 32° and 37°C. Gas concentrations were monitored in a manner described in section 1.1.1. of this report.

Insects were exposed to 75, 90 and 99% CO₂ in air. Three replicates were tested at each of the three temperatures and four exposure periods of 16, 24, 48 and 72 h for normal air and the three CO₂ concentrations. At the end of the exposure period, the test chambers containing the larvae were transferred to an incubation chamber at normal atmosphere, 26°C and 65% relative humidity. Mortality counts were made three weeks after the termination of each exposure period,

by placing the larvae on a hot plate at 45°C to examine for movement. A correction for mortality was made by comparison with the relevant untreated groups (Abbott, 1925). A factorial design was adopted using analysis of variance and the data were analyzed using arcsin transformation.

1.4.2. Results and Discussion

Mortality of the active and quiescent larvae of T. granarium exposed to different CO₂ concentrations in air at three temperatures are shown in Tables 1, 2 and 3. At 26°C the tested CO₂ concentrations failed to cause complete mortality during 72 h exposure (Table 1). Moreover, at 32°C and 37°C complete mortality was observed only when active larvae were exposed to 99% CO₂ (Tables 2 and 3). Multiple range test performed for each level of CO₂ and each exposure time clearly indicate a significant difference between active and quiescent larvae, the latter showing increased resistance to the treatments.

The conventional methods of controlling T. granarium are largely based on the use of fumigants and contact insecticides. The quiescent larvae of this species are known to have a significant resistance compared with the active larvae when exposed to fumigants (Vincent and Lindgren, 1960). The available literature on the action of MAs is based on experiments undertaken with active larvae, and no serious attempts have been made to determine the relative resistance of this species when quiescent larvae are exposed to MAs. Verma and Wadhi (1978) found that T. granarium larvae were considerably less susceptible to 100% CO₂ than three other stored product insect species. Le Torc'h (1983) also found that larvae of this species were less susceptible to pure CO₂ than five other stored product insect species. Exposure for 17 d at 30°C was effective in causing 100% mortality of T. granarium larvae in an atmosphere containing 60% CO₂ (Bailey, 1965). In all the above mentioned experiments the response of active larvae to CO₂ was shown, without considering the possibility that quiescent larvae may respond differently. In a recent study Spratt et al. (1985) attempted to test the effect of 60% CO₂ in air on active and diapausing larvae. However, since these investigators were unable to induce diapause other than by lowering the temperature, they suspect that the state of diapause may have been broken during tests

at 30°C. Although the active larvae proved to be more tolerant to the gas mixture than larvae in diapause, it appears that this was due to a sudden temperature increase from 20° to 30°C while larval food reserves were low. Conversely active larvae exposed to the gas mixture at 20°C probably tended to enter diapause during the experiment when both the atmosphere and temperature were unfavourable. The most tolerant larvae at 20°C were those which had been kept at that temperature for 2 weeks before the test.

In the present study, quiescence was induced by a method different from that in the work carried out by Spratt et al. (1985). Therefore, the quiescent larvae so obtained were not dependent on the influence of temperature. Results shown in Tables 1, 2 and 3 indicate that except for short exposure times the tolerance of quiescent larvae was significantly higher than that of the active larvae.

According to Spratt et al. (1985) survival at 30°C, 75% CO₂ and 60% RH was 75% when T. granarium larvae were exposed for 3 d. In the present study exposure to 75% CO₂ and 55% RH for three days caused 41.6% and 28.4% mortality of active and quiescent larvae respectively (Table 2). If 75% survival can be considered as 25% mortality, the result obtained by Spratt et al. (1985) is closer to our results obtained for quiescent larvae.

In the present work an attempt was made to obtain information on the control of T. granarium that can be achieved using an atmosphere rich in CO₂. Since control of this pest is important in the frame work of the activities of quarantine authorities, short exposure periods being desirable in such cases the application of CO₂ as a control method may favor. The results obtained so far indicate that at temperatures higher than 32°C the application of CO₂ at concentrations close to 99% would be a practical proposition for the control of active larvae in less than 3 d exposure. However, in cases where it is suspected that quiescent larvae also contaminate the product, temperatures should be kept closer to 37°C to ensure a successful treatment.

To demonstrate the relationship between various CO₂ concentrations and the time needed to obtain 99% mortality at three different temperatures, a multiple regression analysis was performed. The results shown in Fig. 1 are the theoretical calculated lines to produce 99% mortality for the active larvae exposed at each

temperature. Although this type of calculation involves the extrapolation of exposure times longer than those actually experimented on, it enables one to assess the limits for effective use of CO₂ to control active larvae of T. granarium.

On the basis of information presented here, it is clear that T. granarium larvae should be grouped among those species resistant to the effect of CO₂. However, the application of CO₂ in an environment in which the temperature is raised to 32°C or higher may provide a promising combination for the control of this pest.

Table 1. Percent mortality of active and quiescent larvae of Trogoderma granarium exposed to three CO₂ concentrations in air at 55% relative humidity and 26°C.

Exposure time (h)	Larval condition A=active Q=quiescent	% CO ₂ in air		
		75	90	99
16	A	4.3a *	2.1a	3.2a
	Q	3.3a	2.2a	13.1b
24	A	8.6a	6.5a	17.7a
	Q	2.2b	2.0a	4.6b
48	A	37.2a	43.0a	50.5a
	Q	6.6b	8.6b	8.8b
72	A	68.3a	77.5a	67.1a
	Q	13.3b	12.9b	26.5b

*) Each level of CO₂ and each exposure time was compared by Duncan's multiple range test and means with the same letter are not significantly different at p=0.05.

Table 2. Percent mortality of active and quiescent larvae of Trogoderma granarium exposed to three CO₂ concentrations in air at 55% relative humidity and 32°C.

Exposure time (h)	Larval condition A=active Q=quiescent	% CO ₂ in air		
		75	90	99
16	A	5.4a*	6.5a	18.6a
	Q	1.1a	6.7a	4.4b
24	A	7.9a	13.5a	76.3a
	Q	3.3b	4.5b	24.4b
48	A	25.8a	60.4a	100 a
	Q	18.4b	28.2b	93.6b
72	A	41.6a	84.0a	100a
	Q	28.4b	59.3b	88.3b

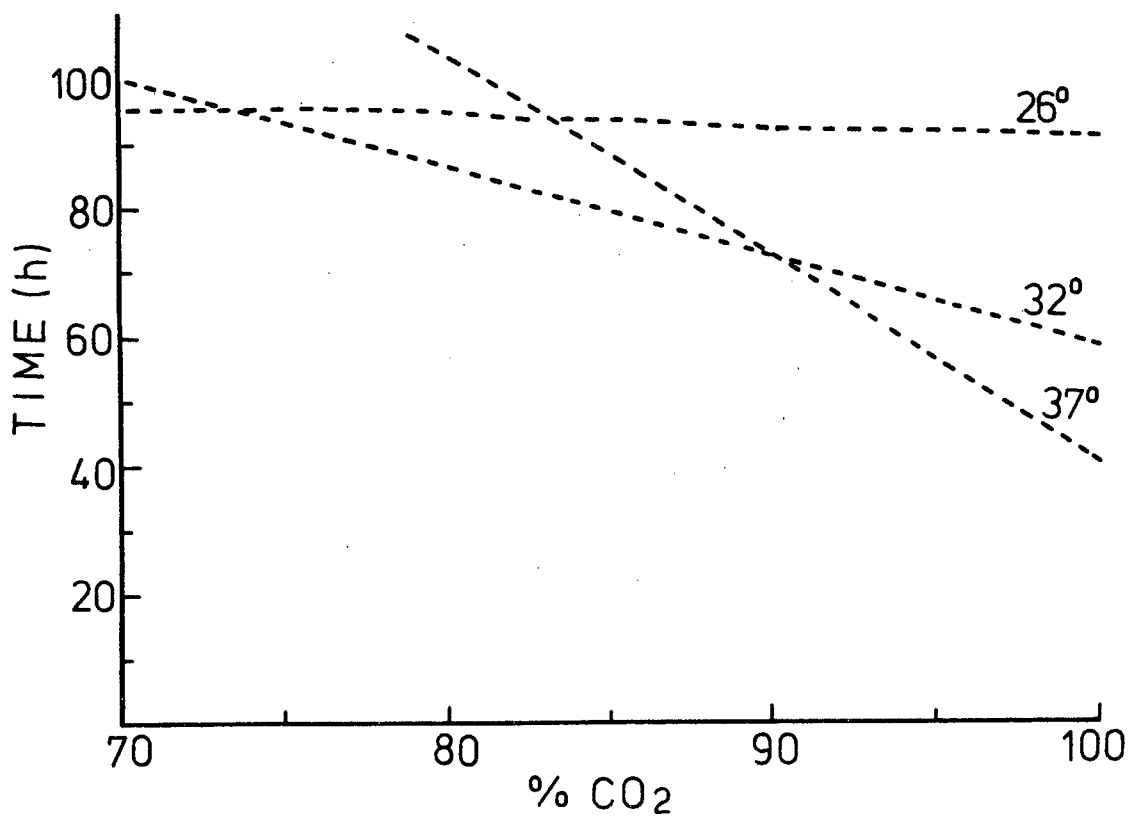
* Each level of CO₂ and each exposure time was compared by Duncan's multiple range test and means with the same letter are not significantly different at p=0.05.

Table 3. Percent mortality of active and quiescent larvae of Trogoderma granarium exposed to three CO₂ concentrations in air at 55% relative humidity and 37°C.

Exposure time (h)	Larvae condition A=active Q=quiescent	% CO ₂ in air		
		75	90	99
16	A	4.4a*	27.7a	83.3a
	Q	4.4a	9.8b	43.0b
24	A	18.0a	66.3a	100 a
	Q	4.4b	16.0b	55.0b
48	A	27.5a	74.9a	100 a
	Q	18.6b	46.5b	100 a
72	A	37.2a	98.0a	100 a
	Q	23.0b	78.5b	100 a

* Each level of CO₂ and each exposure time was compared by Duncan's multiple range test and means with the same letter are not significantly different at p=0.05.

Fig. 1 -Regression lines to demonstrate the relationship between CO_2 concentration and the time needed to obtain 99% mortality of Trogoderma granarium active larvae exposed at three temperatures and 55% relative humidity.



1.5 Trogoderma variabile and T. glabrum

1.5.1. Materials and Methods

Larvae of Trogoderma variabile (Ballion) and of Trogoderma glabrum (Herbst) were obtained from cultures routinely reared at 26.7°C and 60% RH on CSM (corn-soybean-powdered milk; a fortified cereal). Larvae of T. variabile were 39 to 46 days old when tested and weighed 6.2 ± 2.3 mg. (mean \pm SD), larvae of T. glabrum were 38 to 40 days old when tested and weighed 5.3 ± 1.9 mg. (mean \pm SD). Ten larvae were placed in 35x35 mesh screen cages (wire: 0.036 cm. diameter) measuring 1.67 cm. in diameter x 6.5 cm. in length. A small amount of rearing media was added to each exposure cage. The exposure chambers were similar to those described by Harein and Press (1968). They consisted of 2.8 liter glass jars that were partly submerged in laboratory baths filled with water. Four such baths were used, and they were individually set to maintain water temperature at 32° or 38°C.

The gas mixtures were released from the cylinders through two-stage regulators and flowed through a micrometering valve and flow meter into gas washing bottles that contained a glycerin-water mixture that adjusted the RH of the gases to ca. 50%. The gases then flowed into the exposure chambers. A flow rate of 100 cc./min. was used during the exposure periods. The RH was monitored with an electric hydrometer (Model 15-2001 humidity indicator and narrow range humidity sensors, Hydro-dynamics, Inc.), and the temperature was recorded daily.

The insects were exposed to gases from cylinders containing air, 100% CO₂, or mixtures of CO₂ and air containing ca. 60, 75 or 90% CO₂ (balance air). The actual concentrations measured during the tests are given in Tables 1-3. Small leaks in the system reduced the actual concentration of 100% CO₂ to ca. 99%; this was expected. Exposures were from 16 to 168 h. Depending on the temperature.

At the end of the exposure periods, the contents of the cages were placed in 70 ml. plastic containers with holes drilled in the lids and held at 27°C and 60% RH for 3 weeks. At this time the insects were examined and those that had turned black, dried out or did not respond to gentle probing or vibration were considered dead.

Quiescent Larvae

Larvae were sifted from cultures of T. glabrum which were ca. 28-35 days old and from cultures of T. variabile which were ca. 41 days old. These larvae were held without food in 1-pint jars with screen lids for periods of 15 or 30 days prior to exposure. They were then exposed to 5 modified atmospheres having the approximate concentration of the major component of the atmospheres shown in Table 3. These larvae were exposed simultaneously with active larvae of about the same age (minus the 15 or 30 days quiescent period) from standard laboratory cultures to determine the differences in the effects of the atmospheres on the two groups.

Prior to exposure selected groups of active and quiescent larvae were weighed to determine the effects of starvation on the body weight of the quiescent larvae.

Pupae

Pupae for testing were obtained from laboratory reared cultures of Trogoderma spp. and were 45 to 53 days old when exposed to the modified atmospheres. These pupae were exposed in the same manner as were the larvae.

Modified atmosphere concentrations used were similar to those used in the tests with larvae. However, pupae were also exposed to an atmosphere of 100% nitrogen (N_2) for comparison with the high CO_2 atmospheres. Exposures were conducted at 32° and 38°C and RH was maintained at 45 to 50% during the exposure of pupae. Mortality was determined by failure of the adults to emerge from the tested pupae.

1.5.2. Results

Pupae

Table 1 presents the results of exposure of T. glabrum pupae to 5 modified atmospheres at ca. 32° and 38°C. Mortality was high (92 to 100%) when these pupae were exposed to atmospheres containing either 99% CO_2 or 99.5% N_2 for 24 and 48 h. At 32°C it took 72 h to obtain 100% mortality of pupae exposed to atmospheres containing 62, 76 or 89% CO_2 although a 48 h exposure to 62% CO_2 resulted in 96% mortality. At 38°C the exposure required time to attain 100% mortality of T. glabrum pupae when exposed to atmospheres containing

99% CO₂ or 99.5% N₂ was 16 h or less while it took from 48 to 72 h to attain this level of mortality when pupae were exposed to atmospheres containing either 62, 76 or 89% CO₂.

Results of comparable exposures of T. variabile pupae are given in Table 2. The mortalities of this species were very similar to those of T. glabrum pupae when exposed to atmospheres high in CO₂ (94%) or N₂ (99.4%); at 32°C 100% mortality was obtained within 48 h. Also, 90 to 100% mortality was observed when pupae were exposed to concentrations of CO₂ of 63, 77 or 89% for 72 h. At 38°C exposures to a 99.4% N₂ concentration for 16 h resulted in 100% mortality while it took 24 h to obtain total mortality at a CO₂ concentration of 94%. A 100% mortality was observed when pupae were exposed to 89% CO₂ for 48 h and to 72% CO₂ for 72 h. The atmosphere containing 63% CO₂ caused 97% mortality within 72 h.

Quiescent Larvae

Results of the effects of 5 modified atmospheres on active and quiescent larvae of T. glabrum at 32°C and 38°C are presented in Table 3. Generally, mortality did not greatly increase with increasing exposure time and temperature and the quiescent larvae displayed a greater resistance to the atmospheres than did the active larvae. At 32°C a 48 h exposure to ca. 90% CO₂ resulted in 52% mortality of quiescent larvae while 94% mortality was observed for active larvae. Atmospheres containing ca. 99% CO₂ or 99% N₂ caused 100% mortality of both forms of larvae in 48 h at 32°C. Increasing the exposure time at this temperature to 72 h failed to produce the expected increase in mortality.

At 38°C results obtained were comparable with those obtained at 32°C and, in some instance, mortality was lower at the higher temperature when the same exposures were made. This was particularly true of the quiescent larvae. At 32°C, for example, a 48 h exposure of quiescent larvae to an atmosphere containing ca. 90% CO₂ resulted in 52% mortality while the same exposure at 38°C only resulted in 21% mortality.

The mean weight (\pm SD) of 10 quiescent larvae from one replicate of these tests was $3.8 \pm 1.1 \times 10^{-3}$ g. The mean weight of active larvae was $5.1 \pm 1.3 \times 10^{-3}$ g for the group exposed at the same time as the quiescent larvae.

Table 4 presents results of exposures of active larvae and pupae of the two species of Trogoderma under study to either 4 or 5 modified atmospheres. At 32° and 38°C, the time required to obtain 100% mortality of larvae of T. glabrum exposed to modified atmospheres containing ca. 60, 75 or 90% CO₂ was generally longer (more than 3 days) than the time to obtain this level of mortality in pupae (3 days or less). This relationship was not as clear for larvae and pupae of T. variabile at CO₂ concentrations below ca. 99% CO₂, but in some instances such as for exposure to ca. 75% CO₂ at 32°C and 38°C, longer exposures were required to control the larvae than the pupae.

1.5.3. Discussion

The most significant finding in this study is that larvae of both species can be controlled (100% mortality) in 16 to 24 h with CO₂ concentrations approaching 100% and at a temperature of 38°C (Table 4). A near 100% CO₂ concentration could easily be attained and maintained in "truck-ship" type containers and, with the use of adequate vaporization equipment, the CO₂ could be introduced at 38°C. A system consisting of hoses connecting a group of containers in series could be easily designed. This system could possibly be equipped with a recovery tank or recirculation system to avoid unnecessary loss of CO₂. In situations where the ambient temperature is below 38°C, continuous flow of warm CO₂ would bring the commodity (and any insects in the commodity) to the temperature of the modified atmosphere.

In earlier field studies Jay, et al. (1972) showed that a 98.6% mortality of larvae of the wax moth, Galleria melloniella (Linnaeus), was obtained in 10 or 12 h when a semi-trailer type van was treated with an average concentration of 98.6% CO₂. Temperatures in this test ranged from 24° to 40°C and averaged 31.5°C. The treatment showed that high CO₂ concentrations can be attained and maintained in this type of container.

A potential advantage of treatment at high temperatures is that the heat involved may break the diapause of any larvae in this state. Karnavar (1967) reports that diapausing larvae of khapra beetle placed at 30°C and 70% RH completed pupation in 35 days in uncrowded conditions. It is assumed that most diapausing larvae encountered in

containers would not be at population levels termed crowded; a condition which, in addition to low temperature, induces diapause in the khapra beetle.

Table 1. Mortality of *T. glabrum* pupae when exposed to 5 modified atmospheres at indicated temperature (\pm SD). Data are the mean of 3 replicates each consisting of 3 cages containing 10 insects each.*

% Atmosphere			% Mortality after indicated exposur period at:						
			32 \pm 0.4°C			38 \pm 0.3°C			
CO ₂	O ₂	N ₂ **	24 h	48 h	72 h	16 h	24 h	48 h	72 h
0	21	78	0	3	3	1	0	3	1
62	8	bal.	30	96	100	13	44	73	99
76	5	bal.	35	85	100	11	49	99	100
89	2	bal.	29	68	100	23	61	100	100
99	0.1	bal.	92	100	100	100	100	100	100
0	0.5	99.5	93	100	100	100	100	100	100

* Relative humidity (\pm SD); 32°C: 51.8 \pm 6.3%; 38°C: 47.9 \pm 3.8%.

**bal.= balance (includes argon and rare gases).

Table 2. Mortality of T. variabile pupae when exposed to 5 modified atmospheres at indicated temperature (\pm SD). Data are the mean of 3 replicates each consisting of 3 cages containing 10 insects each.*

% Atmosphere			% Mortality after indicated exposur period at:						
			32 \pm 0.3°C			38 \pm 0.5°C			
CO ₂	O ₂	N ₂ **	24 h	48 h	72 h	16 h	24 h	48 h	72 h
0	21	78	0	2	1	0	4	1	5
63	8	bal.	22	64	90	9	31	66	97
77	5	bal.	14	92	100	27	45	95	100
89	2	bal.	21	99	99	31	67	100	100
94	1.4	bal.	87	100	100	93	100	100	100
0	0.6	99.4	91	100	100	100	100	100	100

* Relative humidity (\pm SD); 32°C: 49.5 \pm 3.3%; 38°C: 49.5 \pm 3.8%.

**bal.= balance (includes argon and rare gases).

Table 3. Percent mortality of quiescent (Q) and active (A) larvae of T. glabrum when exposed to 5 modified atmospheres at indicated temperature and exposure period at ca. 49% RH. (Quiescent larvae held 30 days prior to exposure).

Approximate Atmosphere* (%)	32°C				38°C			
	48 h		72 h		48 h		72 h	
	Q	A	Q	A	Q	A	Q	A
Air (control)	0	4	4	1	2	3	2	4
60 CO ₂	16	39	25	34	18	36	12	31
75 CO ₂	28	47	22	66	18	46	17	58
90 CO ₂	52	94	69	94	21	82	44	99
99 CO ₂	100	100	99	100	98	100	100	100
99 N ₂	100	100	100	100	100	100	100	100

* Balance of atmosphere containing CO₂ is O₂ and N₂; balance of N₂ atmosphere is O₂.

Table 4. Time in days to obtain 100% mortality of T. glabrum and T. variabile active larvae (L) and pupae (P) when exposed to 5 different modified atmospheres at 32°C or 38°C,

Approximate Atmosphere* (%)	T. glabrum				T. variabile			
	32°C		38°C		32°C		38°C	
	L	P	L	P	L	P	L	P
60 CO ₂	3+	3	3+	3+	3+	3+	3+	3+
75 CO ₂	3+	3	3+	3	3+	3	3+	3
90 CO ₂	3+	3	3+	2	3+	3+	3+	2
99 CO ₂	2	2	0.66	0.66	2	2	0.66	1
99 N ₂	-	2	-	0.66	-	2	-	0.66

*Balance of atmosphere containing CO₂ is O₂ and N₂; balance of N₂ atmosphere is O₂

1.6. References (for sections up to 1.5.3)

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2. Resistance of insects to modified atmospheres
2.1. SELECTION FOR RESISTANCE TO MODIFIED ATMOSPHERES IN THE RED FLOUR
BEETLE (TRIBOLIUM CASTANEUM HERBST.)

Introduction:

Since modified atmospheres (MAS) generally involve a reduction in Oxygen concentration available for insect respiration and an increment in Carbon dioxide concentration (whereas Nitrogen is not considered to be involved in insect metabolism) it was decided to attempt to select concurrently for a strain of Tribolium castaneum resistant to high carbon dioxide concentrations (HCC) and a strain resistant to low oxygen concentrations (LOC).

Water loss due to changes in the regulatory capacity of the spiracular apparatus and possibly integument and respiratory membranes has been shown to be instrumental in raising insect mortality when MAS are applied at low air humidities (Jay et al., 1971, Navarro & Calderon 1973, Navarro 1975, Jay & Cuff 1980). Therefore it was decided to obviate this effect by supplying the exposure gas mixture at 95% RH.

Materials and Methods:

Experimental material

The insects chosen were a Malathion susceptible strain of Tribolium castaneum obtained from the Pest Infestation Laboratory, Slough, England and reared at the Stored Products Department, A.R.O., Bet Dagan since 1979 in a CT room at $26 \pm ^\circ\text{C}$ and $70 \pm 5\% \text{RH}$. The adult stage was chosen for exposure to the MAS since at this stage the insects are of uniform size, culture techniques enable the separation of adults into uniform age groups, and therefore heterogeneity of the insect material at this stage is at a minimum.

The chosen modified atmospheres

Low Oxygen concentration, (LOC): the gas mixture decided upon was $0.5\% \text{O}_2$, $99.5\% \text{N}_2$ and $95\% \text{RH}$. Preliminary exposures of laboratory stock insects indicated that exposures of $1\% \text{O}_2$ resulted in an LT50 (lethal time for 50% mortality) of above 240 hrs thus prolonging the experimental exposures over inconveniently long periods at the outset. These results are corroborated by those obtained by Navarro 1975. Conversely concentrations lower than 0.5% were difficult to regulate at a steady level and produced extremely heterogenous results.

High carbon dioxide concentration, (HCC): The chosen mixture consisted of 65%CO₂, 20%O₂, 15%N₂ and 95%RH. The high carbon dioxide concentration is similar to concentrations obtained in commercial practice by purging and maintenance with CO₂ from pressurised cylinders or tankers while the addition of 20%O₂ instead of 7%O₂ as would occur when CO₂ is mixed with air is destined to obviate the possibility of simultaneous selection for low O₂ concentrations.

Preparation of the initial strain.

The parent generation was selected from the stock culture at the pupal stage by sexing 5 female and 5 male pupae and isolating them in test tubes in an incubator at 30±1°C and 60±5%RH until adult emergence. Upon emergence the adults were transferred to a 90ml jar containing app 35gm flour. They were then held for a period of up to 10 days after which the flour was sieved through a set of 2 sieves (25 and 60 mesh), the adults being retained above the 25 mesh sieve, the eggs above the 60 mesh sieve and the flour passing through to the bottom container. The adults were then returned to the flour for subsequent oviposition periods of 5 days until a period of one month had elapsed when the parents were destroyed. The eggs of the F1 generation were placed in 150ml jars containing 40gm finely ground wheat and 1gm brewers yeast. These jars were held in the incubator until first F1 adults were visible. Five days later the jars were sieved and F1 adults were removed and placed separately in 150ml jars containing flour for oviposition of the F2 generation. Subsequent sievings every 5 days and transfer of fresh adults to the oviposition jars enabled the production of large numbers of eggs of the F2 generation which were then sieved from the flour and cultured in the food medium as described above.

As the population increased in size the insects were transferred to 1 liter oviposition and culture jars to avoid the effects of overcrowding. In this way over about 2.5 months some 20,000 adults of the F2 generation were produced.

Subdivision of the initial strain into three groups:

Eggs of the F3 generation were separated into three groups, one group to be subjected and selected for tolerance to high carbon dioxide concentration, (HCC), one group to be subjected to and selected for tolerance to low oxygen concentration (LOC), and one group that would be selected for neither HCC nor LOC but would serve as a

susceptible strain for comparison with the other strains, and determination of resistance factors at each successive generation.

Standard culture and exposure techniques, and analysis of results.

At this stage uniform culture and exposure techniques were applied and adhered to throughout the selection procedure; they may be summarized as follows:

Oviposition jars: One liter jars containing up to 250gm flour and 3000-5000 adults. Every 5 days these oviposition jars were sieved for egg removal as described above. The adults for oviposition were obtained from surviving populations of the mass exposures of HCC and LOC selected strains.

Rearing jars: Some 2000-3000 eggs from the oviposition jars were placed in 1 liter jars half filled with ground wheat plus 5% brewers yeast. Rearing jars were sieved every 5 days for removal of adults as soon as they became visible and were discarded after no further adults emerged or a maximum of 5 weeks from the date they were initiated.

Pre-exposure jars: Adults, 0-5 days old obtained from the rearing jars were held in 1 liter jars containing flour to facilitate subsequent separation by sieving. These adults were held for 10 days and then exposed to the MAs in the experimental apparatus described previously.

Exposure techniques: Adults, aged 10-15 days were removed from the pre-exposure jars, sieved from the flour and then collected into groups by use of a suction trap. They were then placed in 100ml erlenmeyer flasks together with approx 1gm ground wheat. The flasks were then linked up to the exposure apparatus and the stoppers pressed firmly down.

Experimental Procedure: Exposure to the two MAs was carried out in an apparatus developed from that described by Navarro & Donahaye (1972). For experiments to determine sensitivity of the insects to the MAs the procedure was adapted to enable probit analysis to be undertaken. six groups of 100 insects were placed in 6 exposure flasks. Five flasks were exposed to give a range of 5 exposure times and the sixth flask served as control. Because of the heterogeneity of response, this experiment was carried out 5 times giving a total of 30 results. At the end of each exposure time, the flask was removed from the apparatus and held in a CT room at $26 \pm 1^{\circ}\text{C}$ and $60 \pm 5\%$ RH for a

further 10 days and then mortality counts were made. All insects failing to show movement when touched were recorded as dead.

Probit analysis was carried out using a Probit analysis program written by Dr R.J. Daum and the Mississippi State University Computing Center (revised 1979) and run on the IBM 370/165 of the Weizmann Institute Computer Center, Rehovot and later on the VAX-11/750 of the A.R.O. Bet Dagan. For each repetition of exposure for the five different exposure times, both the selected strain and the MA susceptible strain were exposed simultaneously: this in order to ensure that variance due to experimental error would be similar for both strains.

Mass exposures: Selection of parent insects to produce the next generation was as follows:- all adult insects of the HCC & LOC selected strains not used for probit analysis were exposed to the MAs in groups of 250 - 350 insects per exposure flask. Adult insects were exposed 10 - 15 days after emergence. Exposure time chosen was approximately that at which 50% of the previous generation had been killed (LT50) and from the 22nd generation on, the level was raised to 70%. Immediately after exposure the insects from each flask were transferred to 90ml post-exposure jars containing about 50gm wheat flour. After 10 days mortality counts were made. These results were used to determine the level of selection at each generation. The surviving insects were transferred to oviposition jars for culture of the following generation, while eggs laid by the surviving insects in the 0 - 10 days post-exposure period were sieved from the flour of each post-exposure jar and transferred to a rearing jar for the same purpose.

Culture of the non-selected strain: This strain which was exposed at each generation for comparison of sensitivity to MAs with the selected strains, was cultured from generation to generation by collecting eggs laid in the flour of the pre-exposure jars; thus for the FC strain the pre-exposure jars were used as oviposition jars and these were sieved after 5 days and 10 days (immediately before exposure of the adults) and eggs obtained were transferred to FC culture jars.

Results and Discussion:

Changes in sensitivity of 24 successive generations to HCC and LOC at the LT 50 level are given in figures 1 to 2. From the figures

it can be seen that the general trend of both the HCC and LOC selected strains was one of increased tolerance to the MA in question.

Fig.1: Sensitivity of *Tribolium castaneum* adults to HCC. Dotted lines represent 95% confidence limits.

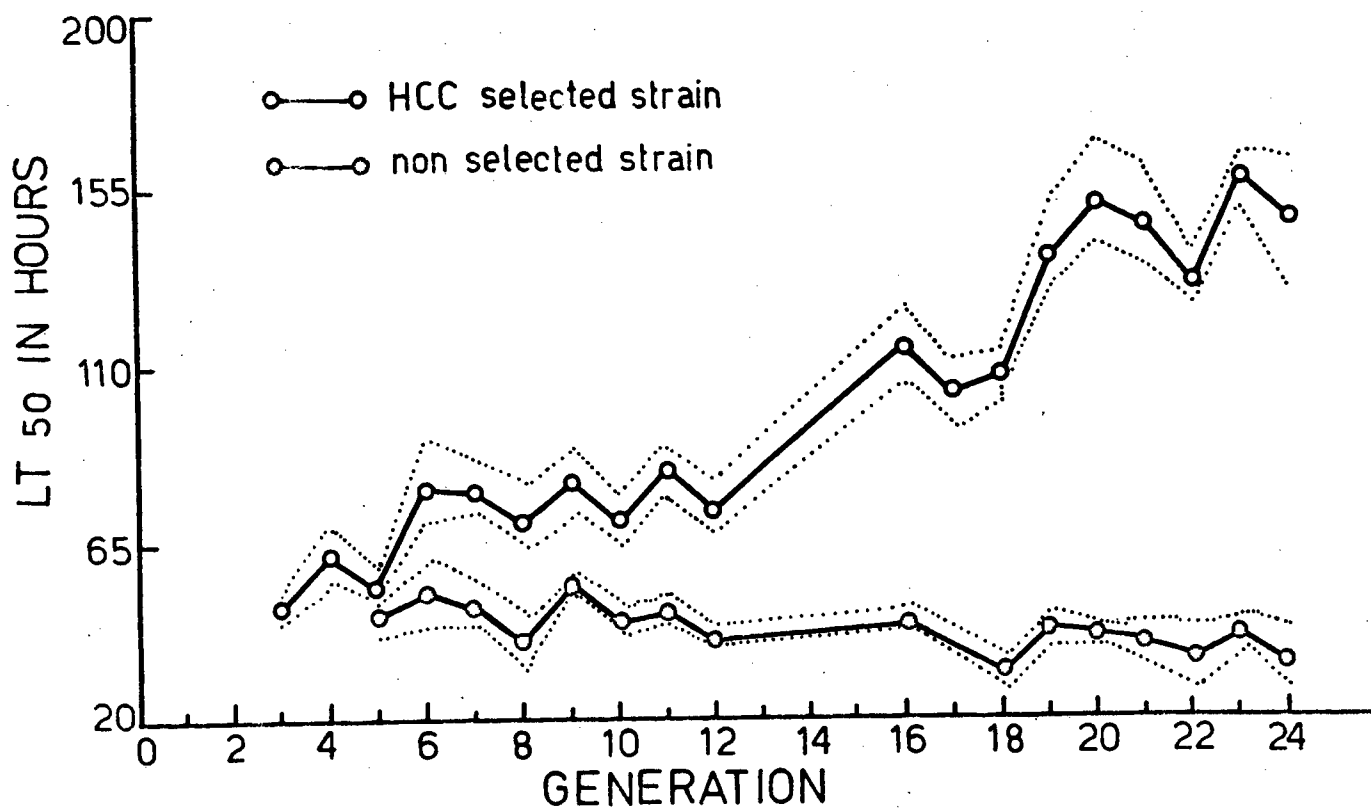
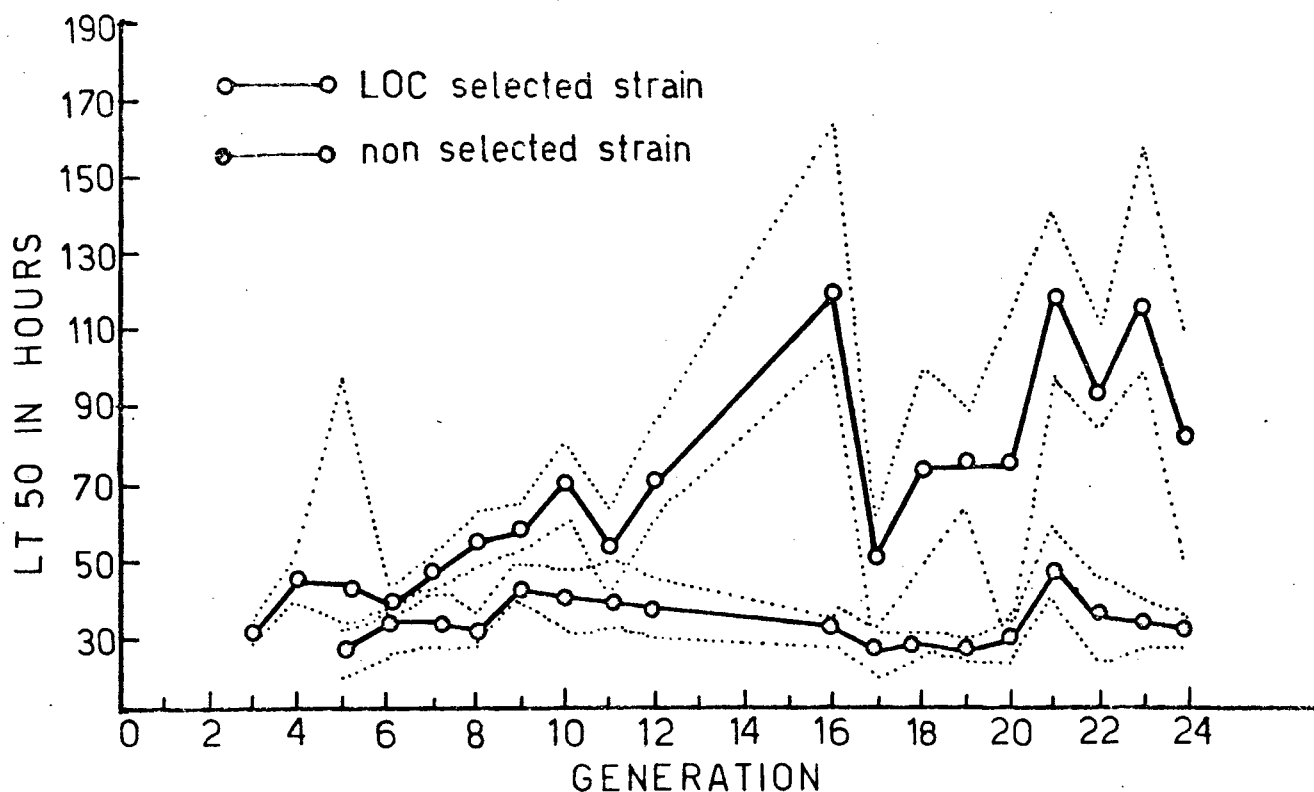


Fig 2: Sensitivity of *Tribolium castaneum* adults to LOC. Dotted lines represent 95% confidence limits.



For the HCC selected strain the general upward trend in resistance is very evident and at the 24th generation there is still no indication of sensitivity leveling towards a plateau, thus suggesting that selection for resistance to HCC is continuing at a rapid if not uniform pace. For the LOC selected strain the large variations in sensitivity between generations of both the selected and

non selected FC strain caused fluctuations in the graph that tend to mask the phenomenon. However the overall upward trend over the 24 selections of the selected strain is clearly demonstrated.

Figures 3 and 4 show the changes in resistance factor of the HCC and LOC strains respectively at each generation at both the LT 50 and LT 99.9 levels. Slopes of probit-lines for the selected strains (HCC and LOC) were generally lower than for the non-selected strain, and non-parallelism prevented relative potency analysis from being carried out.

Fig 3: Changes in resistance factor of the HCC selected strain of *Tribolium castaneum* over 24 generations.

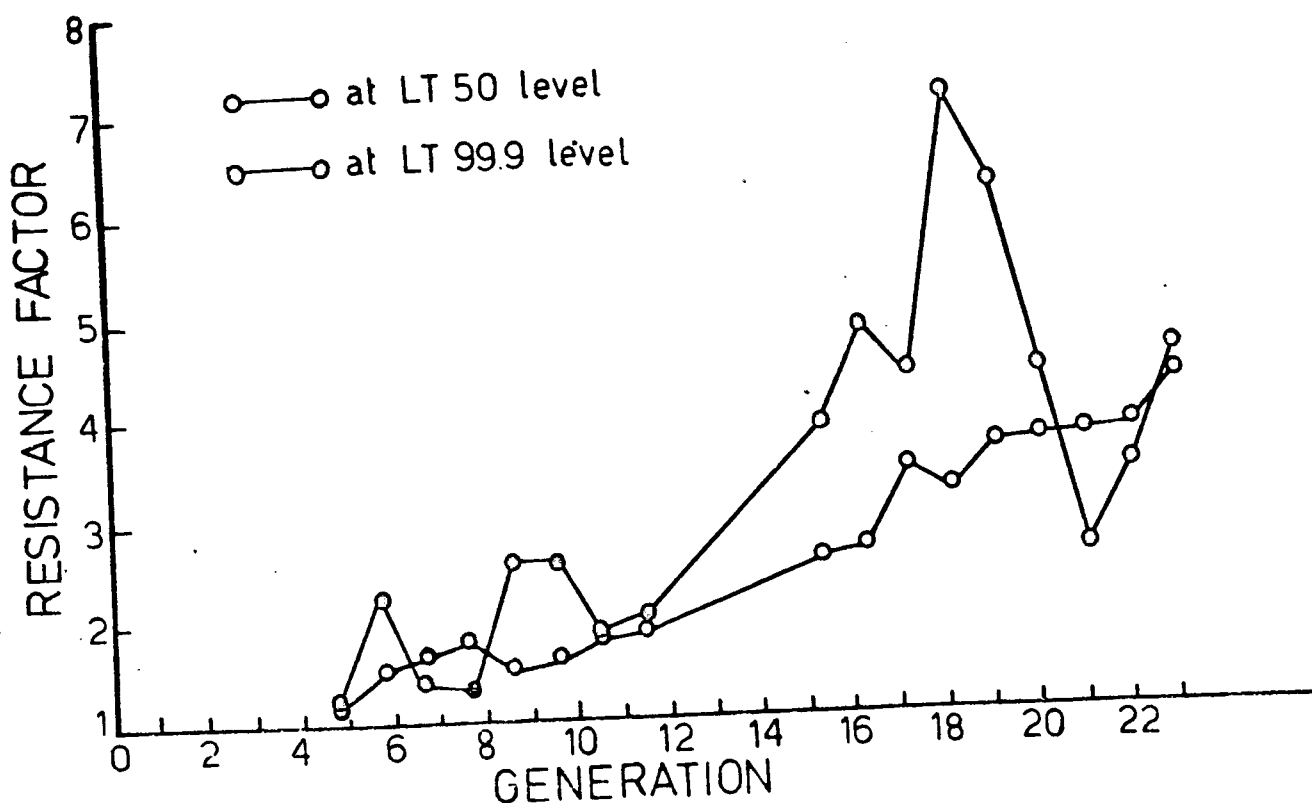
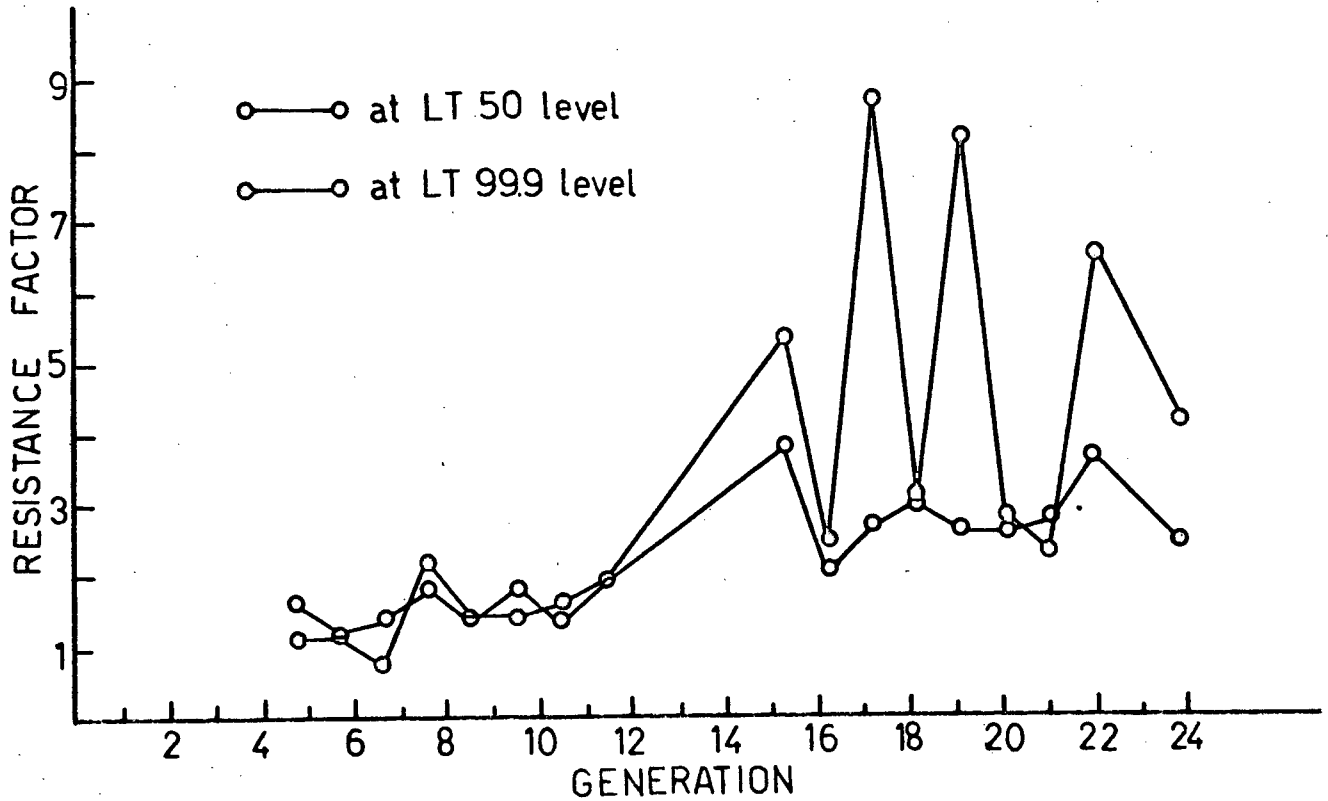


Fig 4: Changes in resistance factor of the LOC selected strain of *Tribolium castaneum* over 24 generations.



For HCC, slope of the selected strain showed no tendency to increase with selection and ranged from 3.33 (19th generation) to 5.63 (23rd generation). This continued high level of heterogeneity over 24 selections indicates a condition of polyfactorial inheritance as found by Monro (1964) for MB induced resistance in *S. granarius*. The steeper slope of the unselected strain resulted in higher resistance factors at the LT 95 level than at the LT 50 level. Although the increase in

tolerance to HCC after 24 generations of selection was low (X 4.24 at LT 50 and X 4.51 at LT 99.9), such a level of tolerance in commerce would demand an exposure period with HCC of 557.3hrs (23.2 days) to produce a 99.9% kill of T. castaneum adults. This would render the method unfeasible in practice.

For LOC, increase in resistance factor over the 24 generations of selection resulted at the F23 generation of resistance factors of X 2.50 at LT 50 and X 4.18 at LT 99.9. Here again slope of probit-mortality lines in the selected strain was frequently lower than that of the unselected strain producing higher RFs at the LT 95 level. The practical implications at this level of tolerance are that 846 hrs (35.3 days) of exposure to 0.5%O₂ would be required to produce 99.9% kill of the selected strain. This again would be both difficult and expensive to obtain in commercial practice and render the economic feasibility of the method questionable.

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2.2. INDUCED TOLERANCE OF SITOPHILUS ORYZAE ADULTS TO CARBON DIOXIDE *

Abstract: Sitophilus oryzae adults were exposed to 40% carbon dioxide (CO_2) in air for seven successive generations and to 75% CO_2 in air for ten successive generations at 26°C and 100% relative humidity (r.h.). The tolerance factor (LT_{95} selected generation/ LT_{95} non-selected generation) at the seventh generation of insects exposed to 40% CO_2 was 2.15 and at the 10th generation exposed to 75% CO_2 , was 3.34. Reduction of the r.h. to 60% in the 75% CO_2 atmosphere caused shortening of exposure times required to obtain the same mortality values, but the tolerance factors remained stable. Removal of selection pressure for five generations of the 40% CO_2 and four generations of the 75% CO_2 selected strains caused significant reduction in tolerance. Augmentation of oxygen concentration to 21% in atmospheres containing 40% and 75% CO_2 did not markedly alter the tolerance factors from those obtained with atmospheres containing the same CO_2 concentrations in air.

INTRODUCTION

The application of modified atmospheres in grain conservation programs has received considerable attention recently (Bailey and Banks, 1975; Banks and Annis, 1980; Navarro et al., 1978). The advantages of using CO_2 as a modified atmosphere gas of choice has

been reported by Jay and D'Orazio (1984). According to Jay (1971), exposure for 4 days to 60% carbon dioxide (CO_2) in air is effective for the control of most stored-product insects. It has been shown that mortality of Tribolium castaneum adults exposed to low oxygen (O_2) atmospheres is also dependent on the concentration of CO_2 present (Calderon and Navarro, 1979).

In field populations of stored-product insects, resistance to the most commonly used fumigants such as methyl bromide, phosphine (Champ and Dyte, 1976; Monro, 1964) and ethylene dibromide (Bond, 1973) has been demonstrated. In a similar way, extensive use of CO_2 in insect control programs might be expected to favor selection of resistant strains. To elucidate the genetic potential of insects to develop resistance to CO_2 alone, Bond and Buckland (1979) investigated the response of Sitophilus granarius to selection. In their study, treatment of successive generations of S. granarius adults produced insects with a 3.3 fold increase in resistance (based on LT_{99}) at 42% CO_2 in seven generations, and a 1.8 fold increase in resistance at 75% CO_2 in four generations. A low degree of resistance to fumigants is all that is required to give rise for concern over insect control. This is because increased dosages are liable to produce excessive residues in fumigated food. Although the risk of residues in the application of modified atmospheres is negligible, the extended exposure time needed for effective control as a result of a marked increase in resistance may render the method impractical. The present study aims at determining the potential of Sitophilus oryzae adults to develop resistance to CO_2 -rich atmospheres.

MATERIALS AND METHODS

Adults of the rice weevil, Sitophilus oryzae (L.), were obtained from cultures reared on wheat with 12% moisture content kept under constant temperature ($26 \pm 1^\circ\text{C}$) and relative humidity (r.h.) ($70 \pm 5\%$). Two-week-old adults were exposed in groups of 200 to gas concentrations in Erlenmeyer flasks of 100-ml capacity containing ca 1 g of wheat. Two different levels of CO_2 in air, a low level (40% CO_2) and a high level (75% CO_2), were chosen for selection of the insects.

Subsequent selected generations were obtained from each CO₂ concentration by rearing from adults that survived treatments in which over 60% mortality was reached. Successive generations reared in a normal atmosphere at 26±1°C and 70±5% r.h., served as control groups.

Since the influence of atmospheric gas concentrations on insects is dependent on humidity (Jay *et al.*, 1971; Navarro, 1978), these experiments were carried out at ca 100% r.h. to eliminate the effects of desiccation. In addition, a series of experiments was carried out in atmospheres with 60% r.h. and 75% CO₂ in air. The gas was humidified by passing it through a gas-washing bottle containing either water (for 100% r.h.) or an appropriate concentration of H₂SO₄ that adjusted it to 60% r.h. (Solomon, 1951).

To test the effect of removal of selection pressure, insects from the last selected generation of strains obtained with 40% and 75% CO₂ were reared without selection for five and four generations, respectively. They were then exposed again to the respective gas mixtures to determine their tolerances. These selection-suspended strains were also tested at the two CO₂ levels (40 and 75%) in atmospheres containing 21% O₂.

Different gas compositions were obtained by preparing mixtures in a 3-liter container supplied with gases from pressurized cylinders of O₂, CO₂ and N₂. These mixtures were passed continuously into the test flasks at a rate of 10-15 ml/min. The whole apparatus, as described by Navarro and Donahaye (1972), was kept in a constant temperature room at 26±1°C. Gas samples of 100 microliters were withdrawn from the flasks once a day and analyzed on a gas-chromatograph equipped with a thermal conductivity detector. The measured atmospheric concentrations in the test flasks are given in Table 1. These compositions varied marginally from the intended concentrations due to minor variations in proportion of the supply gases. For convenience, only the nominal concentrations will be mentioned here.

After treatment, the insects were kept in 100-ml flasks at 26±1°C and 70±5% r.h. for 10 days until mortality counts were made (Lindgren and Vincent, 1970). In all experiments, at least four exposure intervals were used to permit probit analysis of the data (Finney, 1971).

RESULTS AND DISCUSSION

The exposure times required to obtain LT_{50} and LT_{95} values for successive selected generations of S. oryzae adults exposed to 40% and 75% CO_2 , in comparison with non-selected (control) generations are shown in Fig. 1. Non-selected generations showed stability in their response as expressed in the time required to produce 50% and 95% mortality at both 40% and 75% CO_2 . However, for the selected generations there was a gradual increase in the exposure time required to produce the same mortalities. Fig. 1 also provides confidence limits to show significant differences in LTs between the selected and the non-selected generations.

According to Dyte and Blackman (1967), "to demonstrate that a population of insects is resistant, the $LD_{99.9}$ of the resistant strain must be shown to be significantly greater than that of the susceptible strain". Such calculations carried out with our data indicate that the level of resistance obtained meets this requirement. Clearly the $LT_{99.9}$ of both selected strains, for 40% CO_2 (7th generation) and 75% CO_2 (10th generation), were significantly higher than the $LT_{99.9}$ values obtained for the respective non-selected generations. However, low levels of resistance recorded in stored product insects exposed to conventional fumigants have been referred to as tolerance (Bond, 1973; Monro et al., 1972; Hole, 1981). Since the resistance levels obtained in this study with S. oryzae exposed to CO_2 concentrations were also low, the term tolerance is used.

The calculated tolerance factors (based on LT_{95}) for S. oryzae exposed to 40% and 75% CO_2 are shown in Fig. 2. The steady increase in tolerance factors obtained for 40% CO_2 up to 7 generations were greater than those obtained for 75% CO_2 . Although not shown, calculated tolerance factors based on LT_{50} at the 7th generation gave similar results. In the study by Bond and Buckland (1979) with S. granarius adults exposed to 75% CO_2 at LT_{99} level, 1.8-fold resistance was found after four selections at 25°C; in the present study tolerance of S. oryzae after four selections but at 26°C and LT_{95} , was 1.5-fold (Fig. 2). The highest tolerance factor obtained in our work with S. oryzae was 3.34, after 10 generations exposure to 75% CO_2 , and

this may be compared with the highest tolerance factor obtained with S. granarius which was 3.4 after 7 generations exposure to 42% CO₂. These values show the order of tolerance that can be obtained when Sitophilus spp. are selected under the influence of CO₂.

Under normal conditions, the stored commodities may have an equilibrium r.h. ranging from 50 to 70%. Sitophilus oryzae adults suffer increasing mortality as the grain moisture content or the r.h. in the environment is reduced (Longstaff, 1981). Our preliminary results indicate that for this species, low r.h. atmospheres containing CO₂ augments desiccation which affects mortality. This aspect of desiccation under the influence of different modified atmospheres in combination with r.h.'s in the range of 50-55% has been investigated on other stored product insect species (Jay and Cuff, 1981; Navarro, 1978; Navarro and Calderon, 1974). To assess whether the tolerance levels obtained at ca 100% r.h. are also maintained when a lower humidity prevails, the strain obtained after ten successive selections at 75% CO₂ was then exposed to an atmosphere of 75% CO₂ in air at 60% r.h. The results presented in Table 2 indicate that at 60% r.h. both the selected and non-selected strains of S. oryzae were more sensitive than those obtained at 100% r.h. and therefore shorter exposure times were needed to obtain similar mortality levels. At 60% r.h., exposure times to obtain LT₅₀ and LT₉₅ of the selected strain were 67% and 48%, respectively, of those at 100% r.h. For the non-selected strain at 60% r.h., the LT₅₀ and LT₉₅ were 68% and 55%, respectively, of those at 100% r.h. Thus, at the LT₅₀ level, the shorter exposure times due to the low r.h. effect were maintained at a similar ratio for the selected and non-selected strains, while at the LT₉₅ level there was a slight change. Similarly, it is clear that the tolerance factors calculated at LT₅₀ remained at the same level (2.26 at 100% r.h. and 2.22 at 60% r.h.), while at LT₉₅, the tolerance factor ca 100% r.h. (3.34) was slightly higher than that at 60% r.h. (2.87). These results show that the increased tolerance at 100% r.h. was also largely maintained at 60% r.h. This would indicate that the tolerance obtained was mainly in response to the action of CO₂. However, the combined action of CO₂ and low humidity atmospheres on insect resistance, requires further investigation.

To determine whether suspension of the selection pressure would cause any change in tolerance of the test insects, the strain obtained from 40% CO₂ (7th generation) was maintained without selection for five additional generations and the 75% CO₂ strain (10th generation) was maintained without selection for four additional generations. The results given in Table 3 show that there is a partial reversion of the tolerance obtained for the 40% and 75% CO₂ strains after selection was suspended. In experiments carried out by Monro (1964) with S. granarius exposed to methyl bromide, the selection process was suspended at the 14th generation. In his work, there was no reversion of resistance, and he concluded that the strain obtained was isogenic for tolerance to methyl bromide. On the other hand, there are reports where resistance has decreased when the selection pressure with fumigants is removed (Monro et al., 1972; Bond and Uptis, 1972). However, little is known of the mechanisms of insect resistance to fumigants. According to Bond and Uptis (1976), it appears that several genes are involved in the development of their resistant strain of S. granarius to methyl bromide, so more than one mechanism may operate. Investigations with Ephestia cautella (Wlk.) pupae (Friedlander and Navarro, 1979; 1983; Friedlander et al., 1983), have shown that CO₂ has an influence at several metabolic sites, and this may enable the development of a number of resistance mechanisms within the same strain. The results shown in Table 3 suggest that these strains were not completely isogenic, and apparently this was the cause for decline in tolerance.

In commercial application of CO₂, the O₂ concentration is reduced by replacement of a large part of the atmosphere with CO₂. Under these conditions, the toxicity of CO₂ is influenced by the availability of O₂ in the atmosphere. In the selection for resistance, the atmospheric compositions applicable to commercial conditions were simulated by running the tests with CO₂ atmospheres containing low O₂ concentrations (Fig. 1). To obviate the influence of low O₂ concentrations on the toxicity of CO₂, 40 and 75% CO₂ atmospheres supplemented with 21% O₂ and the balance N₂ were also tested on the selection-suspended generations. From Table 4, it is evident that increase in O₂ to 21% did not alter significantly the LT values nor

the tolerance factors obtained for the tested CO₂ concentrations. Therefore, the tolerance factors obtained in these tests are largely due to the action of CO₂.

The above results indicate that S. oryzae has the genetic potential to develop resistance to CO₂. The role of O₂ and that of r.h. at the tested levels were of minor influence on the tolerance obtained and the action of CO₂ was dominant. The suspension of selection caused a considerable reduction in the tolerance factors. In view of the low rate of development of resistance exhibited by S. oryzae, and the fact that under field conditions CO₂-treated insect strains are likely to mix with non-treated populations, the risks of endangering the practical application of modified atmospheres are greatly reduced. Therefore, it would be advisable to integrate the modified atmosphere technique with other control methods (Navarro and Calderon, 1980) to minimize the risk of selection of resistance to CO₂ over successive generations. Furthermore, care should be taken that CO₂ is not used in situations where the treatment is likely to be incompletely effective, so as to minimize selection of CO₂-resistant strains. Since this is a non-toxic residue treatment, an increase in CO₂ concentration and extension of exposure time, would not have a harmful effect on the treated commodities.

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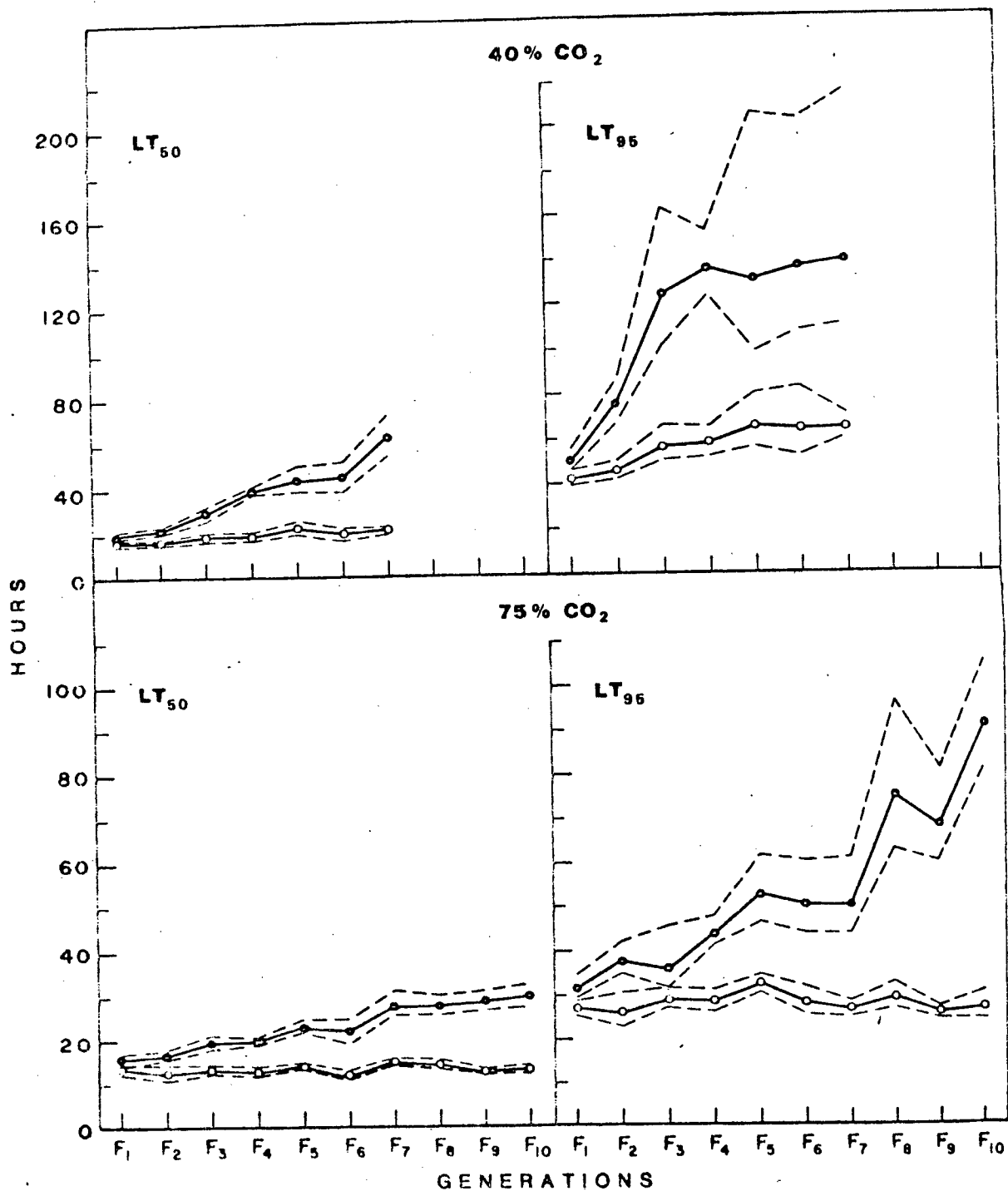


Fig. 1. Response of selected (●—●) and non-selected (○—○) generations of *Sitophilus oryzae* adults exposed to 40% and 75% carbon dioxide in air. Exposure time to obtain 50% mortality (LT₅₀) and 95% mortality (LT₉₅) is represented by a continuous line, while the 95% confidence limit for each treatment is represented by a dotted line.

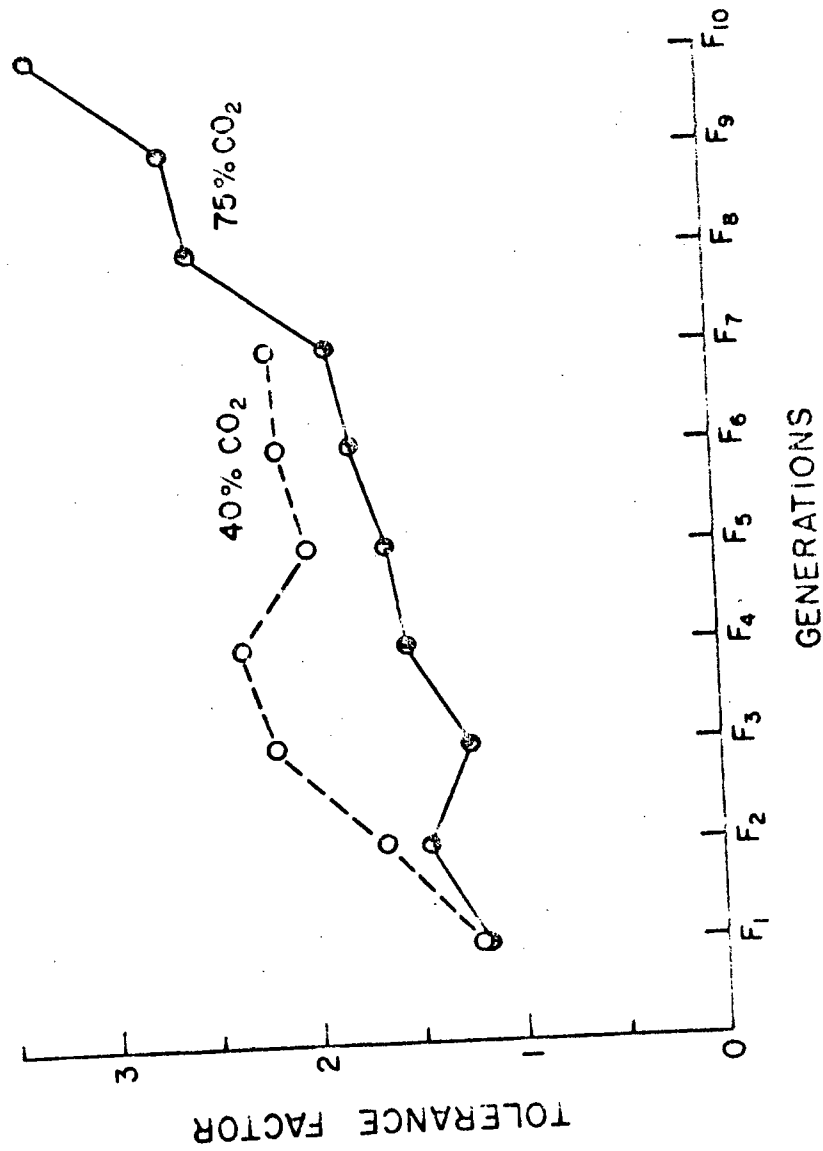


Fig. 2. Tolerance factors (LT₉₅ selected generation/LT₉₅ non-selected generation) obtained in successive generations of Sitophilus oryzae adults exposed to 40% and 75% carbon dioxide in air.

Table 1 - Nominal and measured average atmospheric concentrations to which Sitophilus oryzae adults were exposed for selection of resistance.

Nominal atmospheric concentrations (%)	Actual atmospheric concentrations (% \pm S.E.)		
	CO ₂	O ₂	N ₂
40 CO ₂ in air	40.6 \pm 0.19	12.3 \pm 0.13	47.1 \pm 0.18
40 CO ₂ with 21 O ₂	41.1 \pm 0.34	20.4 \pm 0.28	38.5 \pm 0.34
75 CO ₂ in air	74.5 \pm 0.21	5.2 \pm 0.08	20.3 \pm 0.17
75 CO ₂ with 21 O ₂	74.9 \pm 0.12	20.9 \pm 0.06	4.2 \pm 0.09

Table 2 - Response of selected and non-selected strains of *Sitophilus oryzae* to 75% CO₂ (in air) in atmospheres of 60% RH and 100% RH at 28°C. Confidence limits (95%) for each treatment are presented in parentheses.

Treatment (%RH)	Insect strain	Mortality (h)		Tolerance factor*
		LT ₅₀	LT ₉₅	
100	Selected	30.5 (27.9 - 32.9)	91.1 (81.3 - 105.2)	3.34
	Non-selected	13.5 (12.5 - 14.5)	27.3 (25.2 - 31.1)	
60	Selected	20.4 (15.3 - 24.3)	43.6 (34.9 - 71.6)	2.87
	Non-selected	9.2 (8.7 - 9.7)	15.2 (13.6 - 18.3)	

* Based on LT₉₅ values.

Table 3 - Effect of suspending selection pressure on the tolerance of *Sitophilus oryzae* to 40% and 75% CO₂ in air at 26°C and 100% RH. Confidence limits (95%) for each treatment are presented in parentheses.

Treatment (%CO ₂)	Insect strain	No. of successive selections	No. of non-selected generations after the last selected generation	Mortality (h)		Tolerance factor*
40	Selected	7	-	63.4 (54.8 - 72.9)	138.9 (110.5 - 215.4)	2.15
	Non-selected	-	-	21.5 (20.3 - 22.7)	64.7 (60.2 - 70.3)	
	Selected	7	5	45.7 (42.7 - 48.8)	83.1 (74.9 - 95.6)	1.35
	Non-selected	-	-	21.3 (19.6 - 23.1)	61.7 (51.9 - 78.9)	
75	Selected	10	-	30.5 (27.9 - 32.9)	91.1 (81.3 - 105.2)	3.34
	Non-selected	-	-	13.5 (12.5 - 14.5)	27.3 (25.2 - 31.1)	
	Selected	10	4	37.0 (32.0 - 40.6)	72.0 (65.4 - 82.1)	2.35
	Non-selected	-	-	16.2 (14.6 - 17.7)	30.7 (27.2 - 35.5)	

* Based on LT₉₅ values.

Table 4 - Response of selected and non-selected strains of *Sitophilus oryzae* to 40% and 75% CO₂ in atmospheres supplemented with air or 21% O₂ (at 26°C and 100% RH). Confidence limits (95%) for each treatment are presented in parentheses.

Treatment	Insect strain	Mortality (h)		Tolerance factor *
		LT ₅₀	LT ₉₅	
40% CO ₂ in air	Selected	45.7 (42.7 - 48.8)	83.1 (74.7 - 95.6)	1.35
	Non-selected	21.3 (19.6 - 23.1)	61.7 (51.9 - 78.9)	
40% CO ₂ with 21% O ₂	Selected	42.9 (40.5 - 45.3)	79.3 (72.2 - 90.0)	1.67
	Non-selected	18.4 (16.8 - 20.0)	47.4 (40.8 - 59.1)	
75% CO ₂ in air	Selected	37.0 (32.0 - 40.6)	72.0 (65.4 - 82.0)	2.35
	Non-selected	16.2 (14.6 - 17.7)	30.7 (27.2 - 36.5)	
75% CO ₂ with 21% O ₂	Selected	38.2 (30.4 - 44.1)	73.6 (62.7 - 97.9)	2.19
	Non-selected	15.6 (12.6 - 17.8)	33.6 (27.9 - 49.0)	

* Based on LT₉₅ values.

3. Application techniques for modified atmospheres

3.1. SEALING EFFICIENCY ASSESSMENT IN MODIFIED ATMOSPHERES STORAGES

Abstract - The leak area that would permit air infiltration into an experimental silo of 665.7-1 capacity was assessed with constant pressure tests on orifice cross-section areas varying between 22.99 and 1005.96 mm². The relationship between orifice cross-section area and its length on the variations of the empirical constants that describe the constant pressure test was also demonstrated. Based on a series of tests an empirical equation to estimate leak rate area was proposed as a guideline.

The experimental silo, which was filled to 92% of its volume with ca. 500 kg of wheat, was tested for carbon dioxide (CO₂) loss using different sized orifices. The measured CO₂ concentrations were compared with the calculated values based on equations that consider initial CO₂ adsorption by wheat, diffusion of CO₂ through the leak, and variations in temperature and barometric pressure. Under the experimental conditions, close agreement between the measured and the calculated values was obtained. The influence of temperature and barometric pressure variation on infiltration of air into the silo was also analyzed. The information obtained from the constant pressure tests and from analysis of the weather conditions provided guidelines on the time that a certain concentration could be maintained in a CO₂ treated structure. Leak rates for any structure may be assessed by the method developed in these experiments.

INTRODUCTION

A fundamental requirement for the application of modified atmospheres (MA) to control stored-product insects is a well-sealed storage. In such a structure a MA created by the application of carbon dioxide (CO₂) can be maintained at an efficient level by restricting air ingress, which otherwise

would eventually cause a decrease in the CO_2 concentration and an increase in the oxygen (O_2) level. The requirement for gastight storages for the application of MA appears to be more critical than the requirement for the application of fumigants. Fumigants have been used for many years with minimum requirements for structure gastightness, and covering the grain bulk or the storage under plastic sheets was usually considered satisfactory (Monro, 1969). Only recently have the consequences of poorly sealed storages under fumigation been discussed by Banks and Desmarchelier (1979) and Banks (1981) and the necessity for adequate gastight structures been recognized as the primary factor responsible for the successful application of MA to insect control (Banks and Annis, 1977).

Methods to determine gastightness of a system have been investigated for different purposes. For the analysis of the energy requirements of buildings, air infiltration is a primary source of energy loss and this infiltration can be measured experimentally. Hunt (1980) has reviewed some tracer gas techniques to measure air infiltration into buildings and compared fan-pressurization-evacuation procedures to estimate the comparative tightness of these structures. Carbon dioxide has also been used experimentally as a tracer gas. Hill and Kusuda (1975) studied the dynamic characteristics of air infiltration into buildings in order to calculate the heating and cooling load for the prediction of seasonal energy requirements. The ASHRAE Handbook of Fundamentals (1972) describes the air change method and the crack method in predicting air infiltration rates. The crack method is usually regarded as more accurate as long as the leak characteristics can be evaluated properly (Hill and Kusuda, 1975). With this method airflow is expressed:

$$Q = c \cdot \Delta P^n$$

where Q = the volumetric flow rate of air; c = the proportionality constant;

n = the exponent between 0.5 and 1; and ΔP = the pressure difference exerted on an enclosure.

In silage systems, a sealed shell is designed to limit the entry of O_2 in order to minimize losses in quality. Meiering (1982) investigated two types of pressure tests; the constant pressure test and the variable pressure test for measuring specific silo permeabilities. The effect of variations in environmental temperature and atmospheric pressure on the O_2 intake of silos was simulated and the permeability limits required for proper O_2 control in silos containing silage were defined.

Gas interchange in freight containers and factors leading to gas interchange between containers and the external atmosphere were detailed by Banks et al. (1974). Their observations, using the constant pressure test, indicated that the relationship between the applied pressure and the gas leak rate gave a useful measure of gastightness. Banks and Annis (1977) developed a practical guide for the storage of dry grain under MA and specified requirements for silo gastightness. Their specifications correspond to pressure decay times needed to maintain the atmospheric composition in the silos. Sharp (1982) using the constant pressure test, quantified the gastightness of sealed structures.

The decision on what degree of gastightness should be satisfactory for MA storage or what gas concentrations can be maintained under given environmental and structural conditions should be weighted against the investment involved in improving gastightness to prevent excessive gas usage in poorly sealed storages. The present work studies an approach to assess gas losses from grain bulks treated with CO_2 under varying temperatures and atmospheric pressures.

MATERIALS AND METHODS

Equipment:

Pressure measurements were carried out using a Pace Wianko pressure transducer kit with a model CD25 indicator and model KP15 transducer having interchangeable diaphragms. The transducer source was calibrated against a Dwyer model 400-23 inclined manometer.

Temperatures were measured using thermistor probes connected to a Yellow Springs Instrument Co. Model 47.1 scanning tele-thermometer,. Two Yellow Springs model 80A continuous recorders were connected to the equipment; one to the pressure indicator and the other to the scanning tele-thermometer. These recorders have a constant chart speed of 2.54 cm/hr, so quicker transducer responses were obtained on a 25.4 cm scale, using a Hewlett-Packard model 7128A recorder.

Barometric pressure changes were recorded on a Belfort Inst. Co., No. 5-800A microbarograph, with a weekly chart record. This microbarograph was calibrated and periodically checked against a Fortin type Fisher Scientific Co., model 02-380 mercury barometer .

Air flow was measured using either a Gilmont K-3200-00 float type variable-area flowmeters having several ranges or a Sierra Instruments, Inc., model 715 mass flowmeter. Flowmeters were calibrated against a bubble type flow meter. The mass flow meter was used for air volumes up to 50 l/min while for higher air volumes an Alnor Thermoanemometer Model 8500 was employed.

Atmospheric gas composition was analysed with a Fisher-Hamilton Model 29 dual column gas-chromatograph equipped with a thermal conductivity detector. A Hewlett-Packard integrator model 3390A was used to measure the areas under the peaks.

Different length and diameter orifices were used to simulate leaks used during the experiments. These were prepared either by using hypodermic needles having measured diameters (for diameters less than 3 mm), or by drilling holes on removable flanges mounted on a laboratory scale silo. The silo was 665.7 l in capacity and was 259.5 cm high with an internal diameter of 57.15 cm. The silo was constructed by welding three commercially available metal drums together. The wall thickness of the drums was 0.825 mm.

Constant pressure test for determination of leak area: Small and large leaks were studied in 2 series of tests. In the series of experiments with small leaks needles having openings of 0.5687 mm^2 , 1.1373 mm^2 , and 1.4233 mm^2 dia. with orifice lengths of 5, 20, and 40 mm were used. Since variations in the ambient temperature and barometric pressure affected the results obtained when these small leaks were located on the 665.7 l silo wall, a parallel series of experiments were carried out while the leak was mounted on a 0.95 l glass container.

The large leaks were made on a removable side plate of the silo which was 3.15 mm thick and the orifices sizes ranged between 22.99 mm^2 and 1005.96 mm^2 in cross-section area. During a test, air was applied from a pressurized cylinder into the silo and the ratio of the volumetric flow to the final equilibrium pressure reached was recorded. The general equation:

$$Q = KA \Delta P^n \quad [1]$$

was used to obtain the value for proportionality constant (KA). Since at equilibrium the airflow into the silo equals the total air leakage out of the silo the pressure difference (ΔP) could be correlated to K which is the empirical constant parameter and A is the effective leak area. Sharp, 1982 and Dickson, 1981 found that the value of n close to 0.5 for a sharp-edged orifice and near 1 for cracks.

CO₂ decay tests:

Silos having a volume of 665.7 l, and the same dimensions as the one described above were used for CO₂ decay tests. These tests were carried out with the silos containing 499.4 kg of soft red winter wheat having a 12.5% moisture content. The wheat occupied 92% of the silo volume.

The CO₂ gas was supplied from pressurized cylinders. After a certain CO₂ concentration was attained 2 to 3 days were allowed to elapse so that the gas could reach uniformity in the silo. Gas samples were taken periodically using a 10 ml Hamilton model 1010 gastight syringe fitted with a 5 cm long side hole model 6-90224 needle. Gas sampling ports were located at 42, 131, and 218 cm from the bottom of the silo (Fig. 1). Carbon dioxide concentrations were analyzed on the gas chromatograph.

The silos were equipped with 8 temperature measuring points as indicated in Fig. 1. Silo pressure was measured from the center of the silo through a tygon tube of 5.7 mm i.d. connected to the transducer. All experiments were carried out in a controlled temperature room and attempts were made to maintain the temperature at 25±1°C in the experiments.

Calculation of CO₂ loss:

The calculation of CO₂ loss was based on a series of assumptions:

1. The effect of wind was negligible, since the leak was protected from any direct air circulation in the chamber which could have been caused by the air conditioning system in the room where the silos were held.
2. Any CO₂ produced by grain respiration was negligible and the grain was free from insect infestation.
3. The volume occupied by the grain was measured according to the method proposed by Day (1964) and the calculated void space of 288.71 l remained constant.

4. The diffusion rate through the leak was $16.1 \text{ mm}^2/\text{sec}$ corrected for 25°C and 760 mm Hg (Roberts, 1963).
5. The concentration of CO_2 in the silo was uniform.
6. The CO_2 concentration outside the silo was equal to the atmospheric CO_2 concentration.
7. The representative silo temperature was measured in the center of the empty silo and, in the silo containing wheat, the influence of external temperature affected only the headspace volume and the bulk temperature remained constant.
8. The variations in temperature and barometric pressure, and the diffusion through the leak caused air to infiltrate the void space in the silo. The volume of air infiltrating into the silo caused a change in concentration by a uniform diluting effect. The rate in change of CO_2 concentration (C_t) with time was uniform throughout the silo.

Based on these assumptions, if V_a is the volume of air infiltrating the silo and V_e the void volume of the silo, then the change in concentration, dc , as each increment of volume is removed is equal to the concentration, C_o , times the fraction of the volume withdrawn $d V_a$ (Nelson, 1971);

$$\frac{dc}{dt} = \frac{C_o dV_a}{V_e} \quad [2]$$

This integrates to give a resultant concentration C_t at time t .

$$C_t = C_o e^{-\frac{V_a}{V_e} t} \quad [3]$$

The volume of air CO_2 diffusing out of the silo is equal to the volume of air entering the silo from the diffusion rate equation:

$$V_{a_d} = \frac{AXDX \ C_o X 3.6t}{L(X10^8)} \quad [4]$$

where Va_d = amount of CO_2 which diffused through the leak, m^3 ; L = length of the leak, mm; A = cross-sectional area of the leak, mm^2 ; t = time during which a given amount of CO_2 was diffused, h; $\Delta C_o = CO_2$ concentration difference between the silo and ambient in %; and D = diffusion coefficient mm^2/s .

Fluctuations in the gas temperature resulted from solar radiation, ambient air temperature, wind, precipitation, and on the thermal properties of the construction material of the silo. The total number of temperature changes is directly proportional to the frequency and amplitude of temperature fluctuation in the silo void space. Therefore, changes in the silo gas temperature can be approximated by the linear functions (Meiering, 1982) under rising or falling silo void space temperature:

$$T_s = T_1 + \sum_{t=0}^{t=t_i} T \quad [5]$$

where T_s = silo gas temperature, $^{\circ}K$; T = initial silo gas temperature, $^{\circ}K$; T = temperature rate of change $^{\circ}C/h$; and t = time in h.

Since a decrease in temperature is involved in the air infiltration into the silo void space, the proportion of $\frac{T \cdot t}{T_1}$ can be considered to be the influence of temperature change on the volume of air which infiltrates into the silo. In practice, in the field, nighttime temperature affects the headspace of the silo and the wheat to a depth of approximately 0.15 m from the outside wall (Oxley, 1948; Muir, et al., 1980). If the void space portion of the silo under the influence of ambient temperature is V_h , the volume of air entering the silo due to temperature change is:

$$Va_t = \frac{V_h T \cdot t}{T_1} \quad [6]$$

Similarly, the increase in barometric pressure will cause air infiltration into the silo. The changes in barometric pressure may be approximated by the linear function:

$$P_s = P_1 + \bar{P} \cdot t \quad [7]$$

where P_s = silo gas pressure in Pa; P_1 = the initial atmospheric pressure; and \bar{P} = rate of atmospheric pressure change in Pa/h. Since the atmospheric pressure would affect the effective total void volume (V_e) of the silo, the volume of air entering the silo due to atmospheric pressure change is:

$$V_{ap} = \frac{V_e \bar{P} \cdot t}{P_1} \quad [8]$$

In calculating V_{at} and V_{ap} the basic assumption is that the volume infiltrating the silo is not restricted by leaks. It is obvious that in an extremely gastight structure not equipped with a pressure relief valve that air infiltration into the silo during sudden changes in temperature or barometric pressure will be independent of the leak characteristic. When this happens the ΔP required to cause air infiltration is approximately one-half of its value.

The time required for this process is important and, for a given time t_p :

$$\Delta P = \frac{V_{at} + V_{ap}}{2 t_p V_e} \quad [9]$$

by substituting (9) into (1)

$$Q = KA \left[\frac{V_{at} + V_{ap}}{2 t_p V_e} \right]^n \quad [10]$$

$$\text{so that } \log Q = \log KA + n \log \left[\frac{V_{at} + V_{ap}}{2 t_p V_e} \right] \quad [11]$$

If A and t_p are sufficiently small values, then Q will be:

$$Q < \frac{V_{at} + V_{ap}}{t_p} \quad [12]$$

In this case, the the quick volume change would result in a pressure change in the system and then the value Q should be combined into (3) as:

$$C_t = C_{oe} - \frac{V_{ad} + Q}{V_e} t \quad [13]$$

but if A and t_p are large values, then Q is:

$$Q > \frac{V_{at} + V_{ap}}{t_p} \quad [14]$$

and the equation (3) can be transformed to:

$$C_t = C_{pe} - \frac{(V_{ad} + V_{at} + V_{ap})}{V_e} t \quad [15]$$

The validity of this approach was investigated using the 665.7 l experimental silo containing wheat. With this approach, when a silo is purged with CO_2 , the initial concentration change simply due to adsorption is neglected but if the adsorption rate is known, the expected CO_2 concentration drop can be estimated. Unpublished laboratory results indicate that the adsorption isotherm of CO_2 for wheat follows the classic Freundlich equation:

$$\log V_{CO_2} = a + b \log P_{CO_2} \quad [16]$$

where, V_{CO_2} = volume of CO_2 adsorbed by the commodity, ml/kg; a and b = constants at a given temperature; and P_{CO_2} = partial pressure of CO_2 .

Preliminary studies with wheat show the values to be $a = 2.3883$; and $b = 1.7191$ at $25^\circ C$. The volume of CO_2 adsorbed by wheat will cause a negative pressure in the system and the volume of air infiltrating in to the silo will cause a diluting effect which will reduce the CO_2 concentration. This will cause a different rate of adsorption than that obtained at a constant CO_2 concentration. Since the volume occupied by grain may vary from one silo to another, the resulting adsorption rate would also vary in relation to the changing CO_2 concentration. Although this relationship has not yet been accurately determined, the following equation may be used to estimate the

expected CO_2 concentration at the end of adsorption process until more detailed information becomes available:

$$C_{ta} = C_o e^{-\frac{V \text{CO}_2 M}{V_e \times 10^6}} \quad [17]$$

where $V\text{CO}_2$ is the volume of CO_2 adsorbed by grain (from 17) in $\text{ml}/\text{CO}_2/\text{kg}$ grain; C_o is the initial CO_2 concentration in percent; M is the mass of grain in kg; and V_e is the effective void volume of the silo in m^3 . Then C_{ta} equals the CO_2 concentration in percent at the end of the adsorption process.

RESULTS AND DISCUSSION

Constant pressure tests:

The volumetric flow in small leaks or cracks is strongly dependent on the orifice length (L). This relationship as measured for a cross-section area of 1.423 mm^2 with various orifice lengths is shown in Fig. 2. The longer the orifice the higher value of the empirical parameter (n). Kreith and Eisenstadt (1975), found an expression of length-to-diameter (L/D) to be a function of the empirical parameter (n) when determining flow characteristics of short capillary tubes. Using a similar approach, the results obtained for different (n) values in Fig. 2 were plotted against orifice length/diameter (L/D) values for the tested leaks of various lengths and are shown in Figure 3. This relation would clearly indicate that where cracks exist in the structure the flow characteristics necessary to determine the empirical parameter of KA (Equation 1) will be strongly dependent on the orifice depth which eventually will affect the characteristic pressure-drop exponent (n). Figures 2 and 3 show that unless the orifice depth is maintained constant the empirical parameter KA can not be generalized in

relation to exponent (n). Additional research is needed to quantify the relation of these empirical constants.

Various leak sizes were studied in the present work for a fixed orifice length (3.15 mm) to determine the relationship of the empirical parameters of (KA) and (n). The calculated (K) parameter for ten different sizes of cross-section areas under the influence of pressure varying from 1 to 60 Pa was $K = 4.0516 \times 10^{-3}$ (+ S.E. = 0.1248×10^{-3}). Under the same conditions the characteristic pressure exponent was found to be $n = 0.55105$ (+ S. E. 0.00573). These empirical values can be plotted on equation (1) by:

$$Q = A 4.0516 \times 10^{-3} \Delta P^{0.55105} \quad [18]$$

where Q = the volumetric air flow per m^3/h ; A = the leak area per mm^2 ; and ΔP = the pressure difference in Pa. Since these values were tested for ΔP values up to 60 Pa, the resulting Q values of equation (18) can be demonstrated in Figures 4 and 5. The determination of the leak area of a given silo, from a practical view where the empirical exponent $n = 0.55105$ is applicable, would require only a single constant pressure test in order to estimate the approximate leak cross-section area in the silo. Since these values were studied on round orificies having a fixed length, more experimental values are needed to express the relation of the empirical parameters (KA) and (n) for different leak configurations. Therefore, the values given in equation (18) and Figures 4 and 5 apply only to the experimental conditions of this experiment and further experiments are required to obtain more accurate results which would describe the leaks of general cases.

Air infiltration into the experimental silo:

Four sets of experiments with leak cross-section areas of 0.068, 0.586, 1.478, and 22.134 mm^2 were carried out while the temperature, barometric

pressure and gas composition of the experimental silo was monitored. The results in the loss of CO_2 as measured on samples taken from the silo and the calculated concentrations based on equation (15) are shown in Figs. 6 A, B, C, and D. The characteristic changes in barometric pressure and ambient temperature were considered in calculating the CO_2 loss, and they were plotted against each period of time of 20 hr, 10 hr, or less. Figure 6 A, B, C, and D show results obtained for different leak sizes for different initial CO_2 concentrations ranging from 34.1% to 51.9%. Although the experimental silos were maintained in a controlled temperature room with a thermostat setting of 25°C , the changes in temperature outside the room influenced the frequency of thermostat activation due to energy loss from the room to the outside air. This influence caused the thermostat to activate the heating unit for intermittent periods, especially when the ambient temperature was in the range of $0 - 10^\circ\text{C}$. The thermostat was shut off to demonstrate the strong influence of these periodic temperature changes on CO_2 decay. In Fig. 6A and D, the CO_2 loss was observed at the start of the experiment when the thermostat was shut off, and the sharper loss observed toward the end of the experiment was largely due to the influence the periodic operation of the heater to maintain the room at 25°C . This periodic increase in room temperature was accompanied by a decrease in pressure in the silo and these periodic changes in the internal silo pressure caused a pumping effect. The increased and decreased pressure caused by the temperature changes resulted in blowing gas out or sucking air in to the silo. Occasionally the gas flow into or out of the silo was measured by connecting a mass flow meter to the leak. This was done to determine whether the temperature change and the resulting pressure change was maintained for a sufficient time to permit the expected

mass of air to flow into the silo. This was especially important with the smallest leak diameter of 0.0677 mm^2 as shown in Figure 6D.

The typical ambient temperature changes experienced in these observations do not represent field conditions where the changes in ambient temperatures may be much larger and exist for extended periods to time. However, T values (Equation 5) calculated in these experiments ranged between $\pm 0.13^\circ\text{C/h}$ and $\pm 3.56^\circ\text{C/h}$. These values may be compared with typical temperature changes under maritime weather conditions which may vary between $\pm 3^\circ\text{C/h}$ and $\pm 10^\circ\text{C/h}$, as observed by Meiering and Wenner (1970).

The temperature changes which affected the silo internal pressure could be best detected when the silo had a cross-sectional area leak of less than 1.478 mm^2 . Typical silo pressure changes recorded during the observations with a leak cross-section area of 0.586 mm^2 and 0.068 mm^2 are shown in Fig. 7 A and B. This figure shows that the smaller the leak, the greater the influence of the temperature changes on the build-up of internal pressure. This may be especially important in horizontal silos having a large head space volume and the use of a pressure relief valve in tightly sealed silos is recommended by Banks and Annis (1977) to prevent damage to the structure during critical pressure build-ups.

Meiering (1982), working with gastight silos, found that when the temperature rises 3°C/h there is sufficient time for gas flow to create pressure equilibrium with the atmosphere at a specific permeability of $0.2 \text{ mm}^2/\text{m}^3$ of leak cross-section area to the void volume of the silo. Our experiments (Fig. 6 A, B, C, and D) were carried out with specific permeabilities of 0.23, 2.03, 5.12 and $76.67 \text{ mm}^2/\text{m}^3$ and, accordingly sufficient time existed for gas flow to create pressure equilibrium with the

atmosphere for the temperature changes which occurred during our observations on the CO₂ loss tests.

A mixture of 60% CO₂ in air for 4 days at a temperature at or above 27°C to create a MA to control stored-product insects has been recommended by Jay (1971). The application of an initial concentration of 60% CO₂ eventually will be accompanied by a gradual decrease in the concentration due to the grain adsorption of the gas (Banks, et al. 1980). Attempts were made to simulate this initial CO₂ concentration drop using Equations 16 and 17. Since the CO₂ purge was carried out from a single port located at the bottom of the silo an initial CO₂ gradient was obtained between the different sampling ports. This gradient progressively changed due to diffusion until a uniform CO₂ concentration was obtained. Since the adsorption phenomena accompanied this changing CO₂ gradient the approximations made to simulate the initial CO₂ decay departed from the observed average values. Figure 6C shows the results of running a circulation pump at a flow rate of 10 l/min immediately after the CO₂ purge phase to obtain a uniform concentration in 2 h. The resultant CO₂ decay shown in this figure indicates that the initial air infiltration into the silo was due simply to adsorption. Equations 16 and 17 gave an adequate approximation of the simulated air infiltration during the initial purge phase of the CO₂ application. The initial adsorption of CO₂ by the commodity has a significant effect on reducing the initial CO₂ concentration attained. Therefore, in the application of CO₂ to maintain a concentration effective to control insects, the addition of CO₂ in the maintenance phase should be balanced against the expected mass of CO₂ to be adsorbed by the commodity. This aspect of CO₂ loss due to adsorption needs further investigation.

The changes in barometric pressure are important in the infiltration of air into silos which are under MA treatment. The dominant effect causing air infiltration into the silo were the changes in the barometric pressure when the thermostat control of the room temperature was discontinued. Typical barometric pressure changes recorded during the experiments are shown in Fig. 8. This figure shows that variations between 11 and 17 Pa/h were typical of the climatic conditions in the room where the experiments were conducted. Under maritime weather conditions Meiering and Wenner (1970) observed typical atmospheric pressure changes of ± 25 Pa/h. Their data and the results of observations shown in Fig. 8 show the importance of gathering local weather observations for the area where a MA is to be applied to assess the CO_2 loss from the storage structure due to this factor.

Other parameters affecting CO_2 loss such as wind and chimney effects were not considered in these studies. It is obvious that under extreme weather conditions the effects of temperature gradients and gas density differences would have additional effects on the CO_2 loss from treated structures (Banks et al., 1974) and the location of leaks would also be important. These weather parameters seem to require more complicated computations for simulation models. Empirical models to measure air infiltration into structures have been proposed by Sherman and Grimsrud (1980) but the application of this information to grain storage facilities requires further research. Further efforts should be made to render the storage structure gastight if the use of the leak rate estimates given in the present study indicate that a silo is unsatisfactory for the application of CO_2 or other MAs. The expected CO_2 loss from a silo containing grain can be estimated its area from the total leak and gathering information on expected changes in temperature and atmospheric pressure for the location. This

assessment is essential in evaluating the costs for maintaining an adequate CO_2 concentration during the purge and maintenance phase of the treatment and becomes more important when compared to a capital investment to render the storage structure gastight.

LIST OF SYMBOLS USED

A = orifice cross-section area, mm^2 .

D = diffusion coefficient, mm^2/s .

L = orifice length, mm .

C_o = initial CO_2 concentration.

C_t = CO_2 concentration at time t .

C_{ta} = CO_2 concentration (in percent) at time that absorption is complete.

ΔC = CO_2 concentration difference between the silo and ambient.

M = mass of grain, kg .

t = time, h .

P_a = atmospheric pressure, Pa .

P_s = silo gas pressure, Pa .

P_1 = initial atmospheric pressure, Pa .

ΔP = pressure difference, Pa .

\bar{P} = change of atmospheric pressure, Pa/h .

Q = volumetric flow rate, m^3/sec or m^3/hr .

P_{CO_2} = CO_2 partial pressure

V_{CO_2} = volume of CO_2 adsorbed by commodity, $\text{ml CO}_2/\text{kg grain}$

T_s = silo gas temperature, $^\circ\text{K}$.

T_1 = initial silo gas temperature, $^\circ\text{K}$.

\bar{T} = change of temperature $^\circ\text{C/h}$.

V_e = silo gas volume, m^3 .

V_h = silo headspace gas volume, m^3 .

V_a = volume of air infiltrating the silo, m^3 .

V_{ad} = volume of air infiltrating the silo due to diffusion through the orifice, m^3 .

V_{at} = volume of air infiltrating the silo, due to temperature change, m^3 .

V_{ap} = volume of air-infiltrating the silo, due to atmospheric pressure change, m^3 .

c = proportionality constant, empirical parameter.

n = characteristic pressure difference exponent, empirical parameter varying between 0.5 and 1.0.

K = empirical parameter, multiplier of the orifice cross-section area (A) in constant pressure test.

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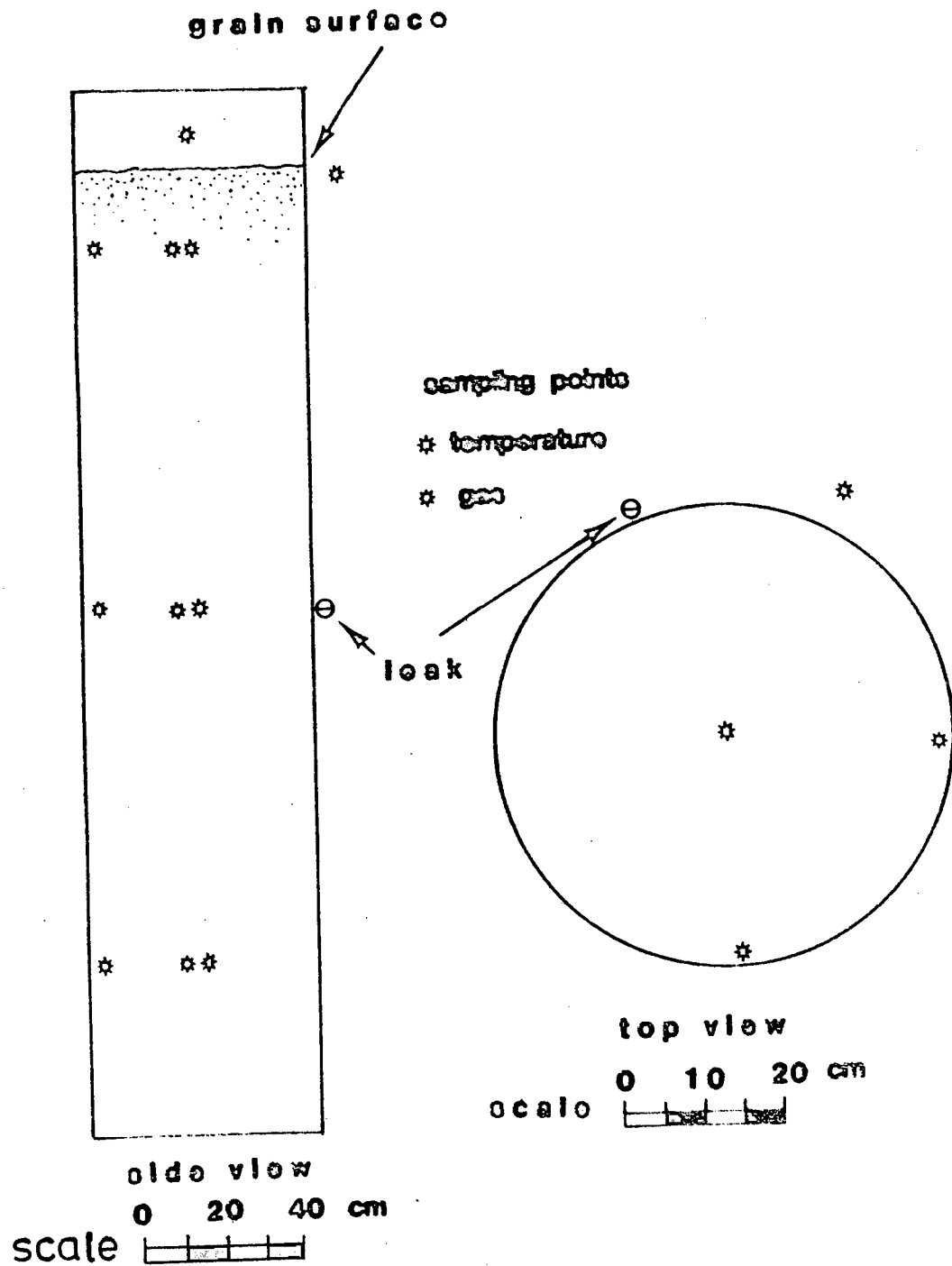


Fig. 1 Experimental silo designed for the assessment of gastightness in modified atmosphere studies.

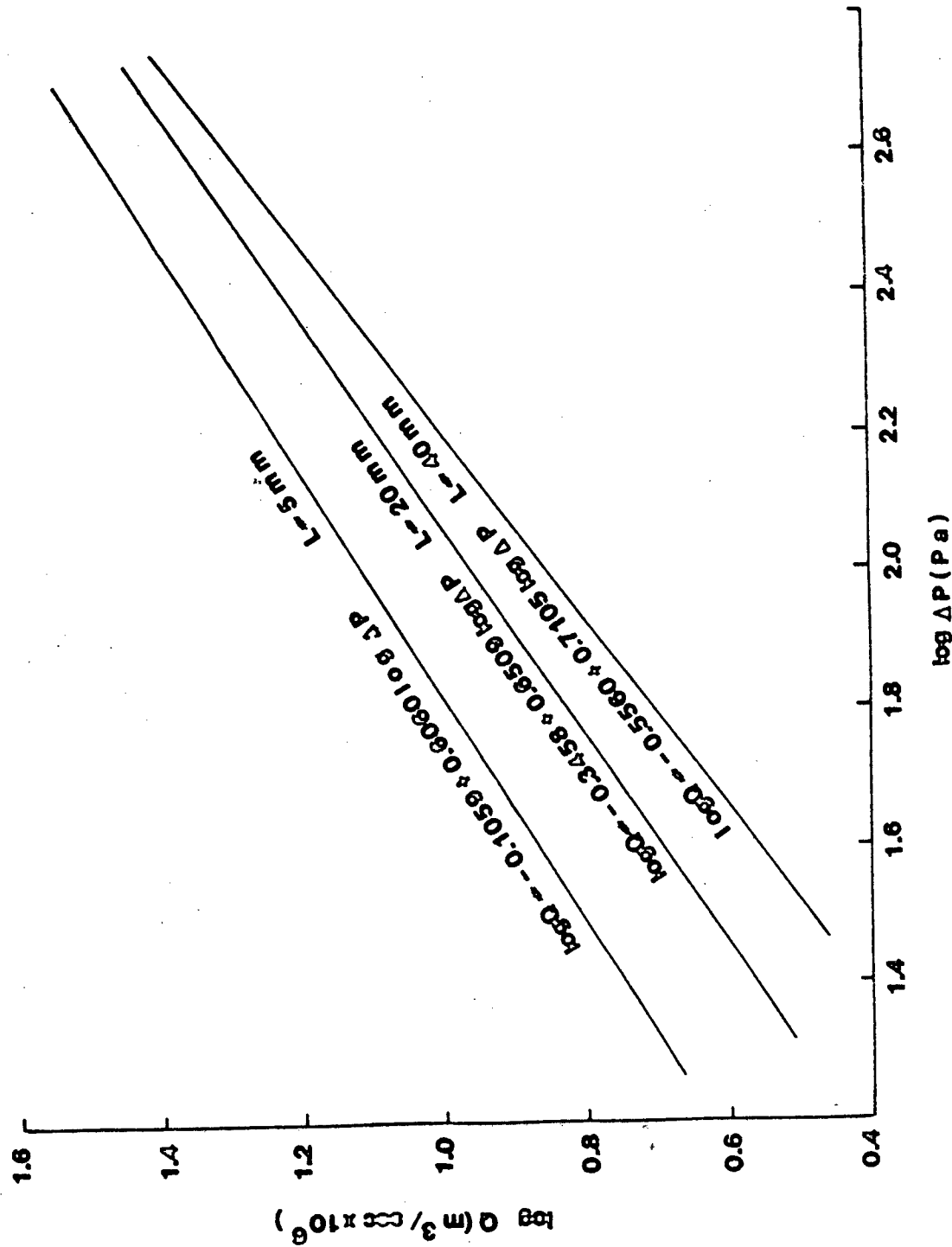


Fig. 2 Linear logarithmic relationship obtained between pressures exerted on the system and the volumetric airflow rates. The orifice cross-section area of 1.423 mm^2 was kept constant and each line represents a different length (L) of orifice.

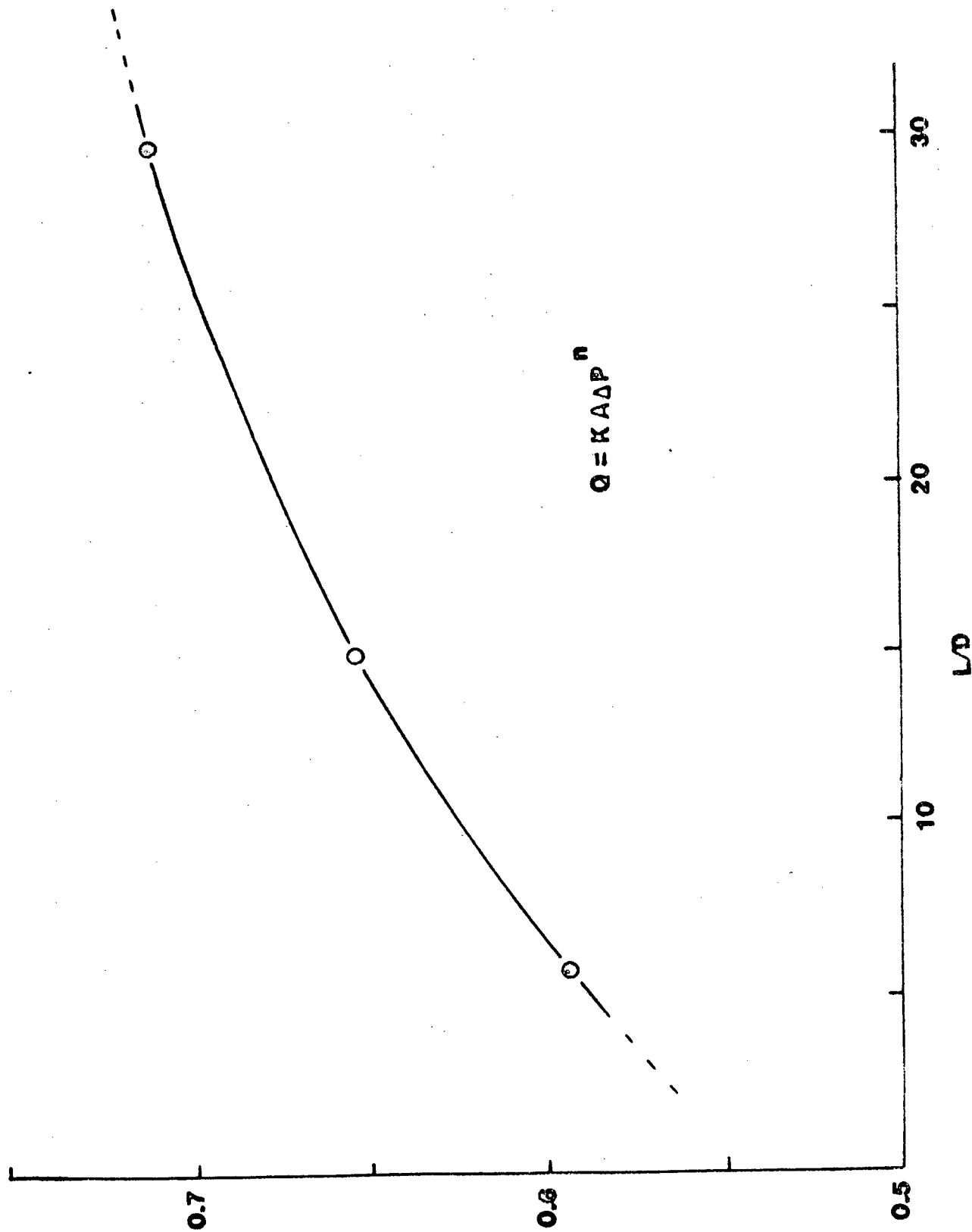


Fig. 3 Relationship between orifice ratio of length to diameter (L/D) and the characteristic pressure difference exponent (n).

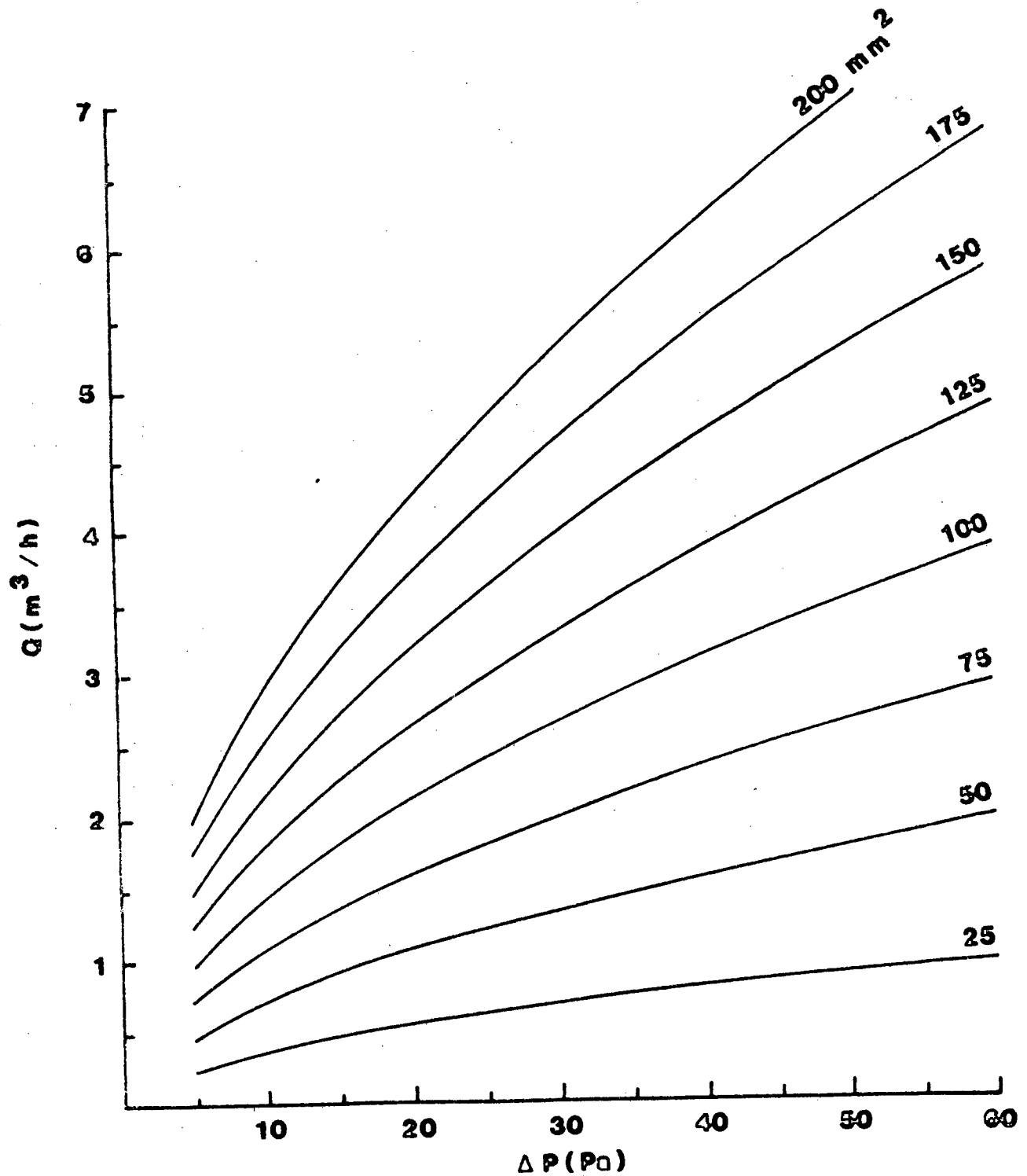


Fig. 4 Calculated family of curves for leak cross-section areas varying between 25 and 200 mm^2 , based on a constant pressure tests using air at 25°C where the empirical parameters were: $K = 4.0516 \times 10^{-3}$, and $n = 0.55105$

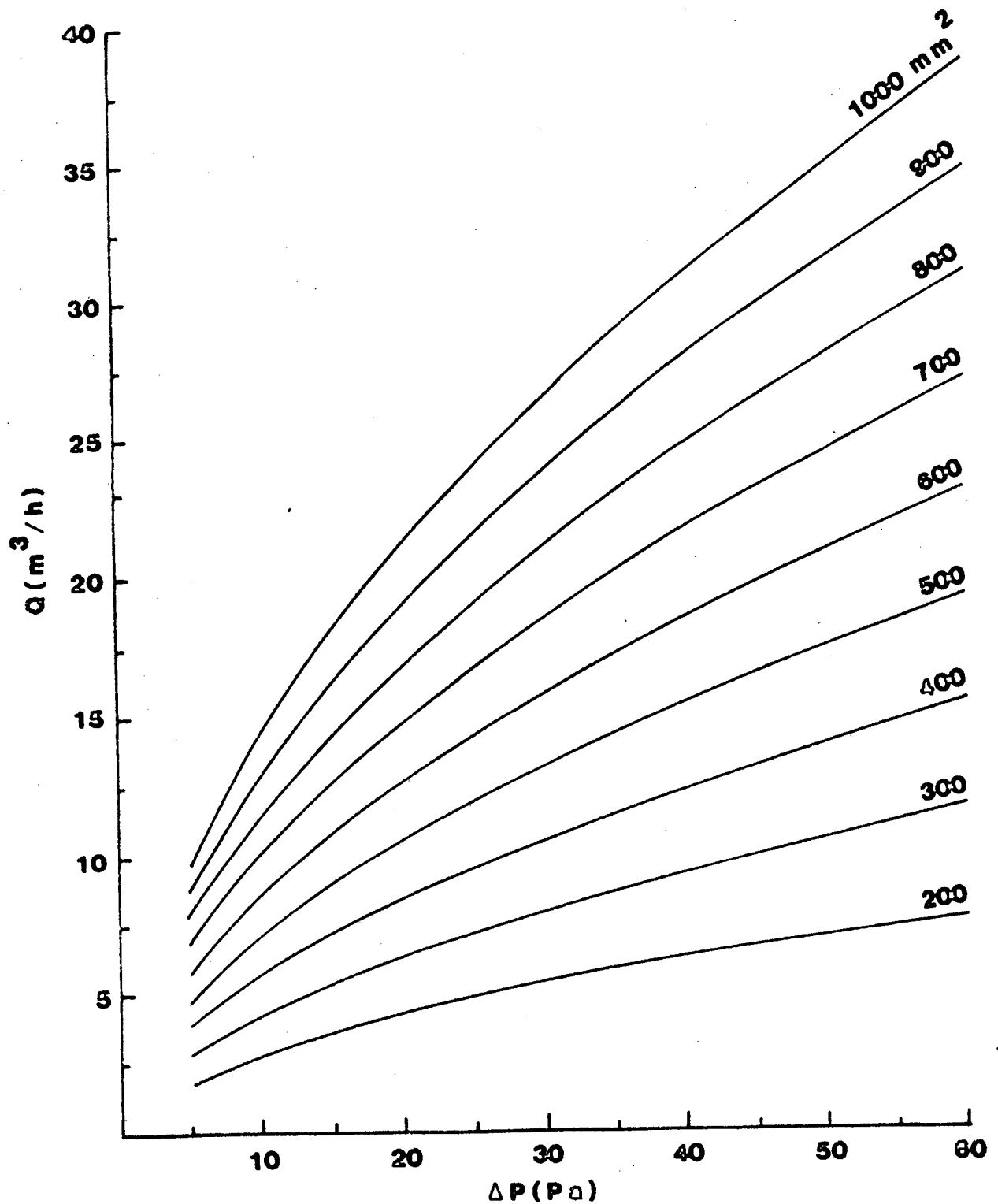


Fig. 5 Calculated family of curves for leak cross-section areas varying between 200 and 1000 mm^2 , based on a constant pressure test using air at 25°C, where the empirical parameters were: $K = 4.0516 \times 10^{-3}$, and $n = 0.55105$.

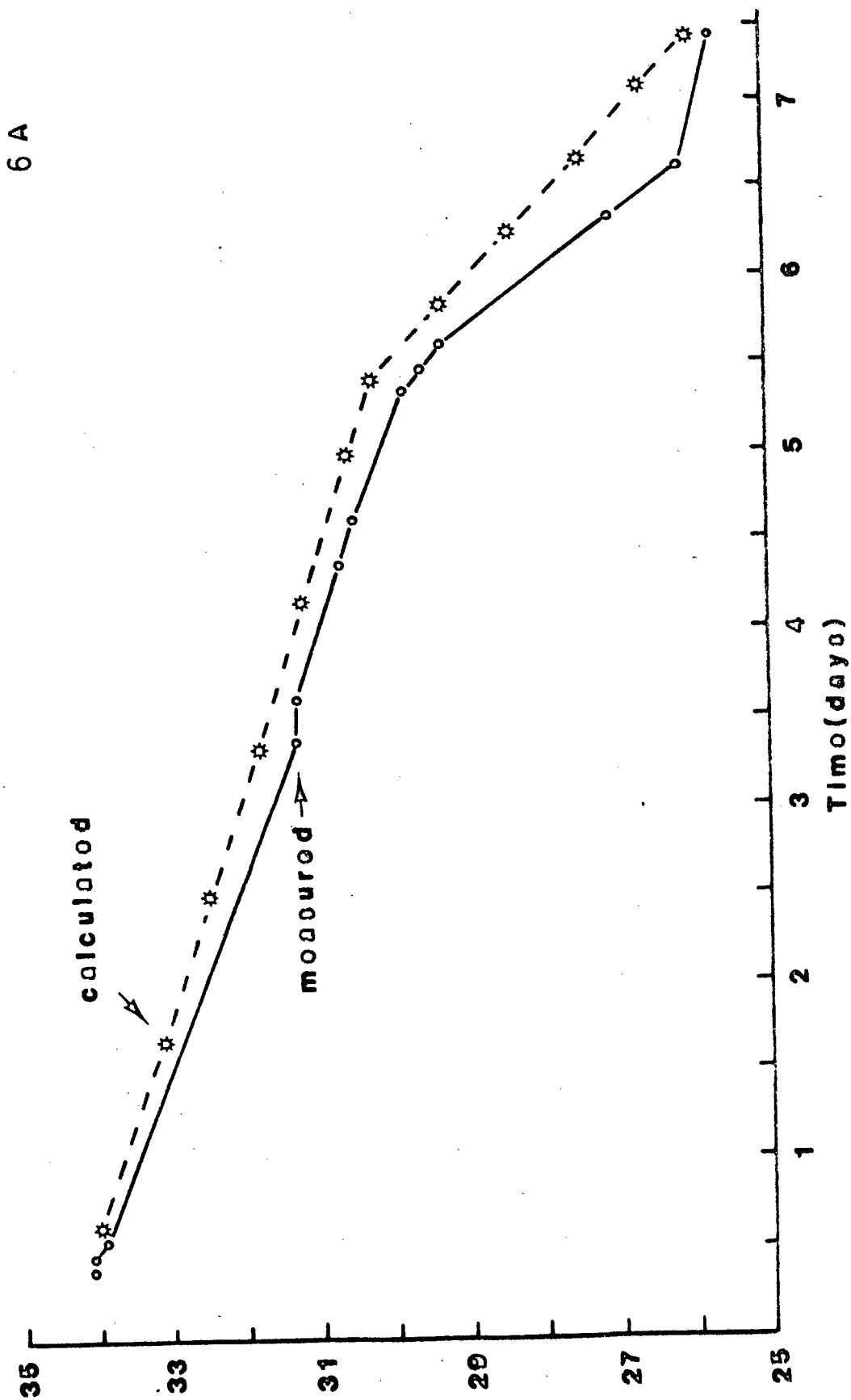
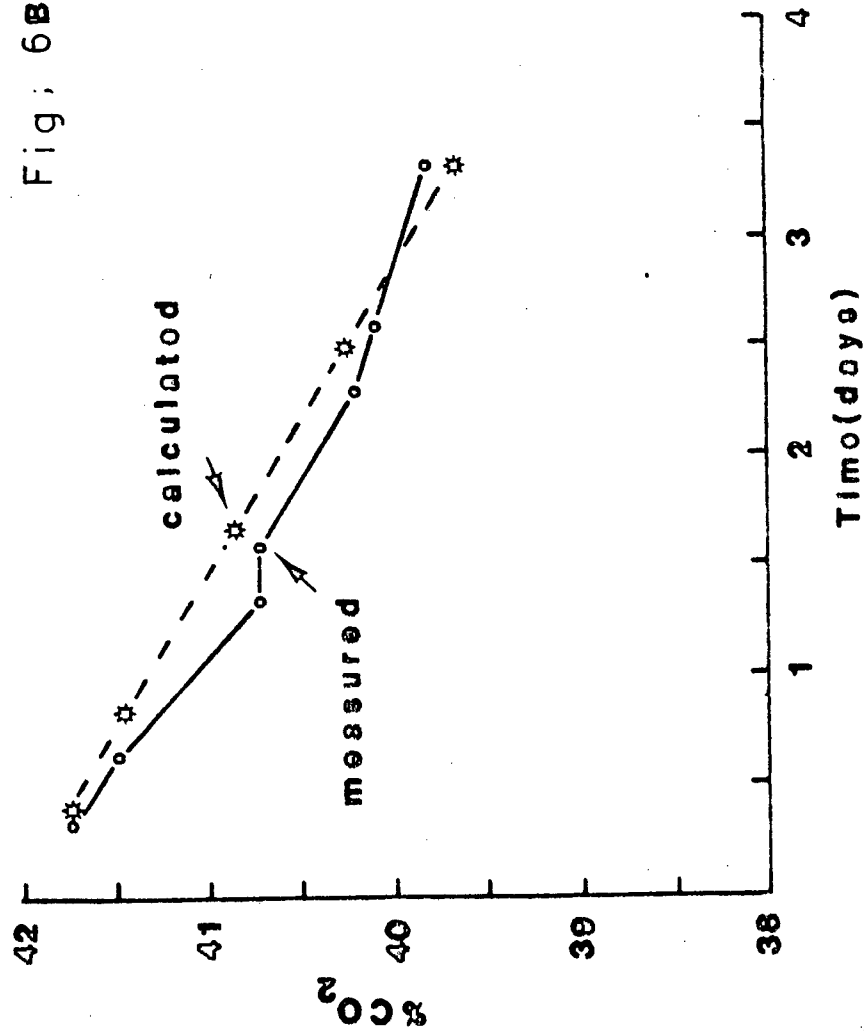


Fig. 6 Measured and calculated CO₂ concentrations obtained in the experimental silo containing ca. 500 kg of wheat equipped with different leak cross-section areas: 6A, 22.134 mm²; 6B, 1.478 mm²; 6C, 0.586 mm²; 6D 0.068 mm².

Fig. 6B



Fig; 6C

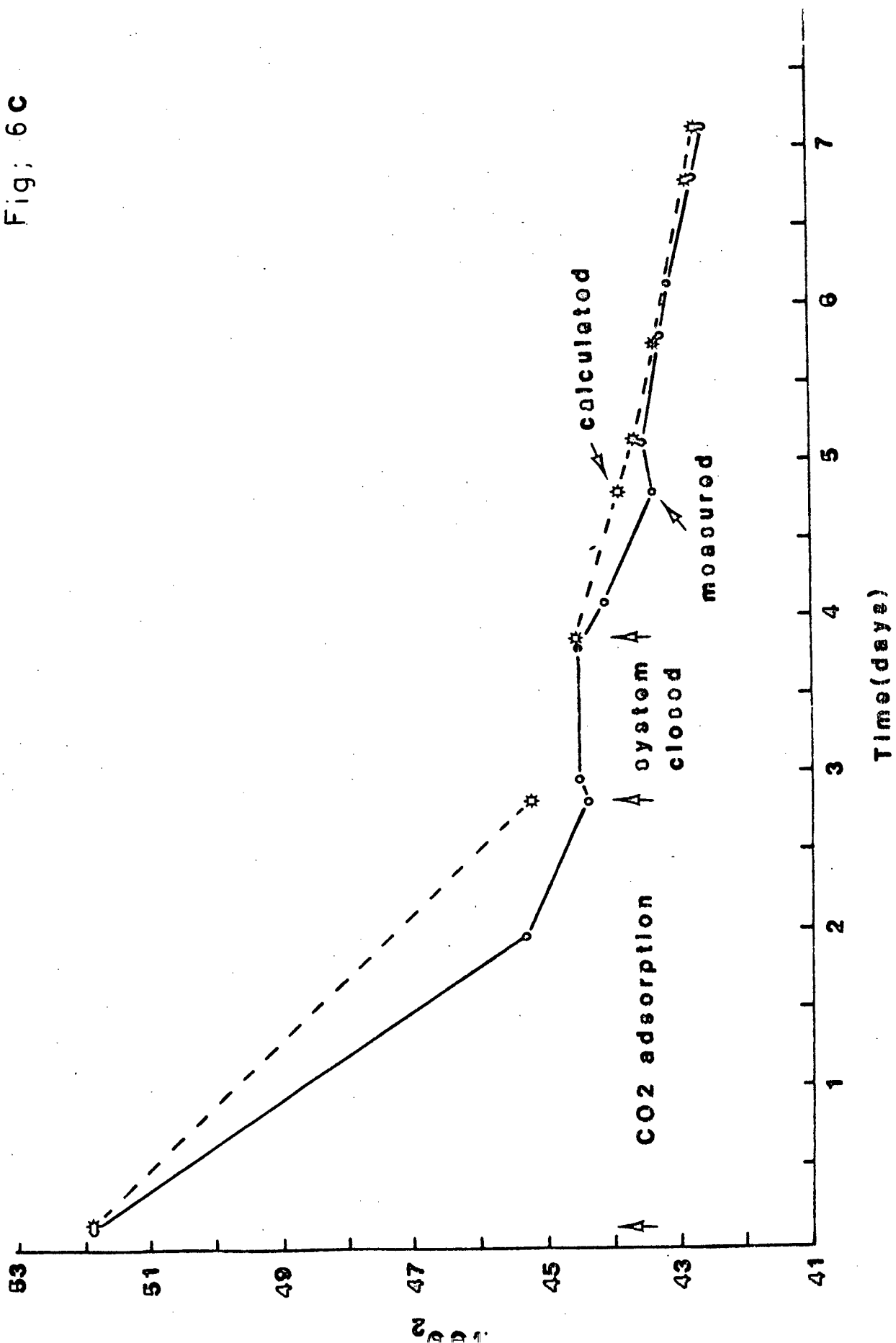
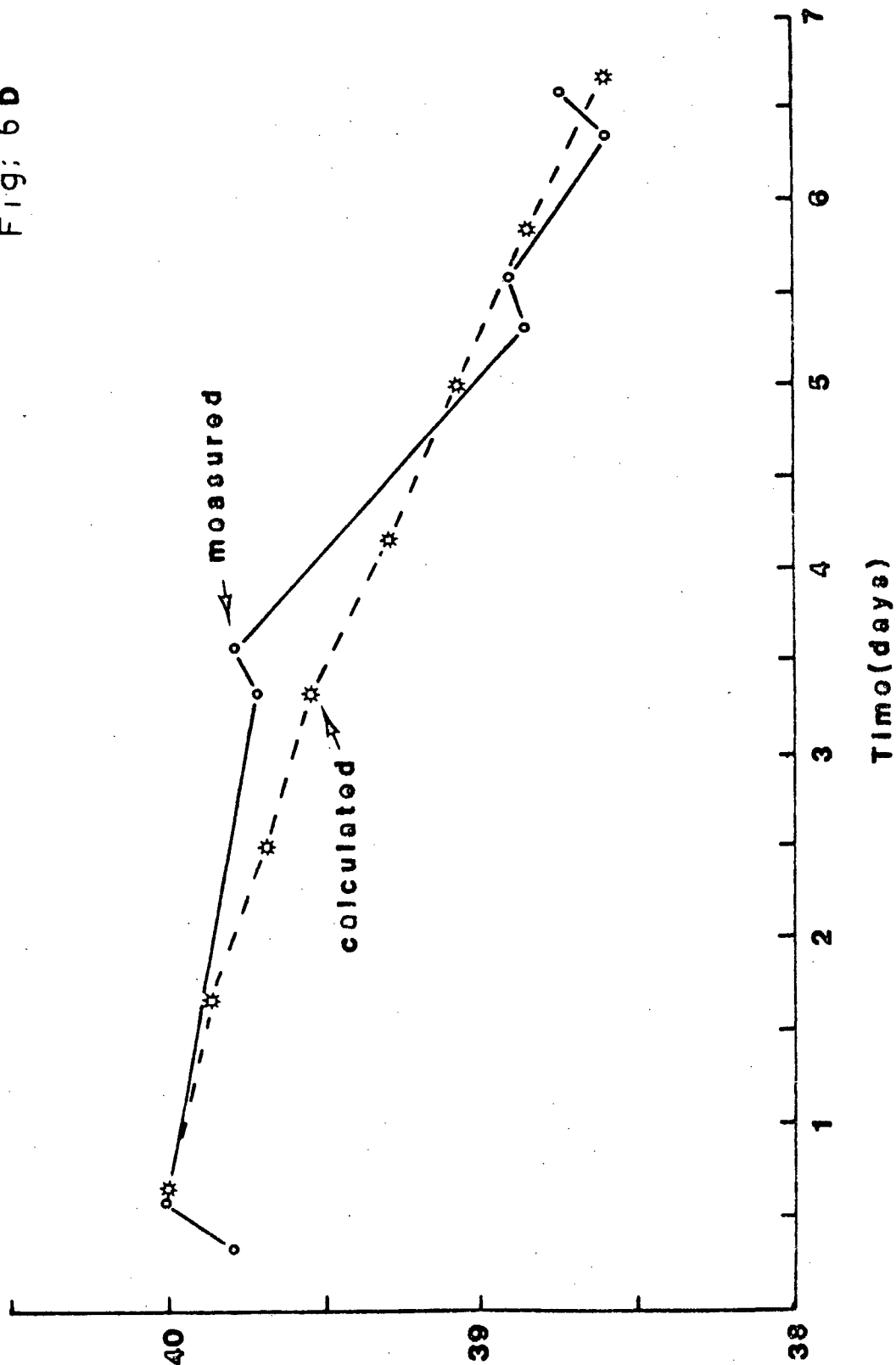


Fig: 6 D



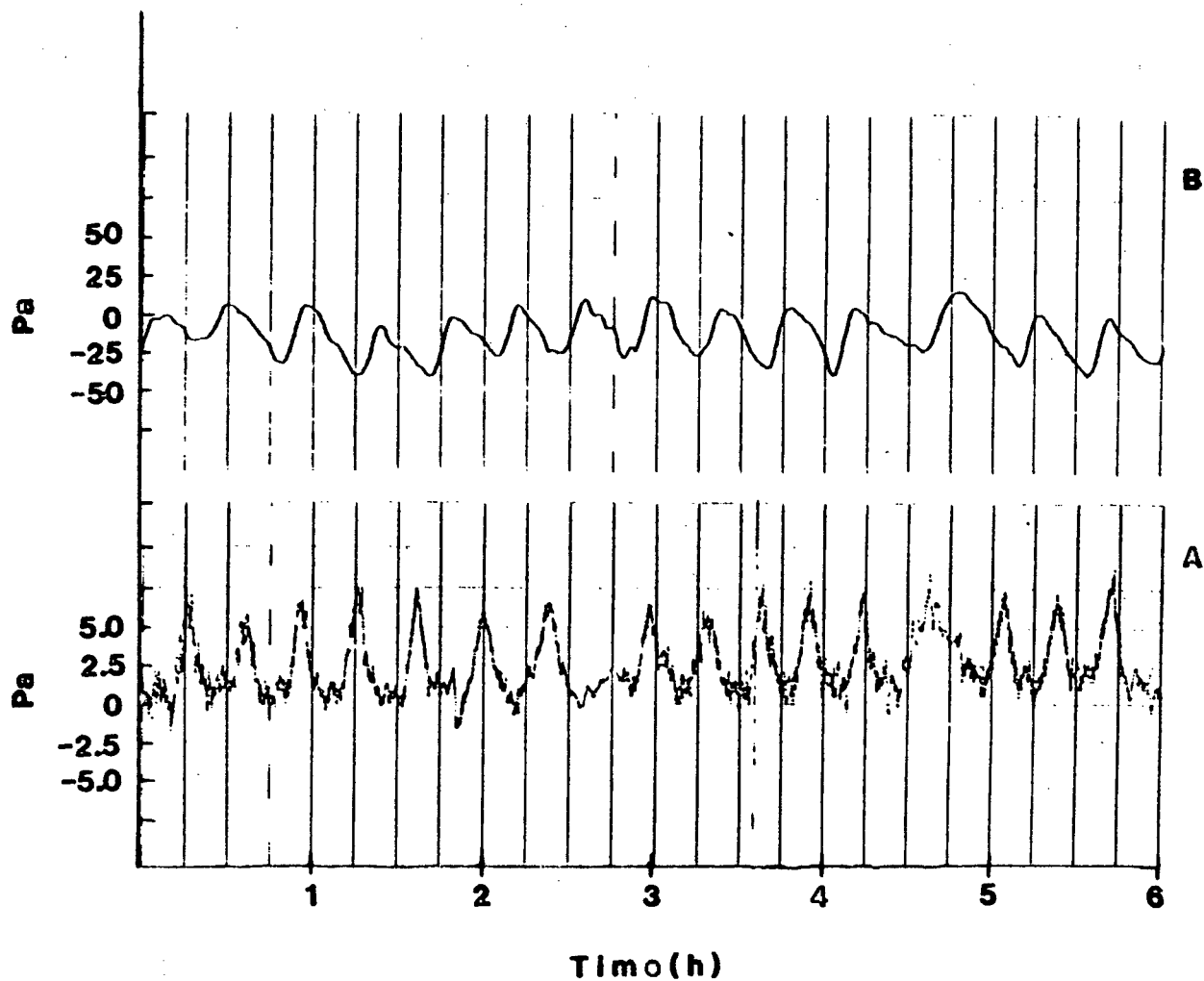


Fig. 7 Typical silo pressure changes recorded under the influence of temperature fluctuations in the experimental silo containing ca. 500 kg of wheat with 0.568 mm^2 (A), and 0.068 mm^2 (B) leak cross-section areas.

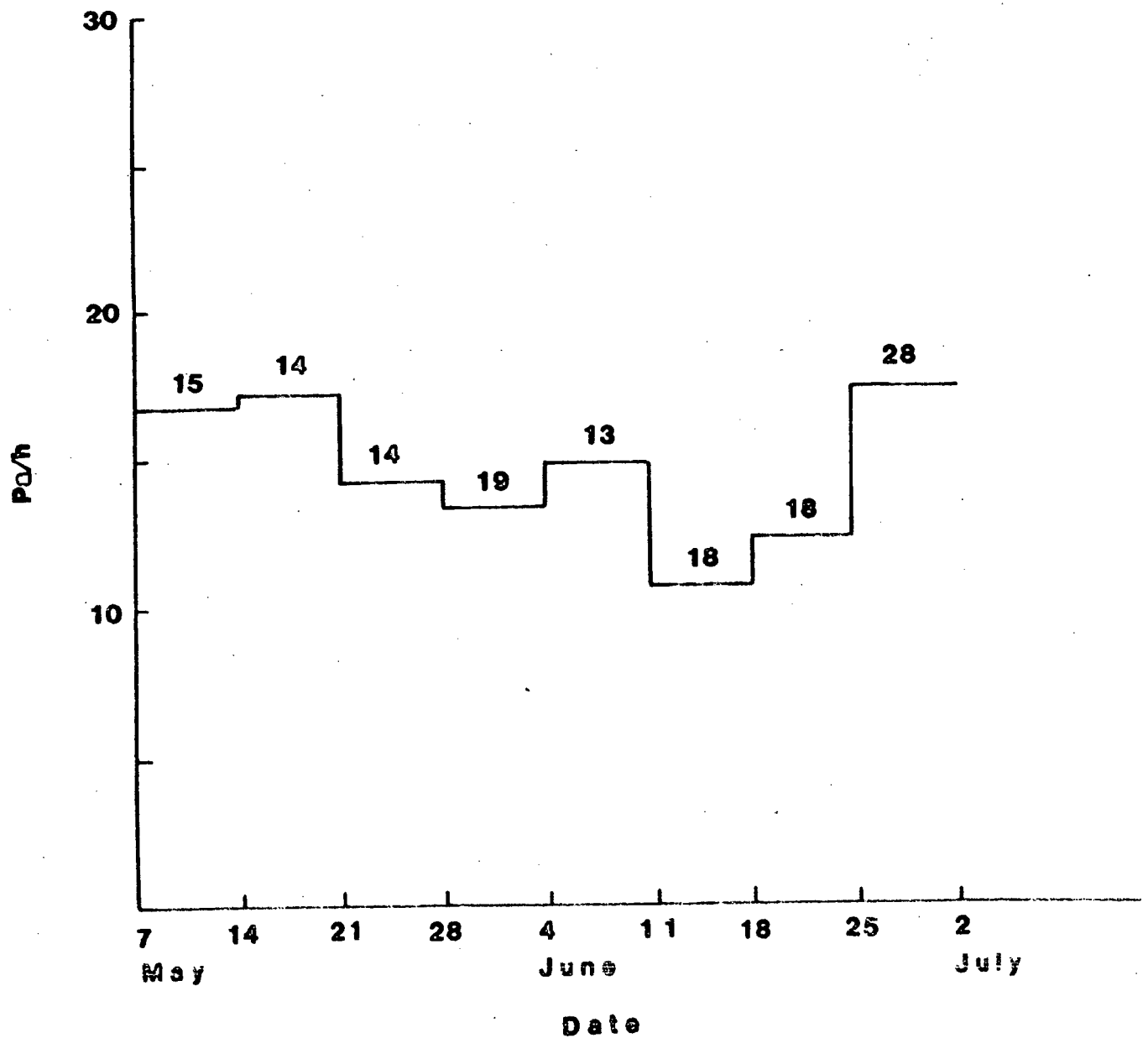


Fig.8 Typical weekly barometric pressure changes recorded during the tests reported in Fig. 6. Number of changes in barometric pressure are given in figures shown for each week period of time.

3.2. SORPTION OF CARBON DIOXIDE BY WHEAT

ABSTRACT

Sorption of CO_2 by 12.97% moisture content wheat was investigated in 960 ml containers. When the initial CO_2 concentration was 99.8% for 93% filled (v/v, grain bulk to jar) containers, the partial pressures dropped until equilibrium was reached. Maximum sorption varied with temperature, and was 259.8, 297.3, 331.3, and 392.9 mg CO_2 /kg of wheat at 30°, 25°, 20°, and 15°C, respectively. Sorption of CO_2 by wheat at constant partial pressures at 25°C was also investigated.

INTRODUCTION

Effects of modified atmospheres (MA) on stored-product insects has been investigated by Bailey and Banks, (1980). The MA method involves the alteration of the concentrations of the normal atmospheric gases present in a storage structure. One technique utilized to obtain a MA is the application of CO_2 to attain a concentration of $60\% \pm 10\%$ (Jay, 1971). Using this technique, Jay (1980) found that sorption of CO_2 by grain makes the gas effective against insect species whose immature stages feed inside the kernel.

The concentration of a gas in equilibrium with a solid is always greatest in the immediate vicinity of the solid. This phenomenon is termed adsorption. This is distinguished from absorption which is the bulk penetration of gas into the structure of a solid or liquid by some diffusion process. In many cases where the two processes occur simultaneously, the term "sorption" is used (Monro 1961; Young and Crowell 1962) and will be used in this paper.

Two processes contribute to the adsorption of a gas on a solid. The first is often called "physical adsorption" and is the result of molecular interaction forces called Van der Waal forces. In the case of physical adsorption, the gas forms a physically adsorbed layer on the solid which is similar to the condensation of a vapor to form a liquid film. The second process which contributes to adsorption is termed chemisorption and involves the transfer of electrons between molecules of the solid and the gas. One way to distinguish between the physical adsorption and chemisorption is to attempt to remove the adsorbed gas using reduced pressure. If the process is primarily physical

adsorption, then desorption of the gas is easily accomplished; however, if the process is chemisorption, much more drastic methods are usually required to recover the gas. The surface area and porosity (or pore volume) have been recognized to play complementary parts in adsorption phenomena in a vast range of solids (Gregg and Sing 1967). The adsorption mechanism of CO_2 into the grain has been found to be very similar to that observed in sorption of gases by charcoal and silica gel (Mitsuda et al., 1973). The sorption phenomenon causes the removal of some molecules of the gas from the free space present in the treated enclosure which causes a progressive lowering of the concentration (partial pressure) of the gas in the free space.

In the application of CO_2 into large bins containing wheat, an initial rapid decay of concentration shortly after purging was observed by Banks et al., (1980). This initial rapid decay seems to be associated with sorption of the CO_2 by grain. Despite the large interest in the subject of CO_2 treatment little information has been published on the sorption mechanism of wheat. The most detailed work on the mechanism of CO_2 sorption by different commodities has been reported by Mitsuda et al. (1973) in relation to skin-packaging. They found that when grain was confined in a bag made of flexible laminated plastic film containing a CO_2 atmosphere, the bag and its contents became rigid after sealing as if packed in vacuo. The adsorption phenomenon responsible for this rigidity has led to the development of the technique of skin-packaging, the "carbon dioxide exchange method" (CEM), for the preservation of rice in storage (Mitsuda and Yamamoto, 1980). Although Mitsuda et al. (1973) were able to demonstrate that wheat at 20°C adsorbs 75 ml CO_2 /kg in 3 hr, the sorption mechanism of the CO_2 on wheat was not fully investigated. The present work was initiated to obtain information on

the sorption mechanism of CO_2 in relation to temperature and the maximum reduced pressures that could be obtained when wheat is maintained in gastight containers.

MATERIALS AND METHODS

Wheat. Soft Red Winter Wheat harvested in 1983 and having an average moisture content of 12.97% ($\text{SE} \pm 0.0609$) was used in the tests. This wheat contained foreign material of different particle sizes, as would be present in storage. The foreign material was composed of 0.15% (W/W) large particles retained by a 5 mesh sieve, 0.27% particles retained by a 30 mesh size sieve, and 0.04% smaller particles (dust). The remaining 99.54% of the tested material was wheat.

Manometry. The sorption of CO_2 was measured using a transducer type manometer (Hastings Vacuum Gauge, Model DNNV-800) connected to a container of ca. 960 ml capacity. Prior to each experiment, the transducer was calibrated against a Fortin type barometer (U. S. Signal Corps model 02-380) and against the very low absolute pressure of < 2 mm Hg obtained from a high vacuum pump. The amount of CO_2 adsorbed by wheat created a negative pressure in the container which was recorded for periods of up to 8 days.

CO_2 concentration. The gas concentration in the headspace of the container was measured by a gas chromatograph (Fisher Model 1200) equipped with a thermal conductivity detector and two columns, one packed with molecular sieve 13X(60/80 mesh) and the other columpak PQ (80/100 mesh). An integrator (Hewlett-Packard Model 3390A) was used to measure the areas under the peaks.

Experimental procedure. The volume occupied by a predetermined weight (ca. 686 g) of wheat grain was measured using a manometric technique described by Day (1964). The grain MC was determined using a capacitance moisture meter, (Motomco Model 919). The grain was then poured into containers having a predetermined volume (ca. 960 ml) and the metal screw-on lid was tightly closed. The lid (68 mm i.d.) was equipped with two 1.15-mm i.d. copper tubes. One tube extended 160 mm into the container and the other 35 mm into the container. These were used for the gas inlet and outlet, and extended 45 mm above the outside edge of the lid.

The containers were submerged in constant temperature water baths equipped with refrigeration and heating systems during the experiments so that the temperature could be adjusted below and above the room temperature. The grain temperature was measured daily by a thermistor placed in the center of a container of the same volume and which also contained 686 g of wheat. This method of temperature measurement in a separate container was adopted to avoid leaks from the lids of the containers which were purged with CO₂. Prior to the CO₂ purge the wheat was maintained for 24 hrs in the baths for temperature stabilization.

Each experiment started with an initial purge of CO₂ supplied from pressurized cylinders at a flow rate of approximately 200 ml/min for 9 min. During this purge phase, gas samples were taken for CO₂ analysis. At the end of the purge, the transducer was immediately connected to the gas inlet tube, the container sealed, and the initial container pressure and the barometric pressure were recorded.

Two sets of experiments were conducted. In the first set, an initial average CO_2 concentration of 99.8% was attained immediately after purge and the pressure changes due to the adsorption were periodically measured. Since the containers were maintained without additional CO_2 supply, in this set of experiments, the pressure drop due to adsorption decreased the partial pressure of CO_2 . These experiments were replicated three times at temperatures of 15°, 20°, 25°, and 30°C. In the second set of experiments, a constant CO_2 concentration was periodically supplied from CO_2 cylinders so that a constant partial pressure was maintained in the containers. The pressure in the containers was subsequently brought to the original pressure based on daily observations by supplying CO_2 at the tested concentration using 30 ml or 1 ml gastight syringes equipped with a three-way Luer-lock attachment. In these experiments five different CO_2 concentrations ranging from 30.64% to 99.81% were tested at 25°C. Sorption of CO_2 was calculated after corrections were made for STP conditions and was calculated in mg CO_2 sorbed kg of grain.

RESULTS AND DISCUSSION

Effect of sorption on changes in pressure. Typical reduced pressures created by sorption in the gastight containers are shown in Fig. 1. Since the amount of CO_2 sorbed is proportional to the amount of grain in the container at a given partial pressure of CO_2 and temperature, the resulting reduced pressure will also be proportional to the the void space of the system. Therefore, the drop in pressure shown in Fig. 1 can only represent the experimental conditions when the grain bulk volume occupied 93% of the total container capacity. Under these conditions the lowest pressure recorded was

520 mm Hg at 15°C. The sorption rate changed inversely with the temperature of the wheat and the highest absolute pressure of 606 mm Hg was obtained at 30°C. Mitsuda and Yamamoto (1980) reported that a 0.8 l container filled with grain (apparently rice) developed a negative pressure of 0.27 kg/cm² after 7 days. This negative pressure in terms of absolute pressure at STP conditions was calculated to be ca. 555 mm Hg. This pressure falls in the range obtained in our experiments (close to the 25°C line in Fig. 1). However, since the commodity and the temperature were not mentioned in their paper these results can not be compared quantitatively.

Sorption of CO₂. The initial sorption rate of CO₂ by wheat was found to follow a linear relationship during the first 3 h of the process when results were plotted on a log-log scale (Fig. 2). In these experiments, the containers in which the sorption process took place were maintained without adding any more CO₂ to compensate for the changing partial pressure conditions. Therefore, the sorption rate in these experiments does not follow the conventional kinetic laws (Daniels and Alberty, 1963) and the rates shown in Fig. 2 have the restriction of being dependent on the intensity of the changing CO₂ partial pressures throughout the sorption process. Since the grain bulk occupied 93% of the container volume, the sorption rates shown in Fig. 2 should be similar to this grain bulk to container volumes. The slopes for the curves for the time-sorption process were almost parallel with values of 0.664, 0.635, and 0.659 for 20°, 25°, and 30°C, respectively. A similar analysis was performed at 15°C, but the initial CO₂ purge probably caused a temperature change in the container so that during the first hour a sorption rate near the values of that found for 20°C was obtained. The calculated slope at 15°C was 0.512 which was markedly different from the slope obtained

at the higher temperatures. The correlation factors calculated for the relationship of adsorption rate were, $r^2 = 0.9994, 0.9994, 0.9995,$ and 0.9982 for $15^\circ, 20^\circ, 25^\circ,$ and 30°C , respectively. Results obtained by Yamamoto and Mitsuda (1980) indicate that at 20°C , wheat adsorbed $75 \text{ ml CO}_2/\text{kg}$ in 3h. This amount of CO_2 is equivalent to $147.2 \text{ mg CO}_2/\text{kg}$ wheat. Our results indicate that at 20°C only $81.6 \text{ mg CO}_2/\text{kg}$ was sorbed. The difference seems to be due to the fact that the sorption rate in our experiments was calculated under a decreasing partial pressure. In practice when a commodity is kept in a container, a decrease in the partial pressure due to the sorption process would be expected and in a completely sealed container the drop in pressure would cause a decrease in the partial pressure of CO_2 . Similarly, in a container having leaks, sorption would cause air to enter into the system as the pressure decreased although the total pressure would be maintained close to that of the surrounding atmosphere. Therefore, the partial pressure of CO_2 would decrease which would result in a lower sorption rate than that reported by Yamamoto and Mitsuda (1980).

The time for the sorption process to reach steady-state equilibrium at varying pressures due to adsorption at different temperatures are shown in Fig. 3. These data show that the higher the temperature, the shorter the time required to reach a equilibrium. Figure 3 shows that the times to required reach equilibrium were 65, 80, 95, and 120 hr at $30^\circ, 25^\circ, 20^\circ,$ and 15°C , respectively. This in contrast to the initial sorption process which showed a higher initial rate at higher temperatures (Fig. 2).

Calculations of pressure drop or air entry into a CO_2 treated wheat container could be made from the results shown in Fig. 2 and 3, provided the

initial CO₂ concentration is ca 99.8% and the filling ratio is 93%. Since a higher adsorption rate was found at 15°C than at 30°C, it would be advantageous to apply modified atmospheres at high temperatures, and the subsequent reduction of a substantial amount of the CO₂ concentration.

Sorption isotherm. Attempts were made in these experiments, to simulate field conditions, where after an initial CO₂ purge sorption would create a partial pressure differential. The amount of CO₂ sorbed to reach equilibrium was measured at 25°C to demonstrate the relationship of sorption and constant partial pressures. This relationship was tested for linearity using the classic adsorption isotherm of Freundlich (Daniels and Alberty, 1963). The calculated correlation factor was $r^2=0.9890$ for the tested CO₂ concentrations varying between 30.64% and 99.81%. This relation was described by the equation: $\log m_{CO_2}/kg = 2.643 + 1.719 \log PCO_2$. A plot of our data and the calculated line is shown in Fig. 4. Using this equation, the adsorption of wheat at 25°C for any given CO₂ concentration in the range of our tests can be calculated. A similar relationship for rice has been described by Mitsuda et al. (1973).

The expected amount of CO₂ sorbed at a constant CO₂ partial pressure may well describe a specific situation, where the supply of CO₂ to the commodity is almost constant. However, since in a field situation or even in a small container there is always a changing CO₂ partial pressure, the rate at which sorption takes place needs further investigation. When the sorption rates at different temperatures and varying partial pressures are known it may be possible to formulate an equation to describe the CO₂ concentration for any given ratio of volume occupied by commodity to the total volume of the container.

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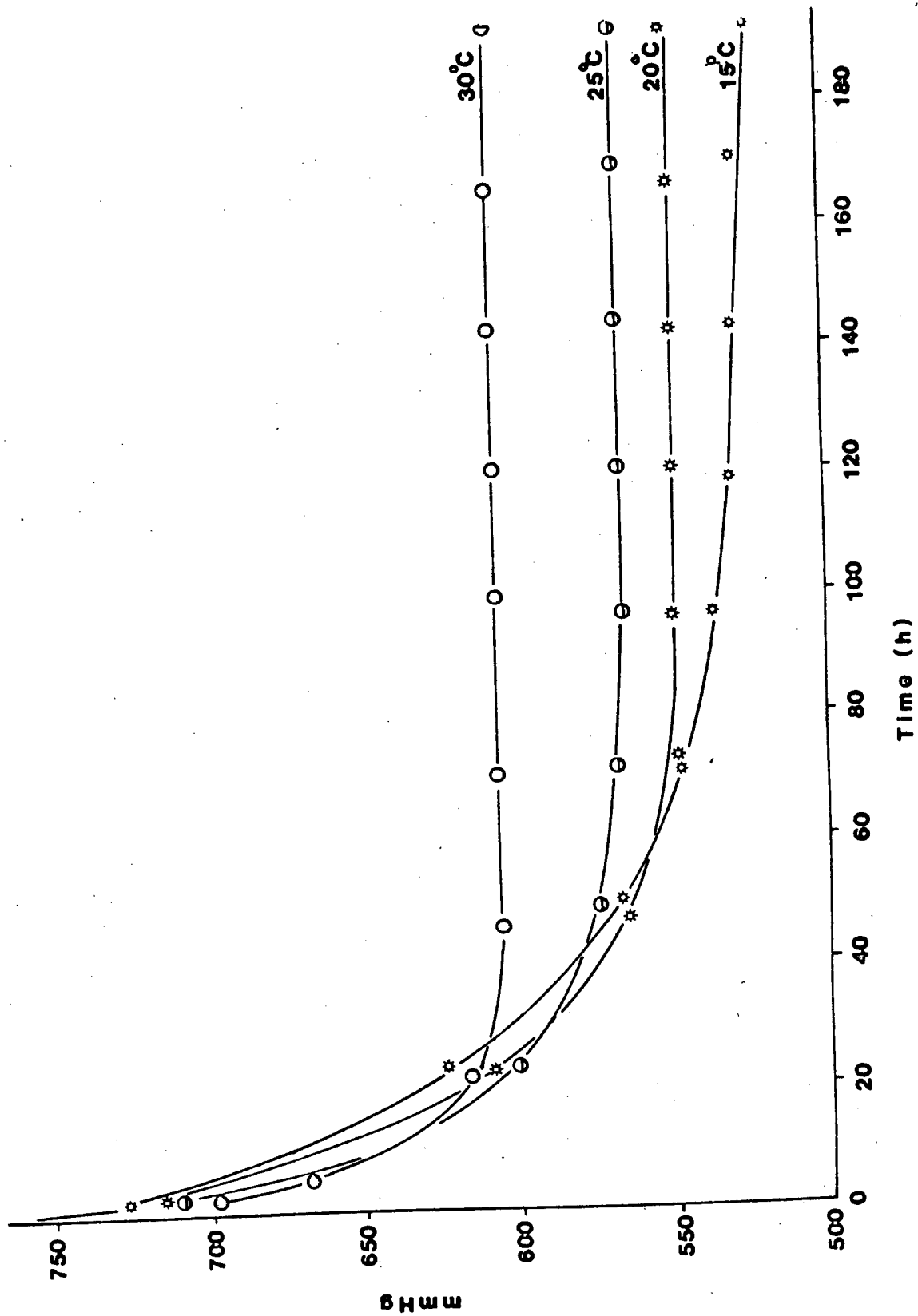


Fig. 1 - Pressure decay due to sorption of CO_2 in gastight containers filled to 93% with wheat (bulk volume to container) at four different temperatures, with an initial CO_2 concentration of 99.8% and an initial pressure 768 mm Hg.

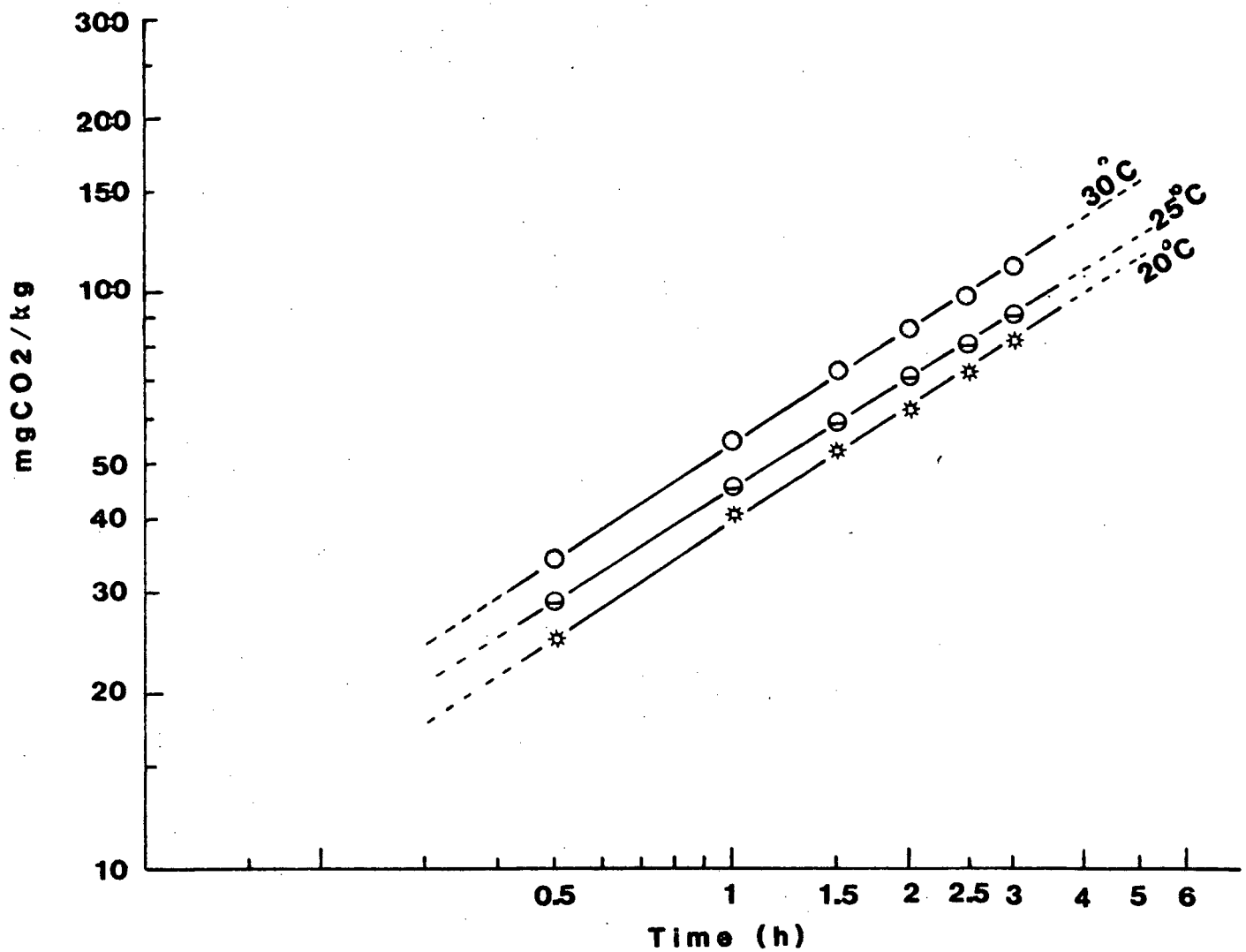


Fig. 2 - Sorption rate of CO₂ by wheat during the first 3 h of the process at three different temperatures with an initial CO₂ concentration of 99.8% and an initial pressure of 768 mm Hg in containers filled to 93% with wheat.

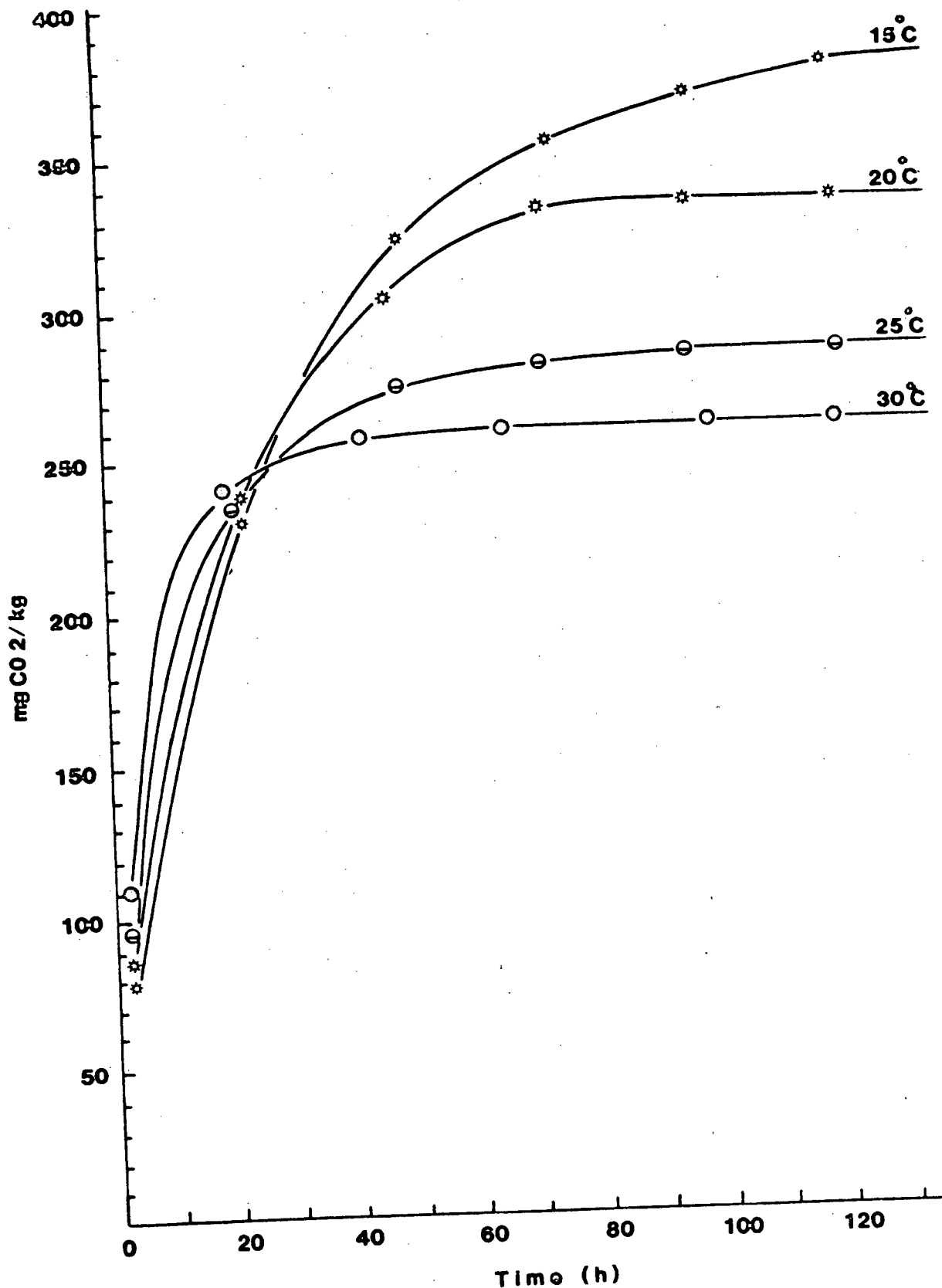
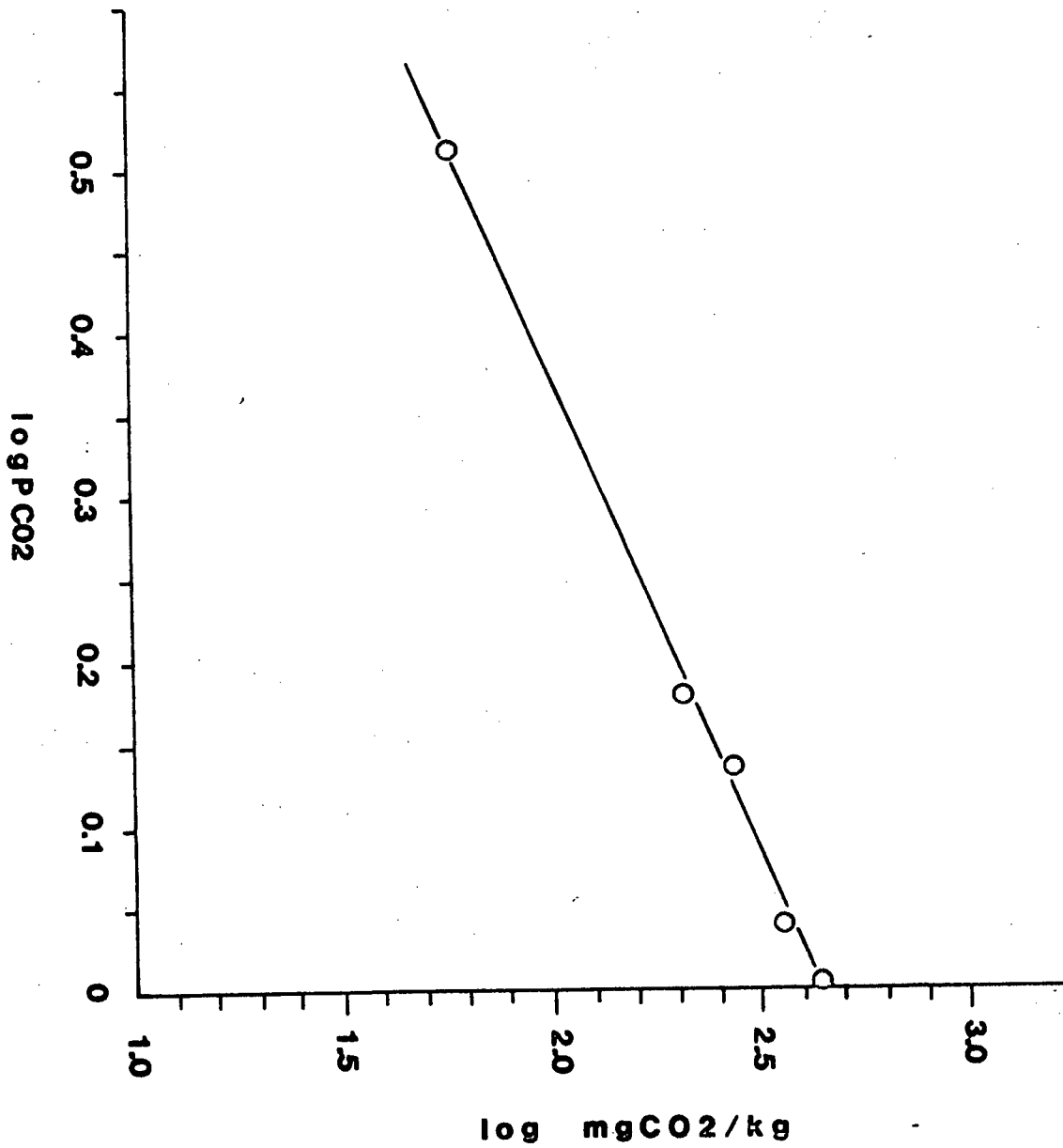


Fig. 3 - Time to reach sorption equilibrium between CO₂ and wheat at four different temperatures, with an initial CO₂ concentration of 99.8% and an initial pressure 768 mm Hg in containers filled to 93% with wheat.

Fig. 4 - Relation between the amount of CO_2 sorbed and the partial pressure of CO_2 until equilibrium is reached between the CO_2 and wheat at 25°C .



3.3. RECIRCULATION RATE REQUIREMENTS FOR ADEQUATE DISTRIBUTION OF CO₂ IN GRAIN SILOS

Abstract

A 665.7 L experimental silo containing wheat equipped with a recirculation system which was used to determine the relationship between the time needed to attain uniform distribution of carbon dioxide (CO₂) and the recirculation rate. A cyclic pressure increase and decrease was observed and this was attributed to the adsorption and desorption processes taking place during recirculation of CO₂. An index based on the ratio of lowest to highest concentration of CO₂ at a given time was used to determine the distribution of the gas. An equation is proposed based on this index to assess the time needed to attain uniform distribution based upon the recirculation rates used.

INTRODUCTION

Recirculation is known as an effective method of application and distribution of fumigant compounds for the treatment of grain stored in bulk. The gaseous-type fumigants may be recirculated upward or downward through the grain bulk to obtain a uniform concentration that will effectively control insect infestations. One of the most important advantages of this method is that lengthy exposure periods are unnecessary (Monro 1969). Recirculation is an effective method of returning the air-gas mixture which has passed through the grain bulk back into a fan so that continuous circulation is achieved (Brown and Heseltine, 1949; Howe and Klepser, 1958).

Monro (1969) recommends that the air and fumigant mixture be recirculated at least 2 and preferably 4 or 5 times to obtain thorough blending of the gas-air mixture in 15 to 20 min and an airflow rate of 1 L of air per min for each 50 L of grain (approximately $3 \text{ (m}^3/\text{h/m)}^3$ of void space (at a porosity of 40%) or $1.54 \text{ (m}^3/\text{h)}/\text{tonne}$ of grain at a bulk density of 0.78 tonne/m^3) is considered adequate in the design of fumigant recirculation systems. The

airflow rate is the basic factor needed for the design of such a system. Using the existing knowledge for the design of aeration systems it is possible to calculate the commodity resistance to flow, the friction loss in ducts, elbows and air distribution systems, and to subsequently determine the fan capacity required in a given silo containing grain (Holman, 1966; Shedd, 1953; Navarro and Calderon, 1982).

Cook (1983) indicated that low airflow recirculation can be effectively used with less sorptive fumigants to obtain even penetration and distribution in grain bulks. This method has been patented (Cook, 1980) for use with aluminum or magnesium phosphide. With this method, one air change in 8 to 12 h or approximately $0.10 \text{ (m}^3\text{/h)/m}^3$ of void space or $0.05 \text{ (m}^3\text{/h)/tonne}$ was found to be satisfactory in recirculating phosphine gas to attain and maintain an even distribution of the gas concentration.

Carbon dioxide (CO_2) can be used to modify atmospheres in grain storage facilities to control stored-product insects (Jay, 1980). After the structure is purged with CO_2 , it may be necessary to recirculate the mixture to attain an even distribution of CO_2 concentrations for effective insect control (Wilson, et al., 1980). Banks and Annis (1980) recommend recirculating the storage atmosphere from the base of the silo into the headspace via external pipework with a small sealed blower to maintain an adequate CO_2 -air mixture. They concluded from unpublished data that a recirculation rate of about 0.1 volumes per day (approximately $0.004 \text{ (m}^3\text{/h)/m}^3$) is adequate for this purpose.

The research reported here is a study on the recirculation rate requirements needed to attain a prescribed gas mixture when CO_2 is used for the control of stored product pests.

MATERIALS AND METHODS

Recirculation system:

A 665.7 L experimental silo (Fig. 1) 259.5 cm high by 57.15 cm i.d. was filled with 499.4 kg of wheat which left a 21.5 cm high headspace. Soft Red Winter wheat of 12.72% moisture content was used in the experiments.

A 16.6 W fan, Dayton Model 4C443, was installed outside the silo to deliver the desired airflow rates by pulling gas mixtures from the bottom and blowing them into the headspace via 15.24 mm i.d. pipe (Fig. 1). The air distribution system was located on the floor of the silo. Thus, gas pulled from the silo entered the return pipe via three ducts and then the gas was conveyed through the fan and the return pipe to the headspace of the silo (Fig. 1). A Sierra Instruments Inc., model 715 mass flowmeter installed 42 cm from the fan on the gas return pipe measured the flowrate of the mixtures.

The CO₂ gas was supplied from pressurized cylinders to obtain the desired gas concentrations by purging the silo either from the top or bottom. The CO₂ concentrations were monitored using a dual column gas-chromatograph, Fisher-Hamilton Model 29, equipped with a thermal conductivity detector, and a Hewlett-Packard Model 3390A integrator was used for quick determination of CO₂ concentrations. Gas samples were taken using a 10 ml gastight syringe fitted with a gastight valve and a 5 cm long needle. Gas sampling ports were located along the silo wall, at 42, 131, and 218 cm from the floor (Fig. 1). The silo was equipped with 7 YSI400 series probes connected to a Yellow Springs Instrument Model 44TD tele-thermometer to measure the temperatures in the silo.

Silo pressure was measured through a 5.7 mm i.d. Tygon^(R) tube which ran from the center of the silo to a transducer. Pressure measurements were carried out using a Pace Wianko Model CD 25 pressure indicator fitted with a

Model KP15 transducer. The transducer was calibrated against a Dwyer Model 400-23 inclined manometer while connected to a pressure source. A Hewlett-Packard, Model 7128A recorder was connected to the pressure indicator to obtain a continuous record of pressures in the silo.

A 15.24 mm i.d. ball-type valve with a teflon joint was used to restrict the gas passage through the return pipe to regulate the gas flow rate. A voltage controller was connected to the fan power supply line for fine adjustment of the gas flow.

The silo was located in a room which had the temperature controlled to $25 \pm 2^\circ\text{C}$. The whole silo recirculation system including the fan was sealed prior to the initiation of the tests. The system was tested under negative and positive pressures of 2500 Pa, and the silo held these pressures for at least 30 min without any marked changes.

Experimental procedures:

The silo was aerated at a flow rate of $0.35 \text{ m}^3/\text{h}$ prior to each test. The recirculation fan was used to aerate the grain by pulling gas from the bottom of the silo and conveying it through an exhaust port located between the mass flow meter and the gas flow control valve. When the gas flow control valve was off and air was permitted to enter the silo through a port located at the top, the CO_2 -air mixture was conveyed outside the silo through the exhaust port (Fig. 1). Using this system, the CO_2 concentrations at the three sampling locations prior to the start of each test were less than 0.5%.

At the start of a test, the silo grain temperature was measured, then CO_2 was supplied either from a top valve (top purge) or from a bottom valve of the silo (bottom purge). With a bottom purge, the top valve was kept open, and vice versa for a top purge to eliminate excessive pressure buildups in the silo. During a top or a bottom purge the recirculation fan was left off. The

CO₂ purge rate was designed to achieve approximate concentrations of 30 or 60% at the end of each test after using the recirculation system. A constant CO₂ flow rate at 10.5 L/min was chosen to purge the silo either for 10 min for a concentration of 30% CO₂ or 20 min for a 60% concentration. The 2.5 mm i.d. exhaust valves restricted the gas flow out of the silo thereby creating a pressure increase during purging. At the end of the purge phase ca 2 min passed while the initial gas samples were taken.

Negative pressures due to adsorption of CO₂ by the wheat were observed throughout the experiments. These negative pressure were partially released by creating a fine leak of 0.3 mm i.d at the exhaust port. This resulted in an adequate air infiltration rate which kept pressure changes in the silo measurable in the range of up to ± 700 Pa.

After the initial gas samples were taken and the 0.3 mm i.d. leak was installed, an additional 4 min elapsed before the fan was started to circulate the CO₂-air mixture. The flow rates chosen were based on number of recirculations needed for the total void space of the silo on an hourly basis. The void space, including the headspace, of the silo was found to be 0.289 m^3 and the flow rates studied were 3.737; 1.869; 0.934; 0.467; 0.234; and $0.117 (\text{m}^3/\text{h})/\text{m}^3$ of the void space. Gas samples were taken every 15 min in most of the experiments, and every 10 to 60 min for the lowest flow rate. The same procedure of CO₂ purging and gas monitoring was followed to observe the CO₂ concentrations that could be obtained without fan operation .

At the end of each test, the grain temperature was recorded and the silo was aerated and prepared for the next test. The aeration time was set proportional to the time the grain was exposed to CO₂ in the silo since the time needed to adsorb the CO₂ was almost equivalent to the time needed to desorb the gas from the wheat.

Calculation of recirculation rate needed for uniform distribution of CO₂:

The calculation of the time needed to recirculate the CO₂-air mixture is based on a series of assumptions. The basic assumption is that the difference between the lowest (C min) and the highest (C max) concentration to be achieved in the silo is expressed as a ratio (Cp). Then, $C_p = C_{\min}/C_{\max}$

and $C_p = 1$, when $C_{\min} = C_{\max}$

Cp is a function dependent on the time (t) needed to achieve a certain concentration ratio (Cp_t), the volumetric flow rate (Q), the initial ratio of concentrations (Cp_o), and the final CO₂ concentration to be achieved (C_f). Also, several other factors such as the temperature gradients which cause convection currents, the diffusion rate, and the rate of adsorption and desorption taking place during the recirculation period are important. An initial experimental R value was examined for all these factors. The R value defines the rate of change of the concentration ratio (Cp). Then the change of Cp with time is:

$$\frac{dC_p}{dt} = R e^{-Rt} \quad [1]$$

This equation is applicable for a large difference between concentrations and where Cp_o approaches 0. In this case when Cp_o = 0, then 1 - Cp_o = 1. In practice when a silo is purged with CO₂ there is always a gradient, so Cp_o ≠ 0. Therefore, equation [1] needs to be modified to:

$$\frac{dC_p}{dt} = R (1 - C_{p_o}) e^{-Rt} \quad [2]$$

This integrates to:

$$C_{p_t} = 1 - (1 - C_{p_o}) e^{-Rt} \quad [3]$$

Where Cp_t is the ratio at any given time (t) after the initial ratio has been established the Cp_t value can be estimated if the R value is known for

any given concentration ratio at a given time. The R values were calculated for the tested recirculation rates used, for different recirculation times and considering the initial concentration proportion (C_{p_0}) using the following equation:

$$R = \frac{1}{\tau} \ln \frac{1-C_{p_0}}{1-C_{p_t}} \quad [4]$$

The validity of R values obtained for different initial concentrations and recirculation rates was evaluated with this equation. Tests carried out with a final concentration of of ca 60% CO₂ and a bottom purge were replicated three times. Two additional series of tests were carried out; one with 30% CO₂ with a bottom purge and one with a 60% CO₂ top purge. These last two series were not replicated. The R factor for each set of test conditions was calculated from the results of these tests.

RESULTS AND DISCUSSION

Mixing process by recirculation

When the initial CO₂ concentration gradients in the silo were not disturbed, diffusion and other forces such as convection currents influencing the grain bulk eventually caused a near uniform concentration of gas. Since CO₂ is 50% heavier than air, the final stage of the advance of CO₂ molecules caused by diffusion was always marked by a higher CO₂ concentration in the bottom layers of the silo. Under the influence of a driving force, such as a recirculation fan, a layer such as this will be removed to a new layer having a different CO₂ concentration. When a high CO₂ level remains layered for an adequate time, adsorption will take place which causes a negative pressure in the silo. Recirculation of the atmosphere will result in this high CO₂ level being replaced by a lower CO₂ level, and desorption will take place which will increase the pressure in the silo. Therefore, adsorption and desorption will be taking place in the silo due to

the movement of the CO_2 gradient and this will be accompanied by increasing or decreasing pressure of the atmosphere in the silo. Recirculation and mixing will cause a decrease in the CO_2 gradient and the pressure changes in the silo will become less pronounced when a uniform concentration is obtained. Fig. 2 shows the cyclic pressure changes for three recirculation rates when the silo was purged from the bottom to obtain CO_2 concentrations of 30% (Fig. 2A) and of 60% (Fig. 2B). Although three replicates were run for the 60% CO_2 test, slight differences in the amplitude of the peaks were observed, apparently due to changes in barometric pressure during each test. Therefore, because of the difficulties in averaging the pressures recorded at each time interval, the curves in Fig. 2B represent a typical single test. These figures demonstrate that the higher the flow rate, the higher the amplitude of the pressure and that as the CO_2 concentration measured in the silo approached uniformity the pressure reached a certain equilibrium. At least three peaks of pressure increase per hour could be observed at a circulation rate of $3.737 \text{ (m}^3/\text{h)/m}^3$ while two positive pressure and one negative pressure peaks were observed at $1.869 \text{ (m}^3/\text{h)/m}^3$. Only one positive and one negative pressure peak were observed at a recirculation rate of $0.934 \text{ (m}^3/\text{h)/m}^3$. These peaks indicate the proportionality of the recirculation rate to the adsorption and desorption processes taking place in the silo. Similar typical pressure change curves were also recorded for a 60% CO_2 purge from the top. This indicates that as long as an initial CO_2 gradient exists, the intensity of the adsorption and desorption processes will depend on the recirculation rate used to mix the gases. The negative pressures in Fig. 2 A and B do not represent the total adsorption by the grain, since to reduce the buildup of extreme negative pressures, the silo was equipped with a leak that allowed air entry into the system. The pressures

shown in Fig. 2 A and B may not be detected in a large silo which is less tightly sealed than the experimental silo used in this study. However, the adsorption and desorption caused by recirculation would have a pumping effect proportional to these pressure changes. This would result in the CO_2 -air mixture being exhausted under a positive pressure and cause air to infiltrate into the silo under a negative pressure. Since the amplitude of these pressure changes is higher at higher recirculation rates, a higher diluting effect would be expected which would cause larger amounts of CO_2 loss when high flow rates are used.

Changes in CO_2 concentration during recirculation:

Measurements taken during the tests indicate that when the mass of the CO_2 -air mixture moving under the influence of the fan arrived at a layer of grain where a higher CO_2 level was present as compared to the CO_2 - air mixture then desorption takes place and the CO_2 concentration of the mass of gas purging the new layer will rise. The grain column in this case reacts to air passage similar to a gas-chromatographic column and this phenomenon has been demonstrated for fumigants applied in the vapor phase by Berck and Solomon (1962). Conversely, when the gas mixture met a layer having a lower CO_2 level, the grain retained part of the CO_2 in the CO_2 - air mixture by adsorption and the resulting CO_2 concentration of the mixture was lower. It was difficult to follow the process of increasing and decreasing concentrations of a given gas mixture, but it could be observed by periodic measurements through the 3 fixed sampling ports along the wall of the silo (Fig. 1). Typical CO_2 concentration changes at the $3.737 \text{ (m}^3/\text{h)}/\text{m}^3$ recirculation rate when the CO_2 was purged into the bottom are shown in Fig. 3. Although the cyclic changes in CO_2 concentrations were recorded in these tests, since several gas samples were missed in two replicates, a third

replicate with complete sampling every 15 min was chosen for the construction of Fig. 3. These cycles were mainly determined by whether CO_2 was adsorbing or desorbing where the initial concentration was the highest - at the bottom of the silo for Fig. 2 and 3. The frequency of the CO_2 concentration peaks observed in Fig. 3 were in direct proportion to the recirculation rates used. At a recirculation rate of $3.737 \text{ (m}^3/\text{h)/m}^3$ at least 3 CO_2 peaks per hour were observed in each level of the silo.

These changes in concentration of the gas mass in the silo resulted in a gradual dilution caused by the gas mixture passage through the different layers. Thus, the initial high CO_2 concentration observed in the bottom moved through the return pipe to the top, then to the middle, and then back to the bottom of the silo. After this sequence occurred, a certain mass of the gas mixture progressively increased or decreased its original concentration and tended to attain uniformity at a rate mainly dependent on its initial concentration, the recirculation rate, and the amount of CO_2 in the silo. An initial CO_2 gradient resulted that the ratio between the minimum and the maximum gas concentrations to be less than unity but, when the differences between any two extreme layers approached zero, the ratio between their concentrations approached unity.

The assumptions derived from this series of tests can be used to calculate the empirical recirculation coefficient R values shown in equation (4). The calculated R values were found to remain constant for a specific recirculation rate and a final gas concentration for each independent test. Figure 4 shows the relationship between R values and recirculation rates plotted on a log-log scale. The calculated R values for 60% CO_2 bottom purge are averages of three replicates. Linearity was observed when the CO_2 purge was from the top of the silo but this relationship became invalid for flow rates between

0.234 and 0.934 (m^3/h)/ m^3 for both concentrations (30 and 60% CO_2) when the silo was purged from the bottom. In this range of recirculation rates, the calculated R values remained almost constant. The practical significance of this is that the time required to achieve uniformity of the CO_2 concentration is almost the same for recirculation rates in the range of 0.234 and 0.934 (m^3/h)/ m^3 when the CO_2 was initially applied at the bottom. When the purge was applied from the top, the initial purge created a less pronounced gradient than when the purge was from the bottom. This difference in gradients was apparent when the initial concentrations were measured. With 60% CO_2 bottom purge the average CO_2 concentrations and their standard error for samples taken from the top, mid, and bottom were $7.7\% \pm 1.76$, $92.8\% \pm 0.59$, and $96.3\% \pm 0.67$, respectively. Whereas with 60% top purge the average CO_2 concentrations and their standard error for samples taken from top, mid, and bottom were $40.4\% \pm 0.52$, $77.4\% \pm 0.60$, and $59.9\% \pm 0.68$, respectively. A possible explanation for this effect could be related to the density of CO_2 causing gravitational flow. However, if during recirculation gravitational flow is an important factor one would expect that the R values for top purge to be higher than for bottom purge at the low flow rates. Whereas in the range of 0.234 to 0.934 (m^3/h)/ m^3 the results indicate the opposite (Fig. 4). Since with top and bottom purges the flow is in the direction of gravitational flow, the results could be related to the kinetics of sorption and desorption taking place during recirculation. With bottom purge producing a high CO_2 concentration at the bottom, at a certain flow rate just sufficient gas could be desorbed to produce a uniform distribution in a shorter time than when the purge was from the top.

The calculated R values, except for the lowest flow rate, were found to be higher at 60% CO_2 than at 30% CO_2 , which suggests that a correction factor

should be added to correlate the R values to the average concentrations attainable at the end of the recirculation process. When the purge was from the top during the mixing process, a significant linear relationship between R and Q values was observed. Linear regression analysis resulted in the following equation with a correlation factor of $r^2 = 0.964$. Therefore:

$$R = 0.9692 Q^{0.4540} \quad [5]$$

Rewriting, equation [3] represents the range of recirculation rates tested in the present work:

$$C_{p_t} = 1 - 1 - C_{p_0} e^{-(0.9692 Q^{0.4540} t)} \quad [6]$$

Based on this equation for each tested recirculation rate, the calculated C_{p_t} values are shown as continuous curves and can be compared with the measured C_{p_t} values in Fig. 5. The measured initial C_{p_0} value for $3.737 \text{ (m}^3/\text{h)/m}^3$ was lower than the tested other flow rates. A failure in the gas purging line was detected causing that the final concentration to be ca 50% CO_2 . However, since the calculated C_{p_t} values were in agreement with the measured values, its results were included in Fig. 5. The figures show that the calculated values adequately predict the time needed to reach a uniform concentration dependent on the initial concentration ratio (C_{p_0}) and the recirculation rate (Q). The validity of this approach in estimating the R values in relation to different silo headspace volumes, the rate and the location of CO_2 flow during purging, and the type of commodity, requires further investigation. However, for the experiments described in the present work, the information obtained indicates that even with recirculation rates as low as $0.117 \text{ (m}^3/\text{h)/m}^3$, CO_2 can be recirculated efficiently to obtain uniformity in 6 to 7 h. This information indicates that relatively low fan power requirements are needed to recirculate CO_2 in treated silos. The

recommended recirculation rates based on 5 volume recirculations per hour for fumigants (Monro, 1969) is about 43 times more than the lowest rate tested in these experiments. For large silos where duct size in recirculation systems is a critical economical aspect, the possibility of using small ducts proportional to the chosen recirculation rate may encourage the more efficient use of modified atmospheres.

A similar approach to the reevaluation of the recirculation system requirements for fumigants seems to reveal new information in the application of fumigants in grain stored in bulk. Cook (1983) has suggested the use of a recirculation rate $0.1 \text{ (m}^3\text{/h)}/\text{m}^3$ for the application of phosphine in silos but this recirculation rate has apparently been obtained from field experiments. A follow up of field experiments with laboratory research should provide useful information in the further clarification of the different factors contributing to the efficient distribution of fumigants in grain bulks.

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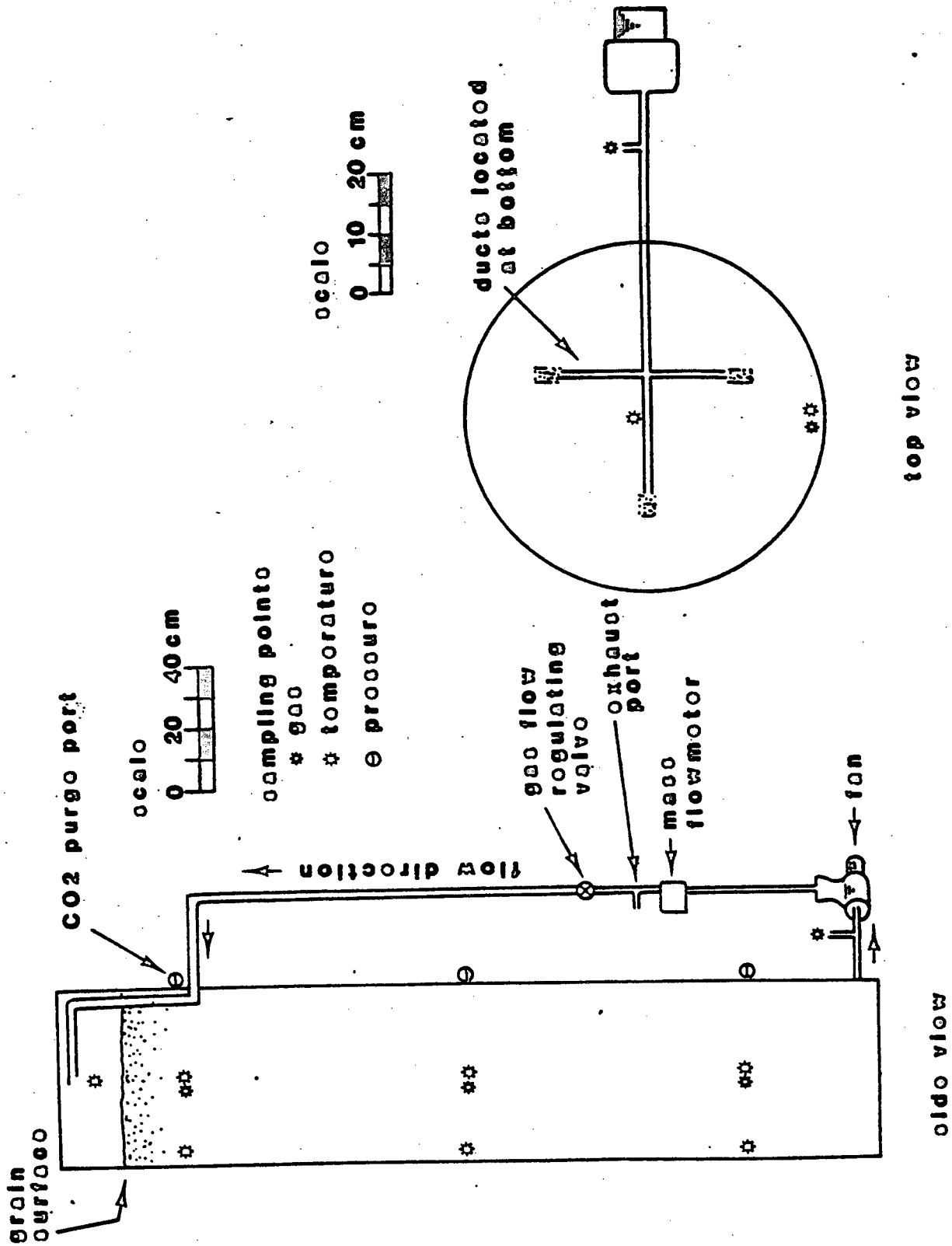


Fig 1. Side and top view of the 665.7 L capacity experimental silo used in testing recirculation rate requirements for the distribution of CO₂.

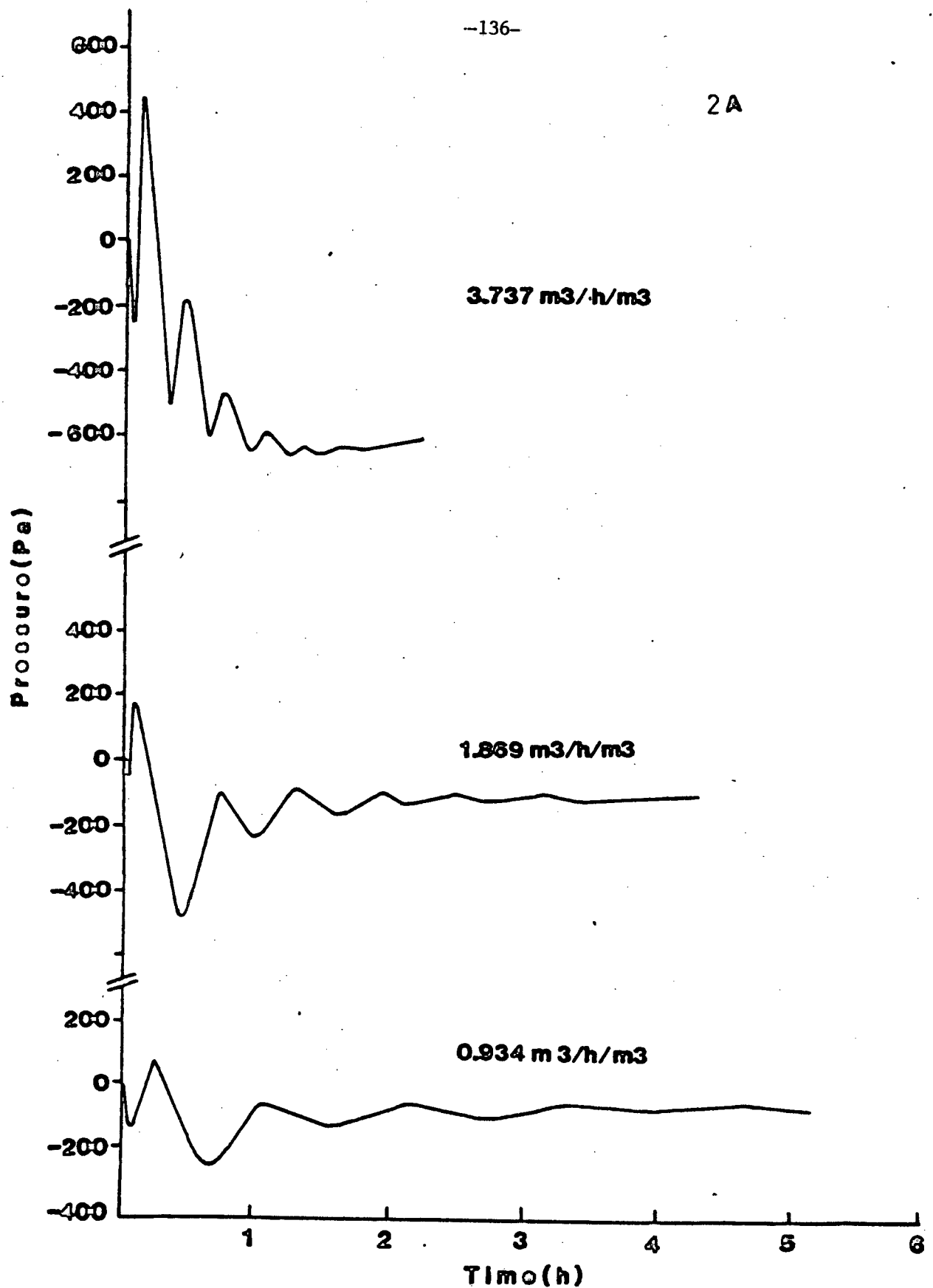
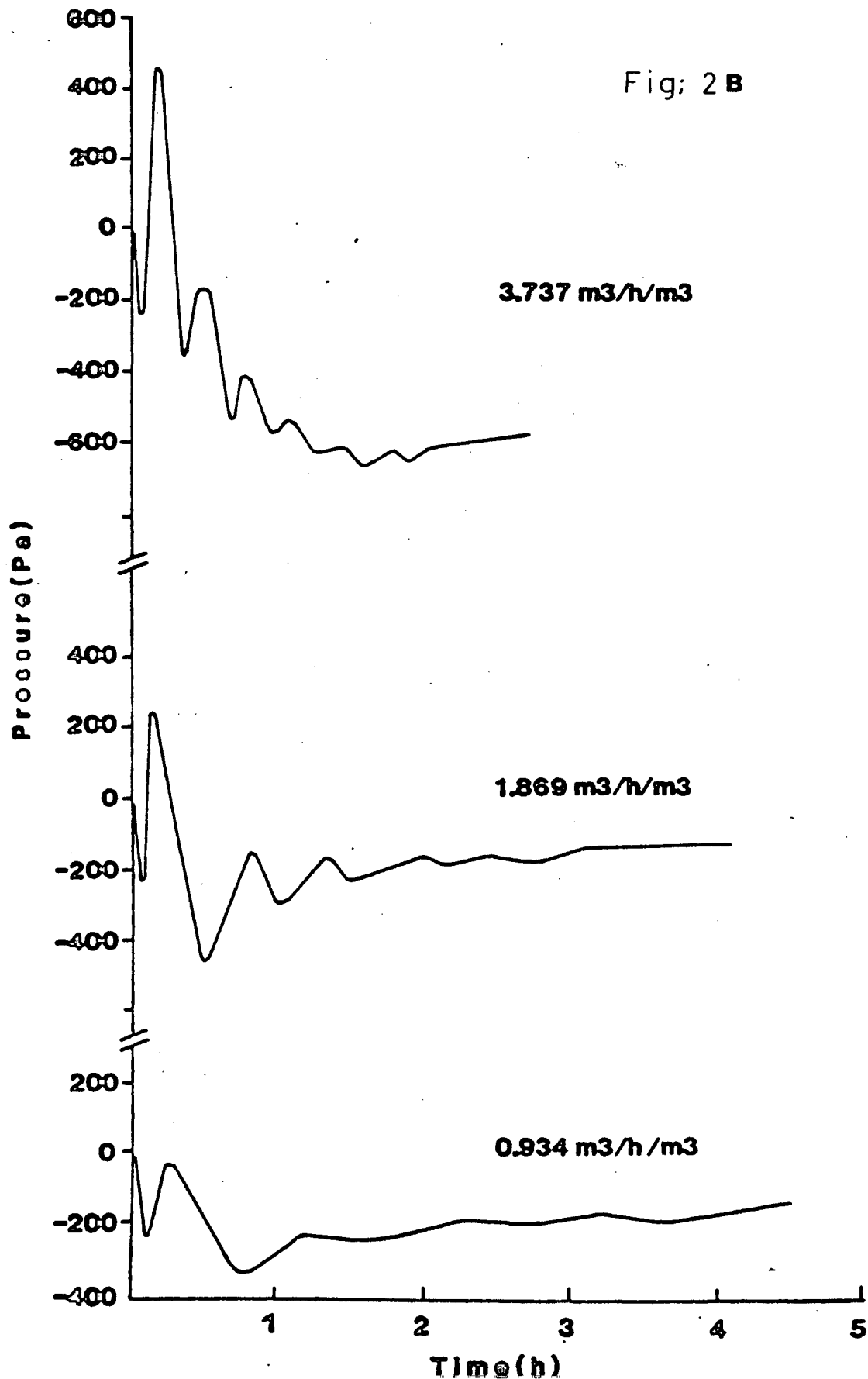


Fig 2. Pressure changes observed under the influence of different recirculation rates to attain final CO_2 concentration of 30% (A) and 60% (B) in the experimental silo containing wheat.



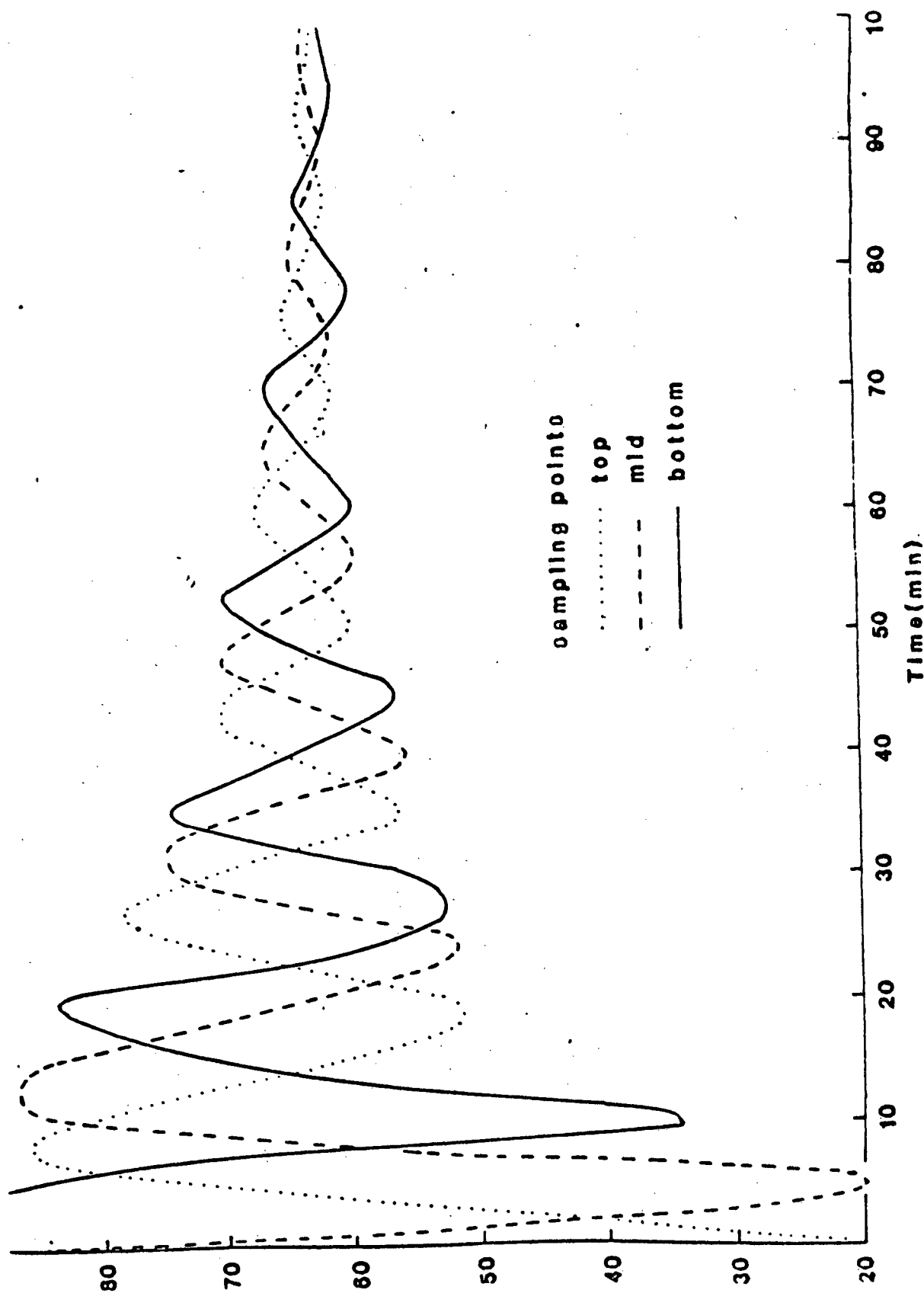


Fig 3. Cyclic CO_2 concentration changes observed with $3.737 \text{ (m}^3/\text{h)/m}^3$ recirculation rate, gas purge from bottom and the final concentration 62%.

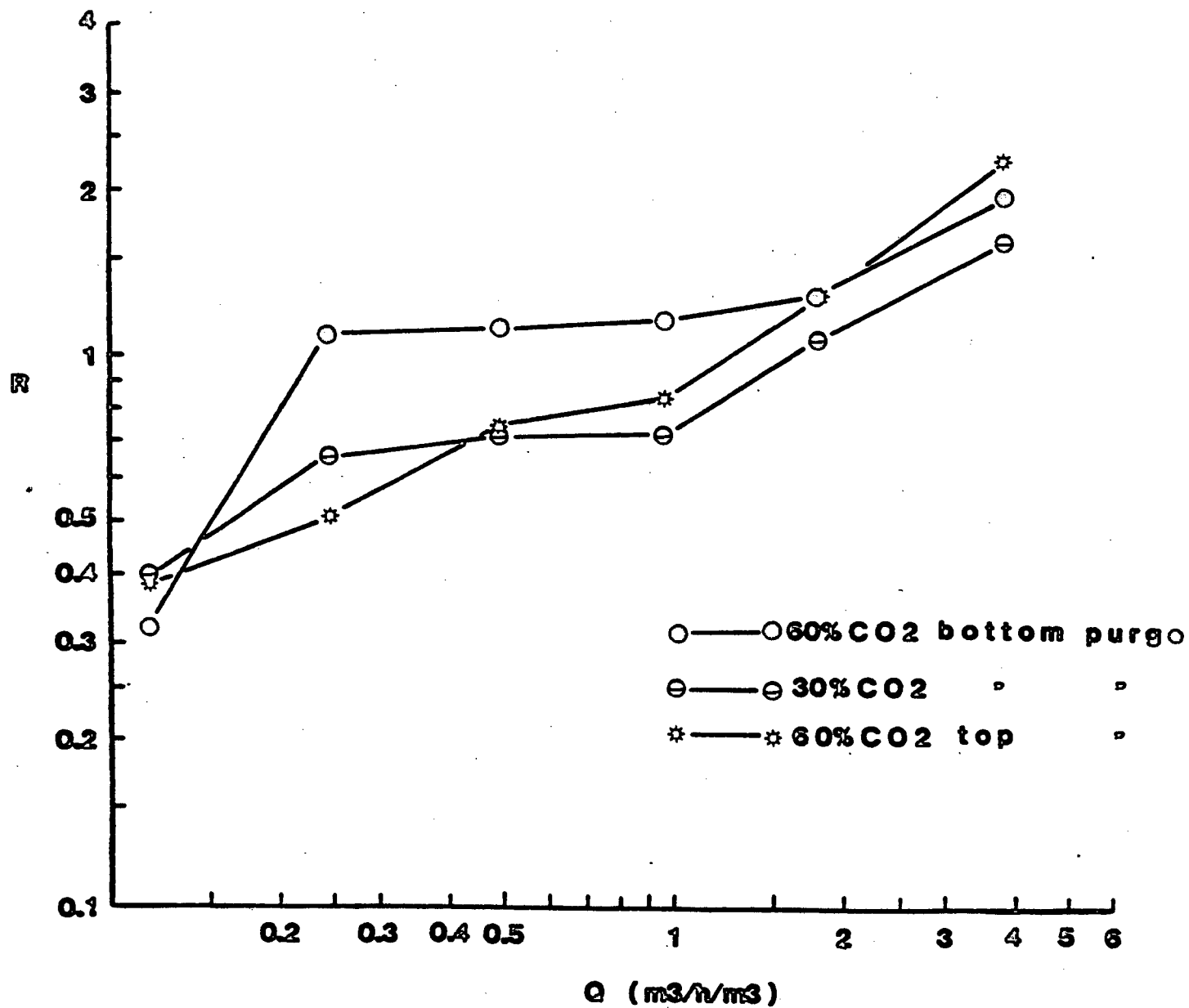


Fig 4. The relationship between different recirculation rates (Q) and the empirical recirculation coefficient (R).

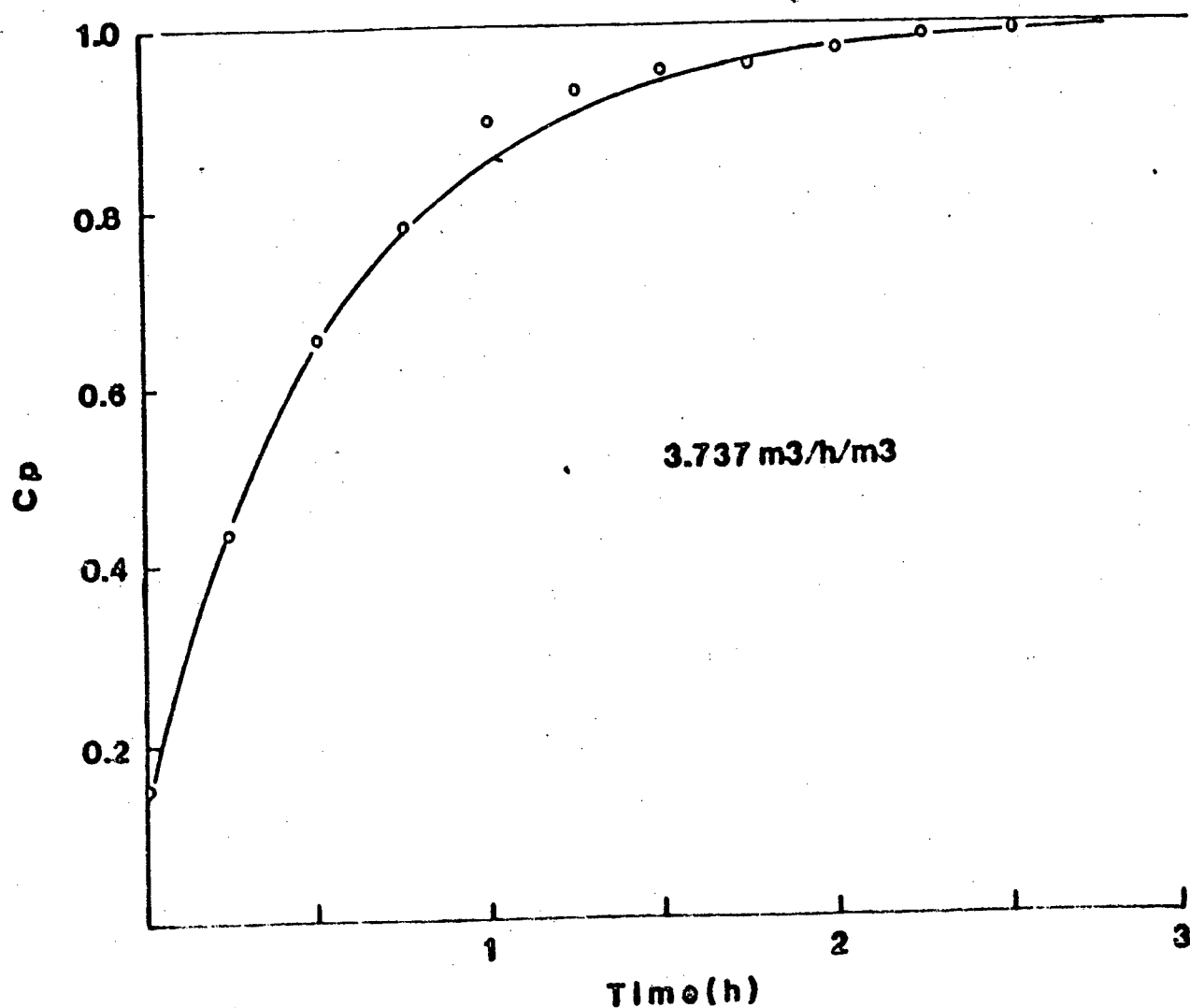
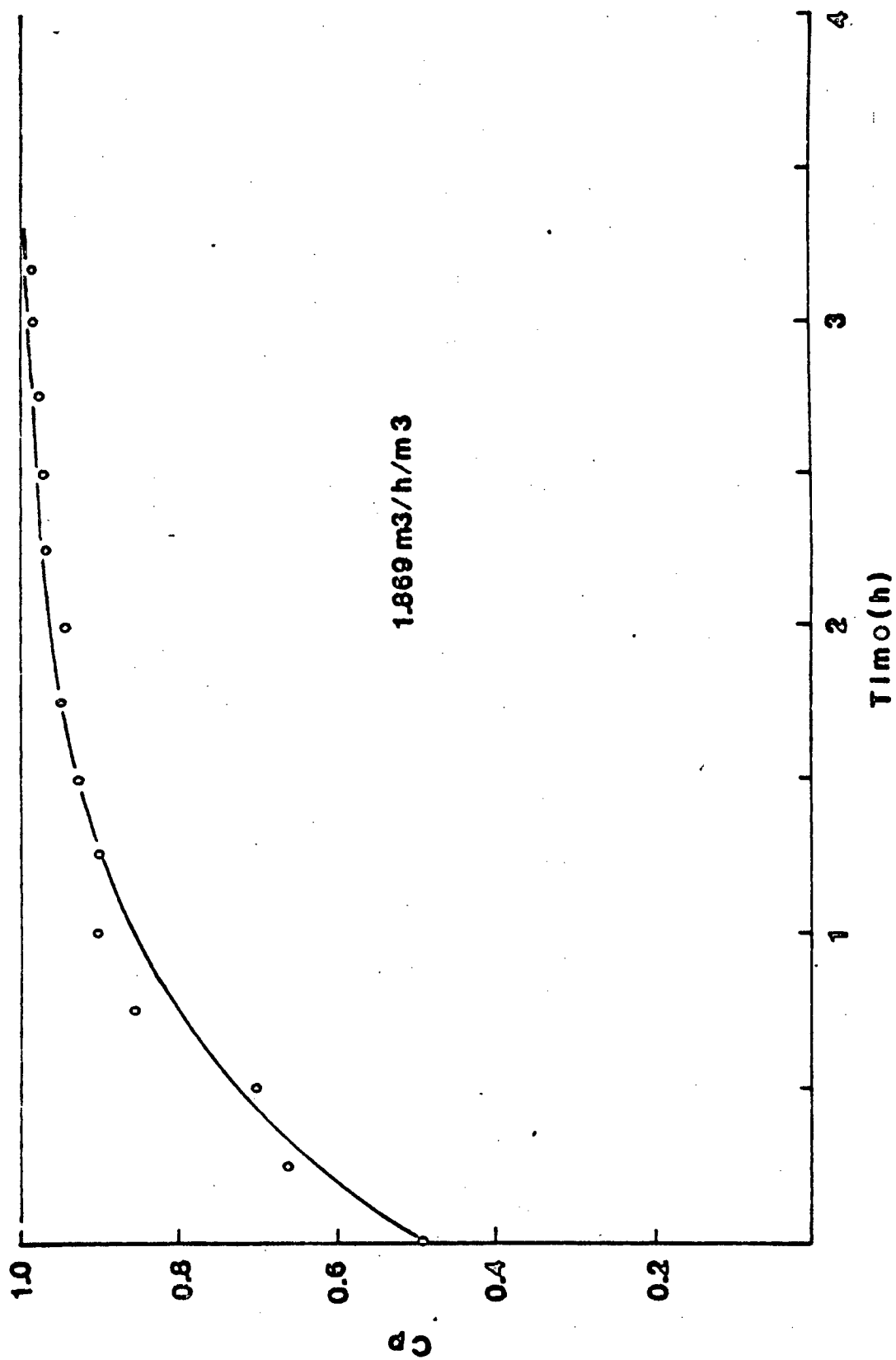
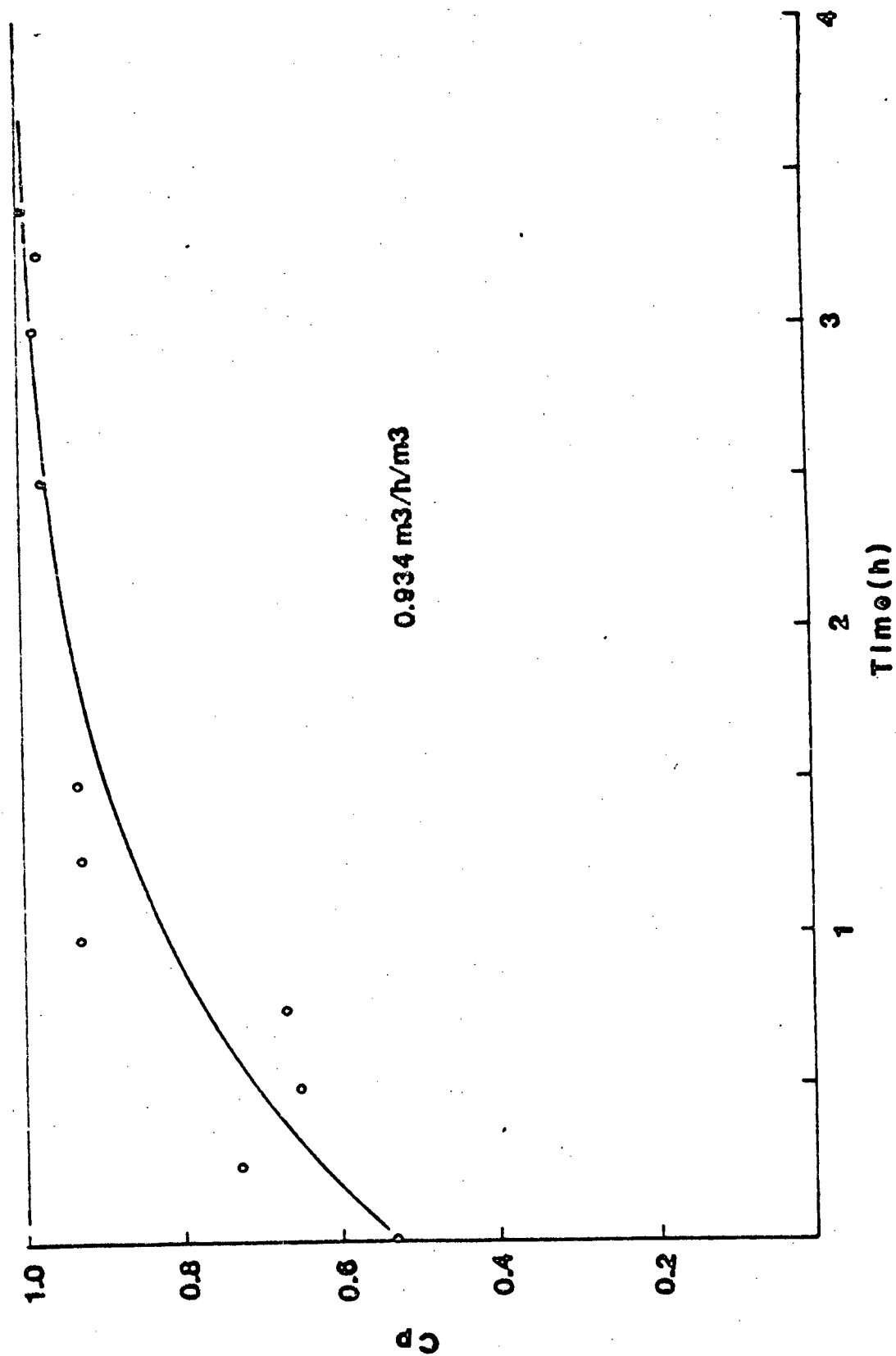


Fig 5. Calculated (solid-line curve from equation [6] and measured (dots) values for the ratio of minimum to maximum concentration of CO₂ (C_p) during recirculation at different rates. The C_p values were calculated based on the recirculation coefficient (R) obtained for CO₂ purged from top of the experimental silo to attain a 60% CO₂ concentration.

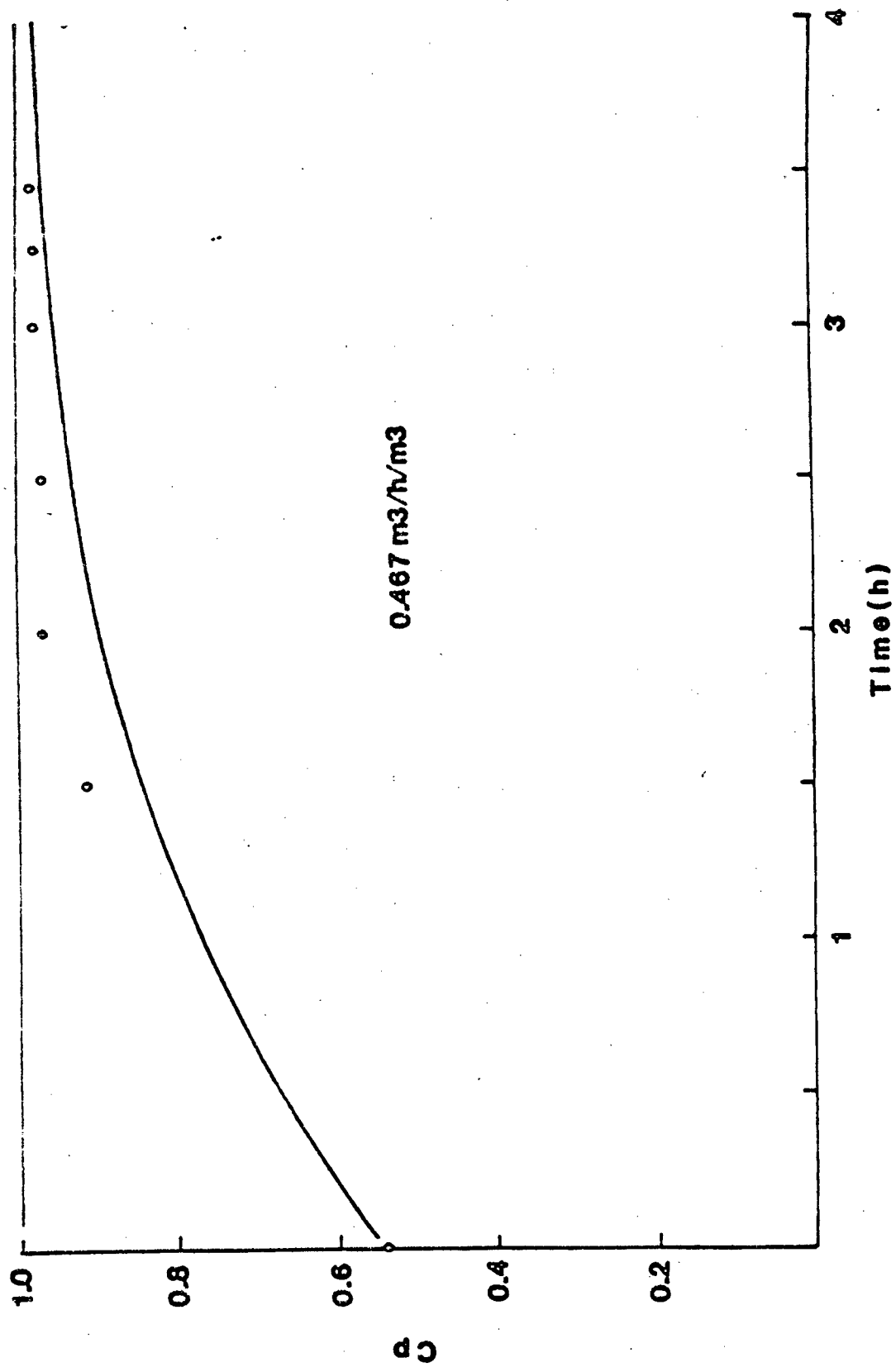
Fig. 5

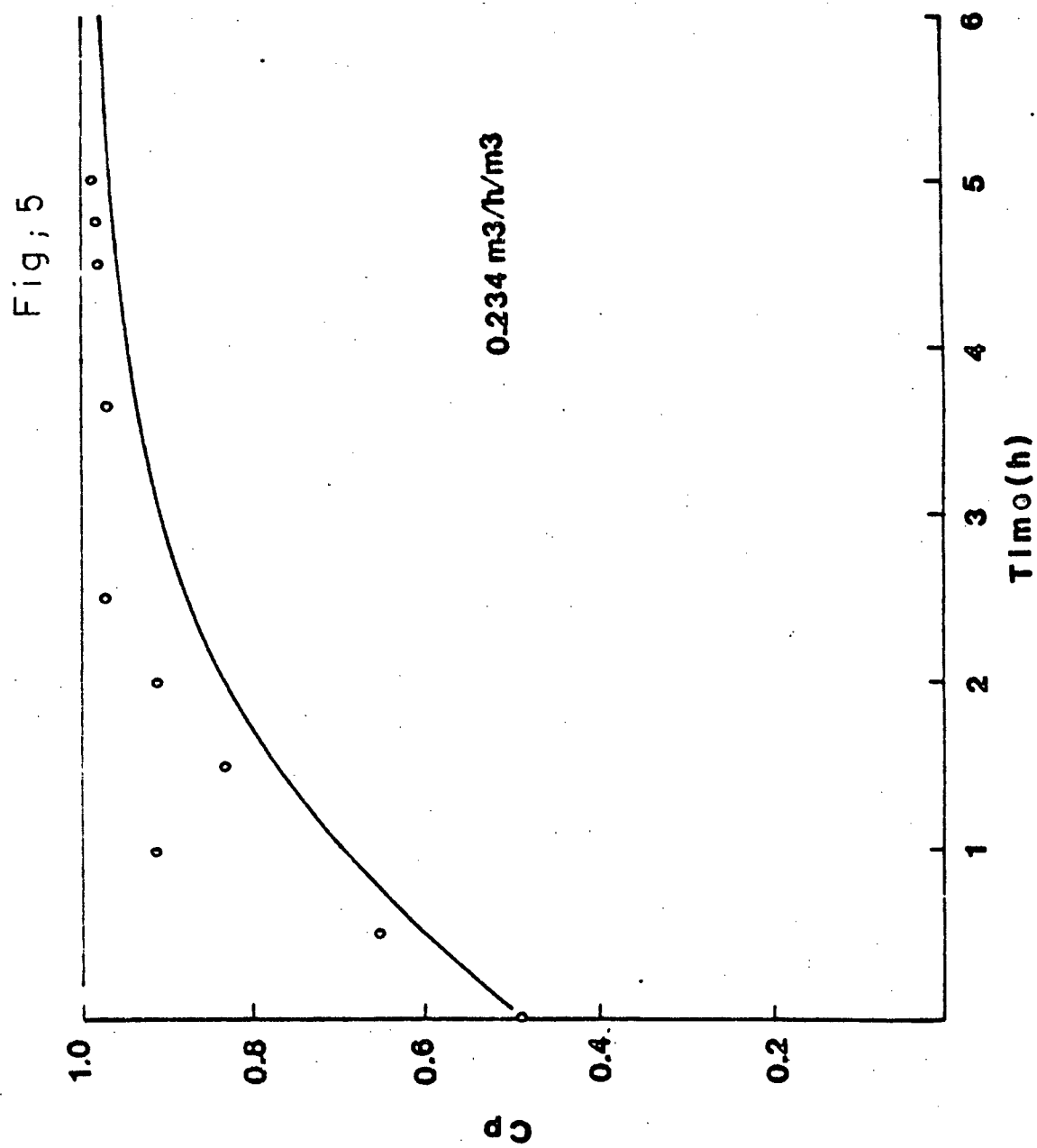


Fig; 5

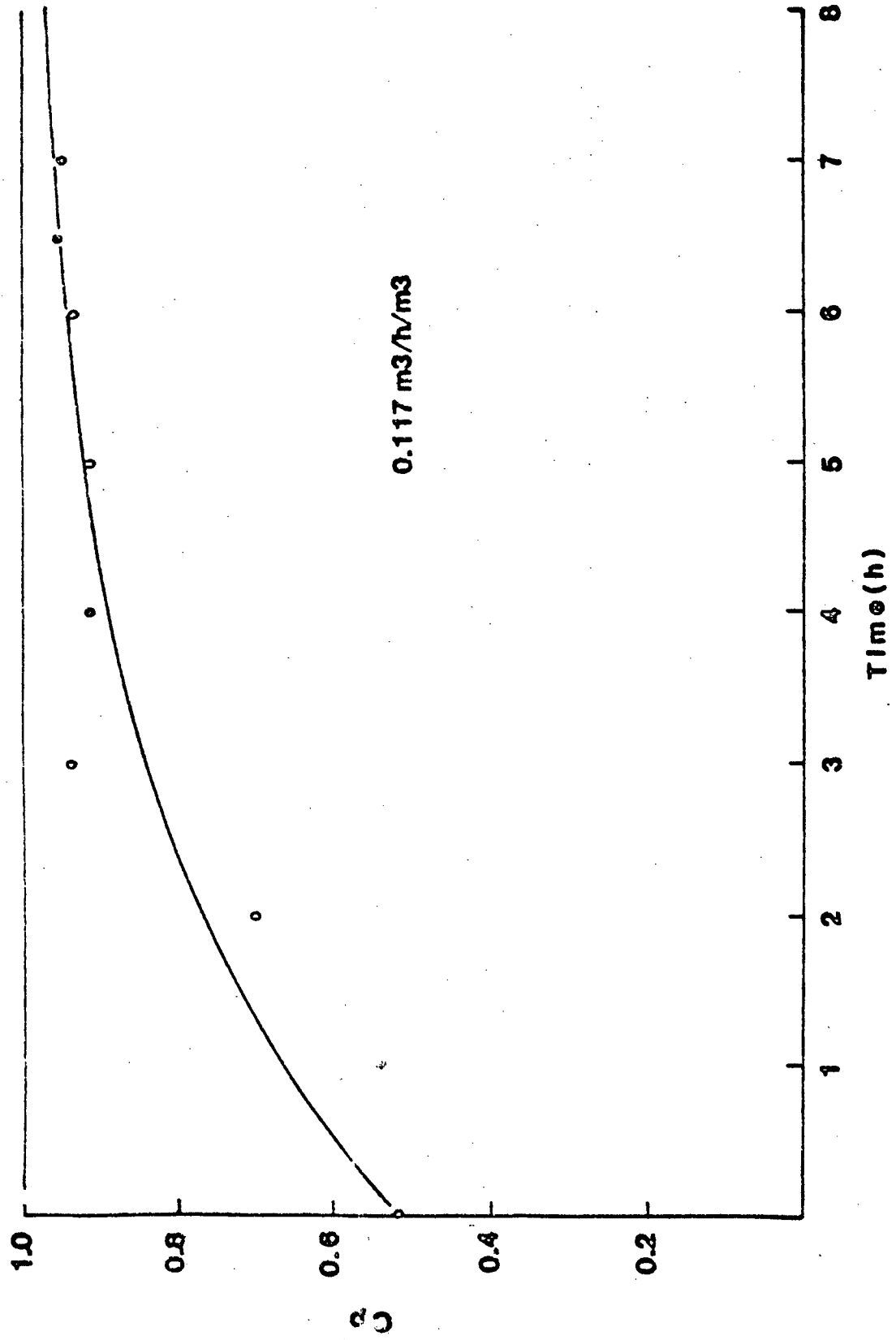


Fig; 5





Fig; 5



4. Field trials

CARBON DIOXIDE: EFFECTS ON NATURAL AND ARTIFICIAL STORED-PRODUCT INSECT POPULATIONS IN WHEAT IN A WELDED STEEL BIN

Abstract - A 11,278 m³ welded steel bin containing 6881 t of soft red winter wheat was treated with carbon dioxide (CO₂) to evaluate the effects of a modified atmosphere on a natural population and on an introduced population of several species of stored-product insects. The bin was purged out from the bottom for 32.5 h and from the top for an additional 6.5 h at a rate of 426.7 kg CO₂/h. Maintenance CO₂ was added from the top for an additional 106 h. The flow was stopped 24 h prior to aeration and subsequent entry and CO₂ concentrations in the bin during the maintenance and holding period ranged from 40 to 100%. The temperature of the grain ranged from 18 to 20° on the surface to 30-33°C in the center of the bulk. Mortality of natural populations ranged from 93.8% reduction-in-population (RIP) for Sitophilus spp. to a 99.4% RIP for Cryptolestes spp. at the 60 day post-exposure observations. Mortality of introduced Tribolium castaneum (Herbst) larvae was 100% while the lowest mortality of introduced Sitophilus oryzae (L.) was 70.0% and the lowest RIP for introduced Rhyzopertha dominica (F.) was 96.4%. Mortality was correlated to the location of the insects in regard to temperature and movement of the CO₂ front from the bottom to the top of the bin. Results of pressure testing the bin prior to treatment are discussed.

INTRODUCTION

Interest in the use of controlled or modified atmospheres (MAs) to protect stored commodities from insect attack has been intensive in the last few years and has resulted in two international symposia being held on the subject (Shejbal, 1980; Ripp, 1984a). However, most published data on bioassay of stored-product pests when exposed to MAs is from laboratory studies or from artificial infestations introduced into the product during field studies. Very little information is available on the effectiveness of MAs against natural populations in actual field situations. When such information is available there seems to be a conflict between it and published laboratory studies on the same species (Jay, 1984a) and the field populations seem to be controlled more rapidly than are those exposed in the laboratory.

In the United States (U. S.), field research has been directed toward the use of carbon dioxide (CO_2) because it is more effective than other MAs (nitrogen or product from a modified atmosphere generator or burner) in storages that have not been properly sealed. This is because CO_2 is effective in controlling insects infesting stored products at concentrations of from 35 to 100% (Jay and Pearman, 1973; Jay and D'Orazio, 1984; Jay et al. 1971). A concentration of ca. 60% CO_2 is recommended for use in the U.S. for a treatment at or above 27°C for a period of from 5 to 7 days (Jay, 1980b). This leaves ca. 8% oxygen in the atmosphere and the concentration can be allowed to drop to ca. 45% CO_2 before additional make-up gas is added. In contrast, if N_2 is used for the treatment the O_2 level must be kept below 1% for the treatment to be effective (Banks et al. 1980). The maintenance of a low O_2 concentration with N_2 would be economically unfeasible in the US because of the non gastight nature of the storage structures and the reluctance of industry to spend the necessary funds to seal these structures.

There also exists the question as to whether or not modified atmosphere generators could economically attain and maintain a lethal concentration of MA in a majority of the storage structures in this country although Storey (1973) successfully accomplished this in large upright concrete silos. In one of the existing studies where natural insect populations were studied Navarro et al. (1978) used a MA generator on wheat stored in welded metal bins. In these tests from 88.1 to 100% mortality was obtained in these insects during exposures of from 22 to 62 days.

Australian researchers have devoted a considerable amount of time to developing sealing and application techniques for the use of CO₂ or N₂ to replace conventional fumigants in grain storage facilities (Banks, 1979; Banks, et al., 1980). Many of these studies were conducted on infested wheat but accurate descriptions of pre-and post-treatment populations are generally not given. However, in Australia, Wilson et al. (1980) sampled two 1900 tonne (t) bins before and after treatment with an 80% CO₂ atmosphere. Prior to treatment they found the grain to be infested with Tribolium castaneum Herbst and Rhyzopertha dominica (F.) at a level of ca 1.2/kg. After an extended treatment of 10 weeks where the CO₂ levels remained above 10% the grain was outloaded and no live insects were found in the grain.

In the U. S., Jay and Pearman (1973) treated 958 kg of shelled maize with CO₂. The maize was sampled as it entered the bin prior to treatment and after treatment by moving it to another bin and taking additional samples. A reduction in the natural population of 99.9% were found when the pre-and post-treatment-samples were compared and 98% of the survivors of the 91-h treatment were Sitophilus spp. Further U. S. studies using CO₂ for controlling natural populations were reported by Jay and D'Orazio (1984). In one of these tests, 1088 (t) of naturally infested wheat was treated for 90 h

after it was purged out to 60% CO₂. A 99% reduction in the natural population was found as a result of this treatment.

This paper describes an additional study on the effects of a CO₂ treatment on a natural population of stored-product insects occurring in wheat in a large welded-steel bin under U. S. conditions. Attempts are made to examine the relationship between bioassay of the natural population and bioassay of an artificially introduced population in the same treatment situation.

MATERIALS AND METHODS

The trial was conducted at a country elevator located in northeast North Carolina, U. S. A., during late September and early October. A welded steel bin 34.90 m in diameter, 9.00 m in side wall height, with a conical roof 17.37 m in height from the base was used for the trial (Fig. 1). The volume of the bin was 11,278.6 m³ and it was partially filled with 6881 (t) of soft red winter wheat.

Temperature, Humidity, and Grain Moisture Measurements

The temperature of the grain bulk was measured using a thermocouple system permanently installed in the bin. The thermocouples were mounted on 18 cables at intervals of 1.5 m making a total of 112 temperature measuring points. In addition, three humidity sensors (Hygrodynamic Inc., wide range) were installed 0.9 m below the grain surface at the south, the north and the peak of the bulk. A Belford Instruments Co. recording hygrothermograph was placed on the surface of the grain during the test. The grain moisture content was measured using a capacitance moisture meter, Motomco model 919.

Sealing This bin had been converted from an oil storage tank to a grain tank and it provided an ideal structure to partially seal with minimum effort. Since the bin was equipped with an aeration system, the air supply ducts and

the roof vents were covered with plywood (12 mm thickness) and sealed with silicone rubber. Silicone rubber was also used to seal openings around the fan switch boxes. The discharge spout was equipped with an auger and was sealed by cutting a piece of plywood to fit the cross section area. A polyurethane base foam sealer was used to secure complete gastightness around this plywood. The flat concrete bottom attachment to the metal wall was not sealed.

Pressure Tests

A series of pressures were applied to the filled bin by reversing the wiring on the 0.61 m fan connected to the central aeration duct (Fig. 1), pressurizing the sealed bin at different flow rates and recording the pressure difference from ambient (Sharp et al., 1976; Navarro, et al. 1978). A Dyer Model 400 inclined manometer and a Alhnor Model 6000 Velometer were used for this test. A pressure decay test was performed by recording the pressure drop against time.

CO₂ Application Equipment

A 10.7 CO₂ storage vessel mounted on a trailer was moved to the grain facility for use in the test. There were two Hex, Inc. CO₂ vaporizers mounted on the trailer, one was 15 kw., the second was 30 kw. The two vaporizers had a maximum conversion capacity of 1.0 t/h of liquid CO₂ to gaseous CO₂. Gaseous CO₂ from the vaporizers was piped through a Flowmetrics, Inc. Series 2600 flowmeter. The CO₂ moved from the flowmeter into the bin through a 5.1 cm (i.d) rubber hose. The hose was run into the bin through a hole cut into the plywood blanking plate over the 0.61 m. fan. This fan was connected to the aeration directing which ran into the center of the bin (Figure 1) and the hose was run into the shaft for ca. 6 m. An additional 2.5 cm (i.d.) rubber hose was run from the CO₂ tank to the top

and down into the center of the bin through the plywood blanking plate located on the grain ingress door below the conveyor. This hose was suspended ca. 2.5 m from the top of the center of the grain mass and was attached to the supply tank, vaporizers, and flowmeter after the initial purge of the bin to provide makeup gas for the maintenance phase of the test.

After the trailer holding the CO₂ application equipment was in place and connected to an electrical source the storage vessel was filled with 11.38 t of liquid CO₂. The vessel was refilled by the tanker 3 additional times during the purge and maintenance phases of the test.

CO₂ Sampling

The gas sampling lines were made of 3 lengths of 6.4 mm o.d. polyethylene tubing inserted into stretched 12.7 mm i.d. hollow core braided polyethylene rope. These sample lines were located adjacent to caged immature insects located inside the hollow core of the polythethylene rope and were measured according to the angle of repose of the grain so that each line in each of the 6 ropes would provide gas samples ca. 1 m from the bottom of the bin, from the center of the grain mass and ca. 0.5 m from the top of the bin. These ropes containing the gas sampling lines were probed into the grain mass with a Prob-A-Vac^(R) grain sampler equipped with a special tip to push the lines down through the bulk. Five of these ropes were probed into the grain mass longitudinally up and across the grain bulk on an axis with the central aeration system (Fig. 1) and were ca. 5.5 m apart. The sixth rope was probed into the grain near the wall of the bin (Fig. 1, line F). Figure 2 shows the actual placement of these lines. Because of the depth of the grain and the inability of the Prob-A-Vac to penetrate to the desired level in some instances only lines A, E and F reached their proper depth.

An additional gas sampling line was placed in the center of the headspace and two lines were run from this line in opposite directions to ca. one-half the distance from the center of the bin to the side wall of the bin. These lines were secured ca. 1.8 m above the grain bulk and the composite gas sample from these two lines was used to measure the CO₂ concentration in the headspace of the bin. The 19 gas sampling lines extended out of the bin through a thermocouple access port, and down to the ground to a central manifold.

Gas samples were pulled out of the bin through these lines with a Universal Electric Co. Model ABOP123 vacuum pump having a suction capacity of 9 ml/min for ca. 30 sec. and were then pulled into a Gow-Mac, Inc. (Bound Brook, NJ, U. S. A.) model 20-600 thermal conductivity CO₂ analyzer. Gas samples were taken ca. 10 times a day around the clock for the duration of the trial.

Aeration: At the conclusion of the test, the plywood covers on the aeration vents on the top of the bin and on the 5 fans (Fig. 1) were removed. The fans were started and the CO₂ concentration in the bin and around the fans was monitored with the CO₂ analyzer during the aeration period.

Bioassay: As the ropes containing the gas sampling lines and caged insects were being probed in with the Prob-a-Vac sampler grain samples were taken for determination of the pretreatment natural infestation. At the conclusion of the test and after the bin had been aerated the Prob-A-Vac was again used to take samples at similar locations alongside the ropes containing the caged insects and sample lines. Figure 2 shows the locations where the samples to estimate the natural infestation were taken. Each sample, in most instances, consisted of two glass containers which held ca. 0.9 l of wheat. When the pretreatment samples were taken only one 0.9 l sample was taken at the

location just above the bottom point and none were taken at the bottom point at the B site. At the C site, a pretreatment sample was not taken at the bottom site and when the pre- and post-treatment samples were collected at this site only one 0.9 l container was collected from the 3 or 4 deepest points, respectively. Therefore a total of 68 0.9 l samples of wheat were collected prior to treatment and 72 were collected after the treatment.

Tribolium castaneum (Herbst) larvae, blended immature cultures of Sitophilus oryzae (L.) and Rhyzopertha dominica (F.) and all stages of Cryptolestes ferrugineus (Stephens) were used for bioassay in cages which were placed in the hollow ropes probed into the grain mass (Figure 2). Cages containing these insects were also placed in cloth bags and lowered onto the surface of the grain through thermocouple access holes (pull cages). All insects were the Savannah Laboratory strains and were reared at $26.7^{\circ}\text{C} \pm 1$ (range) and $60\% \pm 5\%$ r.h. (range). The T. castaneum larvae were 22 ± 2 days old at the initiation of the test and were reared in a 1:1 mixture of white wheat flour and maize meal containing 5% brewer's yeast. Ten larvae were placed in 3 g of rearing media in wire mesh screen cages measuring 2-cm-in dia x 9 cm-in height for exposure. The S. oryzae used were from cultures reared on soft red winter wheat which were seeded with adults for 3 days after which the adults were sieved off. The R. dominica were from cultures reared on cracked soft red winter wheat containing 5% brewer's yeast which were similarly seeded with adults for 3 days. Four cultures of different age of these two species were individually blended in a ball mill for 5-min and then 5g of this blend were placed in wire mesh cages, the same size as used for T. castaneum, for exposure. At the time these cages were probed into the grain mass or lowered onto the surface of the grain for use as daily pull cages, they contained cultures 18 ± 3 , 24 ± 3 , 32 ± 3 , and 41 ± 3 days old. The cages were

filled with the infested grain ca. 6 days before the initiation of the test and were held in a room maintained at the same temperature and r.h. as the room where they were reared. There was the potential of some adults having emerged in the cages during this holding period since the life cycle from egg to adult of these two species under these conditions is ca. 35 days. The C. ferrugineus used were from cultures seeded with adults 60 days before they were probed into the grain mass or used for the daily pull cages. The adults were not removed from these cultures and they were reared on a 1:1 mixture of cracked wheat and rolled oats containing 1.5% brewer's yeast and were blended for 5 min in a ball mill before they were placed in exposure cages. The wire mesh cages used for C. ferrugineus were 1.1 cm-in-dia by 4.5 cm in height and contained ca. 1.5 g of the blended media and insects. The life cycle of this species is ca. 28 days from egg to adult so all life stages should have been present in the cages during the exposure.

Four cages, each containing one of the four species and media, were inserted into the hollow-core ropes adjacent to each of the three gas sampling points in each rope. At the conclusion of the test the ropes were removed from the grain mass, the cages removed and transported to the Laboratory where mortality counts were conducted. Four cages of each of the four species served as controls for the rope cages. These cages were buried in ca. 8.1 kg of wheat which had been removed from the bin and placed in 19 l plastic containers. These containers were placed in a building adjacent to the bin and remained there for the duration of the test.

Cages of these insects contained in small cloth bags were also lowered by nylon line through 4 thermocouple access ports onto the surface of the grain to determine the efficacy of the treatment at daily intervals. Four cages of each species were placed in 4 bags and these were lowered onto the grain

surface at four equidistant points around the bin. One bag was removed at 36, 60, 84, and 108 hr after the start of the treatment (Figs. 1 and 2). Two cages of each species served as controls and were buried in a container of wheat in an adjacent building along with the controls for the rope cages. The controls were removed after the same periods as were those in the treated bin. After removal, the T. castaneum larvae were transferred to Petri dishes while the other three species were transferred to 236 ml glass containers fitted with rings and filter paper discs for covers.

The pre-and post-treatment samples collected to study the natural infestation were returned to the laboratory and the insects were counted as soon as possible after arrival and thereafter at 15, 30, and 60 day intervals. At each examination, the individual 0.9 l samples were sieved and the alive and dead insects were counted. The wheat and alive insects were returned to the containers and the dead insects were discarded. The number of alive insects in the 68 pretreatment samples were converted to the number of alive insects in the 72 posttreatment samples. These figures were then converted to % reduction-in-population emergence (RIP) which evaluated the efficacy of the treatment.

The effectiveness of the treatment on artificially introduced S. oryzae, R. dominica, and C. ferrugineus was also evaluated in this manner for both the rope cages and the pull cages. In this case, the mortality of the insects from these cages was compared to that of those from controls held in wheat in the plastic containers. The effectiveness of the treatment against caged T. castaneum larvae was determined by counting the number of alive and dead insects at each examination and converting this to percent mortality.

RESULTS

Temperature, Relative Humidity and Grain Moisture The ambient temperature throughout the trial varied between 15.5° and 29.4°C. This range is typical

during September-October for the region where the trial was conducted. The temperature of the grain bulk during the trial remained almost constant and a typical isotherm was drawn (Fig. 3) to show the temperature gradients prevailing during the test. Before the start of the trial, an attempt was made to cool the grain using the aeration system. This caused a significant reduction of the temperature on the grain surface and in at the lower parts of the bulk which subsequently created these temperature gradients throughout the bulk. The highest grain temperatures were recorded in the center of the bulk and were ca. 33°C. The temperature of air measured in the headspace above the grain fluctuated between 12.8° and 29.4°C because of the influence of the ambient temperatures and solar radiation. The r.h. in the headspace ranged from 48 to 61% during the test. The average wheat m.c. as determined from the 68 0.9 l samples taken before the CO₂ treatment was 12.8% (S.E. ± 0.055) with a range of 11.4 to 13.9%. The m.c. at the termination of treatment was 12.5% (S.E. ± 0.045) with a range of 11.4 to 13.3%.

Pressure Tests: The results of the pressure decay test are shown in Fig. 4A and indicate that a 50% decay (from 160 Pa to 80 Pa) took 1.05 min and after 3.5 min the pressure had equalized to that of the atmosphere. Based on an applied pressure difference between the bin and the atmosphere (Δp), and the rate of gas flow (Q) a relationship of $Q = 0.0023 p^{0.9631}$ was obtained (Fig. 4B).

CO₂ Concentrations: The CO₂ introduction during the initial purge was through the main aeration duct (Fig. 1) and was carried out for 38 h. During this period, a high concentration of CO₂ was formed at the bottom (Fig. 5, 16 to 30h). These iso-concentration lines indicate that CO₂ gradients expanded progressively towards the upper layer of the bulk. After 30 h of purge (Fig. 5, 30h) a large proportion of the bin volume had a CO₂

concentration in the range of from 80 to 100% but the CO_2 concentration remained at the 0 to 20% level in the top layer and around the peak of the roof. After 32.5 h of purging through the aeration duct in the bottom, the CO_2 supply was connected to the top (2.5 cm) line and a high purge flow was continued for an additional 6.5 h. This caused the CO_2 concentration in the upper layers of the bin to rise to a range of from 40 to 60% (Fig. 5, 36 h) but there was still an area between this layer and the high CO_2 concentrations below it that only contained 20 to 40% CO_2 . During the purge phase, an average of 426.7 kg of CO_2 was applied to the bin/h. After the 39 h purge, the CO_2 flow into the top of the bin was reduced and this eventually brought the CO_2 levels throughout the bin to concentrations of 40% or more after 42 h (Fig. 5, 42 h). Figure 5, (48 h) shows that the 60 to 80% gradient rose higher in the bin during the next 6 hours of applying maintenance gas. Although gradients were still present 72 h after the start of the purge, the upper layers were in the range of from 60 to 80% and the lower layers were in the range of from 80 to 100% CO_2 (Fig. 5, 72 and 96 h). Later, during the maintenance phase of the test, higher CO_2 concentrations were found to form a conical shape and this cone of higher concentrations was in the center of bulk (Figs. 5, 120 and 144 h). After 144 h, the maintenance flow was shut off and the bin was allowed to remain in a stable static situation for an additional 24 h. The average flow rate during the maintenance phase was 79 kg/h.

Figure 6 presents the average CO_2 concentrations which were found throughout the trial. These averages are characterized by large variations especially during the first day of the purge phase when an average of ca. 80% CO_2 was obtained. The continued purge from the top caused these variations to smooth out. This figure also shows that during the maintenance phase the

average CO₂ concentrations were in the range of 71 to 85%. A drop in the average CO₂ concentration was observed in the evenings and this concentration rose each morning.

Aeration: The CO₂ concentration in the bin of from 60 to 90% dropped rapidly when the fans were turned on and were < 1% after 0.5 h of aeration. The tank was aerated for an additional 2.5 h and was then entered. Detectable concentrations of CO₂ were not found in the headspace at this time and the ropes containing the sample lines and cages were removed and the Prob-A-Vac samples taken after this 3 h aeration period.

Bioassay: Table 1 shows the % RIP that occurred when the natural populations of Tribolium spp. (mostly T. castaneum), Sitophilus spp., R. dominica and Cryptolestes spp. were exposed to the CO₂ treatment. The Tribolium population averaged ca. 0.3 insect per pretreatment sample and 65% of these insects were alive. Initial counts on the RIP showed that 92.3% of the population was controlled and the RIP rose to 100% after 30 days post-treatment. However, some reproduction occurred and the RIP dropped to 96.6% after 60 days posttreatment. Reproduction in pretreatment samples was low and the population rose only to ca. 0.4 insect per sample after the 60 day holding period.

This table presents data showing that the natural Sitophilus population averaged ca. 9 adult insects per pretreatment sample and that 58.8% of these insects were alive. The treatment caused an initial 97.4% RIP in the population. This RIP dropped to 84.2% at the 15 day examination but rose to 93.8% at the 60-day examination. After 60 days, the number of adults in the pretreatment samples rose to ca. 180 insects indicating that reproduction in this population was proceeding while an average of only ca. 10 insects were present in the posttreatment samples. Complete control of R. dominica was

observed in the initial comparison of pre-and posttreatment samples for mortality of this insect and ca. 0.44 adult per sample were observed in the initial pretreatment samples of which ca. 67% were alive. Subsequent observations showed that some survived since the % RIP dropped to 96.6 after 15 days. However, the % RIP rose to 99.7 at the 60 day examination. The pretreatment population of alive insects rose to ca 9.6 insects per sample after 60 days postexposure. Table 1 also shows the effects of the treatment on the natural Cryptolestes population occurring in this bin. A 98.7 to 99.4% RIP was observed in this group from the initial observation to the 60 day observation. The pretreatment population rose from an average of ca. 2 insects to an average of 14 insects during this period.

When a portion of this data is divided into the number of insects found at different depths in the silo (Table 2) it can be seen that the majority of the Sitophilus spp. were infesting the top and bottom zones of the bin and that most of the survivors were found in the top zone. The R. dominica infestation occurred throughout the three zones but was larger in the top and middle zones than in the bottom. The only posttreatment survivors of this species were found in the top zone of the bin (Table 2).

Table 3 presents the mortality data for T. castaneum larvae and the % RIPs for S. oryzae, R. dominica and C. ferrugineus which were exposed in cages in ropes which were probed into the bin as shown in Fig. 2. All T. castaneum larvae were killed by the exposure to CO₂ while control mortality did not exceed 3% for this group of insects. An initial 94.6 to 98.4% RIP was observed in S. oryzae from this exposure. However, after 60 days postexposure, the % RIP for the insects exposed near the surface dropped to 70 while the % RIP for those exposed near the bottom of the bin dropped to 77.4. The % RIP for those insects exposed in the middle of the bin did not fall

below 97.1 at any examination indicating more successful control of S. oryzae in this area than at the top or bottom of the bulk. The initial examination of the R. dominica exposed in these ropes (Table 3) shows that a 100% RIP was obtained at all depths. However, in subsequent observations of cages from near the surface of the bulk, the % RIPs dropped to ca. 95-96 while the % RIPs of cages exposed near the bottom remained at or near 100. At all examinations of all cages containing C. ferrugineus exposed at the 3 depths, the RIPs were > 99% with the exception of the RIP of those exposed at the surface and held for 60 days. All exposures of this insect in the middle of the bin produced 100% RIP at all postexposure examinations.

Table 4 presents data from the cages of the same species of insects that were probed into the bulk in rope which were placed in bags, lowered onto the surface of the bulk, and pulled from the bin after varying exposure periods. The mortality of T. castaneum larvae increased from the 36 h exposure to the 60 h exposure and all were dead after an 84 h exposure. In the initial examination of the S. oryzae the % RIPs ranged from 89 after a 36 h exposure to 100 after a 60 h exposure. Subsequent examination of these insects provides erratic results for all but the 30 day posttreatment observation where the % RIPs increased from ca. 67 after a 36 h exposure to ca. 93 after a 108 h exposure. All of the 30 day % RIPs dropped at the 60 day examination indicating that the survivors of the treatment were successfully reproducing. The data for R. dominica in Table 4 show that at the 0, 30 and 60 day examinations, the % RIP increased as the length of exposure increased and that significant reproduction did not occur between the 30 and 60 day examinations. This reduction in the % RIPs between the initial and 15 day examinations is probably due to tolerant life stages emerging as adults and then showing, as can be seen in the subsequent increase in RIPs between 15 and

30 days after the 60, 84, and 108 h exposures, a high mortality without reproducing. The C. ferrugineus exposed in this manner gave RIPs of 95% at the 0, 15 and 30 day examinations after either 60, 84 or 108 h exposures. A 108 h exposure produced RIPs of >98% at all examinations but reproduction was evident after the 36, 60, and 84 h exposures as shown by the reduced % RIPs between the 30 and 60 day examinations.

DISCUSSION

This test was conducted for only 130 h after the desired CO₂ concentration was attained. This is on the lower end of the recommended exposure time of from 5 to 7 days at > 27°C. and a large portion of the bulk was at or below this temperature (Fig. 3). It is apparent that a lethal CO₂ concentration could have been maintained in the bin without the addition of CO₂ for several days since the concentration did not greatly change from that observed at 144 h (Fig. 5) when the gas flow was shut off. The mean CO₂ concentration at that time was 78.82 ± 1.54 (SE)% and at the termination of the test after 168 h it had dropped to only $78.44\% \pm 1.54$ (SE). Simple recirculation would also have to be considered (Wilson et al., 1980) here since the CO₂ would possibly have fallen out of the headspace during any long-term exposure without the addition of make-up of CO₂. However, the decision was made to aerate the bin at this time so that efficacy of this reduced exposure period could be evaluated and to study the time required for aeration.

This reduced exposure period caused a < 100% mortality of all test insects except the introduced T. castaneum larvae (Tables 3 and 4). Table 3 does show that this level of mortality was also obtained in the introduced C. ferrugineus population in the cages probed into the middle and the bottom of the grain mass and was almost obtained at these levels with introduced R.

dominica. The RIP for the introduced populations of the three species which survived this exposure was generally lower than that of those populations which were located on or near the surface of the grain bulk (Table 3). These lower RIPs can be directly related to the low temperature found at the surface of the bulk (Fig. 2) and could also be related to the increased time that the insects located in the middle and the bottom of the bin were exposed to CO₂ (Fig. 5, 6 through 24h). This data can be compared to that in Table 2 for the natural populations of Sitophilus spp. which shows that the majority of the survivors were found in the top of the grain bulk where the temperatures were very low (18 to 20°C, Fig. 3) and that only 3 insects survived in the bottom samples where the population was exposed to CO₂ for a longer time but the temperatures were moderate (20 to 28°C, Fig. 3). There was no survival of the natural population of Sitophilus spp. in the center of the bin where temperatures ranged from 26 to 33°C (Fig. 3). Table 4 also presents data on the natural population of R. dominica and shows that the only survivors were from the samples taken near the surface of the grain bulk. Clearly, the % RIP in the natural populations was influenced by the temperature of the bulk and the RIP for Sitophilus spp. was also apparently affected by the location of the portion of the population in relation to the movement of the CO₂ front during the purge.

A comparative analysis to determine the degree of gastighness was performed using data given by Navarro et al. (1978) based on information obtained from the constant pressure test (Fig. 4A). The air ingress into the structure was estimated to be approximately 1% per day but the volume of air entering the bin atmosphere would have had a diluting effect on the existing concentration so for an average concentration of 75% CO₂, the drop in CO₂ is estimated to be approximately 0.75% per day. Figure 4B shows the slope of

the line in Fig 4A has a value of $n = 0.962$. This value is given in the literature as close to 0.5 for sharp-edged orifices and close to 1 for cracks (Sharp, 1982). The nature of this curve indicates that many small cracks existed in the silo envelope. An attempt was made to seal all apparent leaks found on the silo structure but apparently these small cracks were not detected. A gastightness specification corresponding to a total leak area of about 1.0 cm^2 has been set by Banks and Annis (1977) for the application of MAs. A pressure decay of from 1500 to 750 Pa in 10.5 min is recommended as adequate for a bin of 10,000 t. filled to 80% capacity. The results in Fig 4A show a decay of from 160 to 80 Pa in about one minute which indicates a low degree of gastightness for the bin used in this trial. However, the level of gastightness of a silo used for the application of MAs requires careful consideration and the location of any leaks, the atmospheric temperature, and the barometric pressure may affect the actual CO_2 decay rate. Wilson et al. (1980) conducted tests in a metal bin containing 1900 t of wheat and observed a pressure decay of from 1500 to 750 Pa in ca. 3.5 min. In this test, the gas interchange rate between the bin and the external atmosphere averaged from 2.4 to 40% per day. Since the CO_2 was recirculated in this test and weather conditions were not specified it is difficult to compare their results with those reported here. However, the resultant decay in CO_2 concentration 24 h after the termination of the maintenance phase was only 0.47% in this study which is close to the estimated 0.75% decay based on Fig. 4B.

Table 5 presents data on CO_2 use and costs when the delivered cost for CO_2 at the treatment site was US \$121.22/t. Total CO_2 use was 3.64t/1,000t wheat. This is much higher than that described by Banks et al. (1980) of a mean of 1.05 t/1000 wheat in tests in well sealed welded metal bins in Australia. In the tests reported here, only a minimal amount of

sealing was done on a bin that was already filled and this CO₂ usage falls into the range of previous US tests on upright concrete or welded steel bins where little or no sealing was attempted prior to treatment. The range of CO₂ used in these tests was from 3.1 to 4.5 t CO₂/1,000 t grain (Jay and D'Orazio 1984) and in the two reported tests with welded steel bins the CO₂ use was 3.3 and 4.0 t/1,000 t wheat.

The amount of CO₂ used in the maintenance phase of this test could have probably been reduced to ca. 25 kg/h instead of the 79 Kg/h actually used. This is based on the pressure test and the low (0.47%) decay rate of the CO₂ in the bin during the 24 h when maintenance CO₂ was not being applied. This would have reduced the use (including the addition of 25 Kg/h during the 24 h when CO₂ was not actually applied) of maintenance CO₂ to a total of 3,250 kg, rather than the actual use of 8,369 kg used in this phase of the test (Table 5). This would have in turn reduced the total amount of CO₂ used from 25,011 kg (Table 5) to ca. 19,892 kg, reduced the t/CO₂ used/1000 t grain to 2.9 and also reduced the cost/t wheat to U. S. \$0.350. Increasing the exposure to obtain complete mortality for an additional 3 or 4 days at a CO₂ maintenance rate of 25 kg/h would not have brought the total CO₂ used in the maintenance phase to the 8,369 kg actually used. This additional exposure period would probably have caused complete mortality of both the natural and introduced populations of Sitophilus spp. and R. dominica since Jay (1980b; 1984a) showed that an atmosphere containing 60% CO₂ caused a 100% reduction-in-emergence of mixed S. oryzae and R. dominica during an exposure of < 14 days at ca. 16°C. The cost for this treatment of U. S. \$0.441/t (Table 5) was lower than that of from U. S. \$0.691 to 0.794/t reported by Jay (1980a) for three different application methods studied in an upright concrete silo containing maize. This may indicate that welded steel

bins are inherently more gastight than upright concrete structures.

The amount of CO_2 used t/grain is obviously directly related to the degree of gastightness of the storage structure to be treated. However, in the U. S. grain processors are reluctant to expend the necessary funds to adequately seal structures for the successful application of CO_2 or other MAs. This may be an unfortunate decision on their part since in Australia (Aus.) Ripp (1984b) found that sealing costs, fittings and maintenance for a large (350,000t) horizontal storage to be Aus. \$2.60/t and that the sealing has a life expectancy of 20 years making the yearly cost for sealing to be ca. Aus. \$0.13/year. Costs to treat with CO_2 in sealed storages are estimated by him to be Aus. \$0.05/t in horizontal storages and \$0.04 in vertical storages. Thus, sealing and treatment costs/t Aus. \$ would be 0.19 and 0.18 for a single treatment fill and treatment each year. This cost would be reduced by the number of times a bin was filled and treated each year and the cost is certainly less than the costs in this test or in those reported by Jay and D'Orazio (1984) for similar treatments in the U. S. Also costs for sealing welded metal bins should be considerably less than for horizontal storages or vertical concrete cells.

This test has shown that, under U. S. conditions, CO_2 has the potential of replacing conventional, residue producing chemicals as the treatment of choice. However, additional studies are needed in the areas of economics, sealing, recirculation and pressure testing. There is also a need for education of the personnel using this technique on the exposure time x temperature x CO_2 parameters necessary to obtain adequate levels of control.

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Table 1. -- Average number of insects (dead and alive) per sample and % alive of 4 different species found in the wheat before and after treatment, and calculated percentage of reduction-in-population (RIP) compared with the population before treatment.

Insect sp.	Posttreatment time) (days)	Before Treatment		After Treatment		
		No. of insects per sample	% Alive	No. of insects per sample	% Alive	% RIP
<u>Tribolium</u> sp.	0	0.29	65.0	0.32	4.4	92.3
	15	0.22	73.3	0.08	16.7	90.9
	30	0.34	95.7	0.01	0.0	100
	60	0.43	100	0.01	100	96.6
<u>Sitophilus</u> sp.	0	8.75	58.8	14.18	0.9	97.4
	15	14.97	69.7	2.88	58.0	84.2
	30	31.96	88.9	2.04	72.6	94.1
	60	180.46	95.5	10.65	94.7	93.8
<u>Rhyzopertha</u> <u>dominica</u>	0	0.44	66.7	0.15	0.0	100
	15	0.51	82.9	0.04	33.3	96.6
	30	2.10	99.3	0.01	100	99.3
	60	9.59	98.9	0.03	100	99.7
<u>Cryptolestes</u> sp.	0	2.40	96.3	2.44	1.1	98.7
	15	2.85	94.3	0.86	1.6	99.5
	30	6.10	91.8	0.10	14.3	99.7
	60	14.18	98.1	0.10	85.7	99.4

1/ 0 days indicates the first examination which was conducted as soon as possible after the insects were removed from the bin.

2/ Dead insects were removed from each sample after each examination was made.

Table 2. -- Total number of alive and dead Sitophilus spp. or Rhizopertha dominica found in the top (A), middle (B), or bottom (C) zones in pretreatment and posttreatment probe samples of the natural population. Totals are for all examinations (0, 15, 30, and 60 days after treatment) and dead insects were removed from the samples at each examination. 1/.

Sampling Zone <u>1/</u>	Pretreatmentment		Posttreatmentent	
	Alive	Dead <u>Sitophilus</u> spp.	Alive	Dead
A	8,851	1,196	956	703
B	958	25	0	77
C	5,183	107	3	398
<u>Rhizopertha dominica</u>				
A	392	19	4	13
B	359	4	0	0
C	89	1	0	0

1/ Data for Zone A is from the top 4 probe samples from the bin (Fig. 2), Zone B is from the middle 4 samples and Zone C is from the bottom 4 posttreatment samples and also from the bottom 4 pretreatment samples with the exception of line B where data is from only 1 sample and line C where it was from 3 samples.

Table 3. -- Average percentage (\pm SE) of reduction-in-population (RIP) of different insect species exposed in cages in ropes located at different depths of the CO₂ treated bin. ^{1/}

Insect sp.	Posttreatment time (days)	Location of cages during exposure		
		Bulk surface	Midway surface to bottom	Bottom of bin
<u>Tribolium</u> <u>castaneum</u> ^{3/} larvae	0	100	100	100
<u>Sitophilus</u> <u>oryzae</u>	0	94.6 \pm 1.4	98.4 \pm 0.9	96.4 \pm 2.2
	15	86.8 \pm 3.7	97.1 \pm 1.3	91.2 \pm 6.3
	30	91.8 \pm 2.3	98.5 \pm 2.7	94.7 \pm 3.4
	60	70.0 \pm 10.3	97.1 \pm 2.7	77.4 \pm 13.2
<u>Rhyzopertha</u> <u>dominica</u>	0	100	100	100
	15	95.7 \pm 2.4	100	98.8 \pm 1.2
	30	95.1 \pm 1.9	100	100
	60	96.4 \pm 1.2	99.7 \pm 0.3	99.2 \pm 0.9
<u>Cryptolestes</u> <u>ferrugineus</u>	0	99.5 \pm 0.4	100	100
	15	99.4 \pm 0.3	100	99.7 \pm 0.3
	30	99.6 \pm 0.2	100	100
	60	97.0 \pm 2.5	100	100

^{1/} See Fig. 2 for location of cages.

^{2/} 0 days indicates the first examination which was conducted as soon as possible after the insects were removed from the bin.

^{3/} Data for Tribolium based on observed percent mortality.

Table 4. -- Average percent (\pm SE) reduction-in-population (RIP) of 4 different insect species exposed during the CO₂ treatment in pulled cages.

Insect sp.	Posttreatment time (days)	Exposure period (hr)			
		36	60	84	108
<u>Tribolium</u> <u>castaneum</u> larvae	0	50.0 \pm 10.2	98.5 \pm 0.7	100	100
	15	50.0 \pm 10.8	98.9 \pm 0.2	---	---
	30	60.3 \pm 13.0	99.2 \pm 0.2	---	---
	60	49.4 \pm 28.5	99.7 \pm 0.1	---	---
<u>Sitophilus</u> <u>oryzae</u>	0	89.0 \pm 3.1	100	90.7 \pm 2.0	94.8 \pm 3.3
	15	60.3 \pm 6.4	55.9 \pm 2.7	79.3 \pm 6.8	82.5 \pm 2.8
	30	67.4 \pm 2.6	72.6 \pm 5.4	92.0 \pm 0.9	93.4 \pm 1.3
	60	37.3 \pm 5.3	67.6 \pm 12.0	62.1 \pm 12.3	64.1 \pm 12.3
<u>Rhyzopertha</u> <u>dominica</u>	0	57.5 \pm 14.8	70.9 \pm 11.3	87.5 \pm 12.5	100
	15	72.4 \pm 13.4	66.2 \pm 12.8	72.9 \pm 16.1	83.4 \pm 7.6
	30	60.8 \pm 15.0	77.0 \pm 8.3	89.6 \pm 3.6	92.6 \pm 3.1
	60	61.8 \pm 13.6	75.0 \pm 9.7	85.1 \pm 5.7	94.4 \pm 1.8
<u>Cryptolestes</u> <u>ferrugineus</u>	0	83.0 \pm 6.0	98.3 \pm 0.9	98.8 \pm 0.9	99.7 \pm 0.3
	15	79.5 \pm 7.2	96.2 \pm 2.0	97.1 \pm 1.4	99.7 \pm 0.4
	30	82.2 \pm 6.3	95.7 \pm 2.5	97.4 \pm 1.2	99.4 \pm 0.4
	60	65.9 \pm 4.8	86.8 \pm 6.0	91.4 \pm 4.0	98.1 \pm 0.8

1/ 0 days indicates the first examination which was conducted as soon as possible after the insects were removed from the bin.

2/ Data for Tribolium based on observed percent mortality.

Table 5. -- CO₂ use and cost during the purge and maintenance phases of the trial.

	Purge Phase	Maintenance Phase	Total
kg CO ₂ used	16,642	8,369	25,011
tonnes CO ₂ 1000 tonnes of grain	2.42	1.22	3.64
kg CO ₂ used/hr	426.7	79.0	--
hours duration of each phase	39	106	145
cost of * CO ₂ \$US/tonne of grain	0.293	0.148	0.441

*Based on \$US 110/US ton of CO₂ or \$US 121.22/tonne of CO₂.

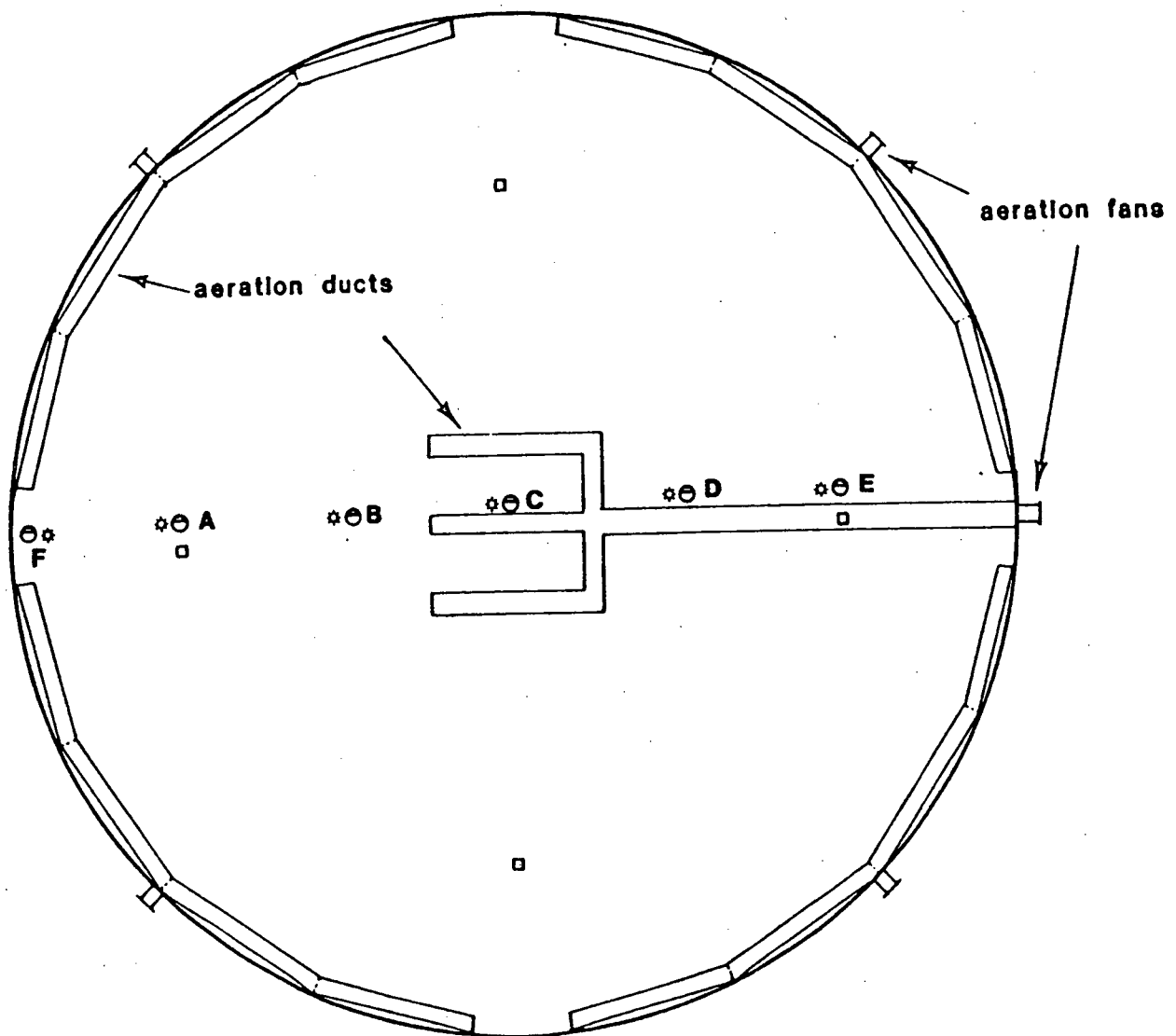


Figure 1. Overhead view of 11,278 m³ welded steel bin showing the location of aeration system; ☆ - probe samples; ⊖ - ropes containing caged insects and gas sampling lines and; □ - daily pull cages.

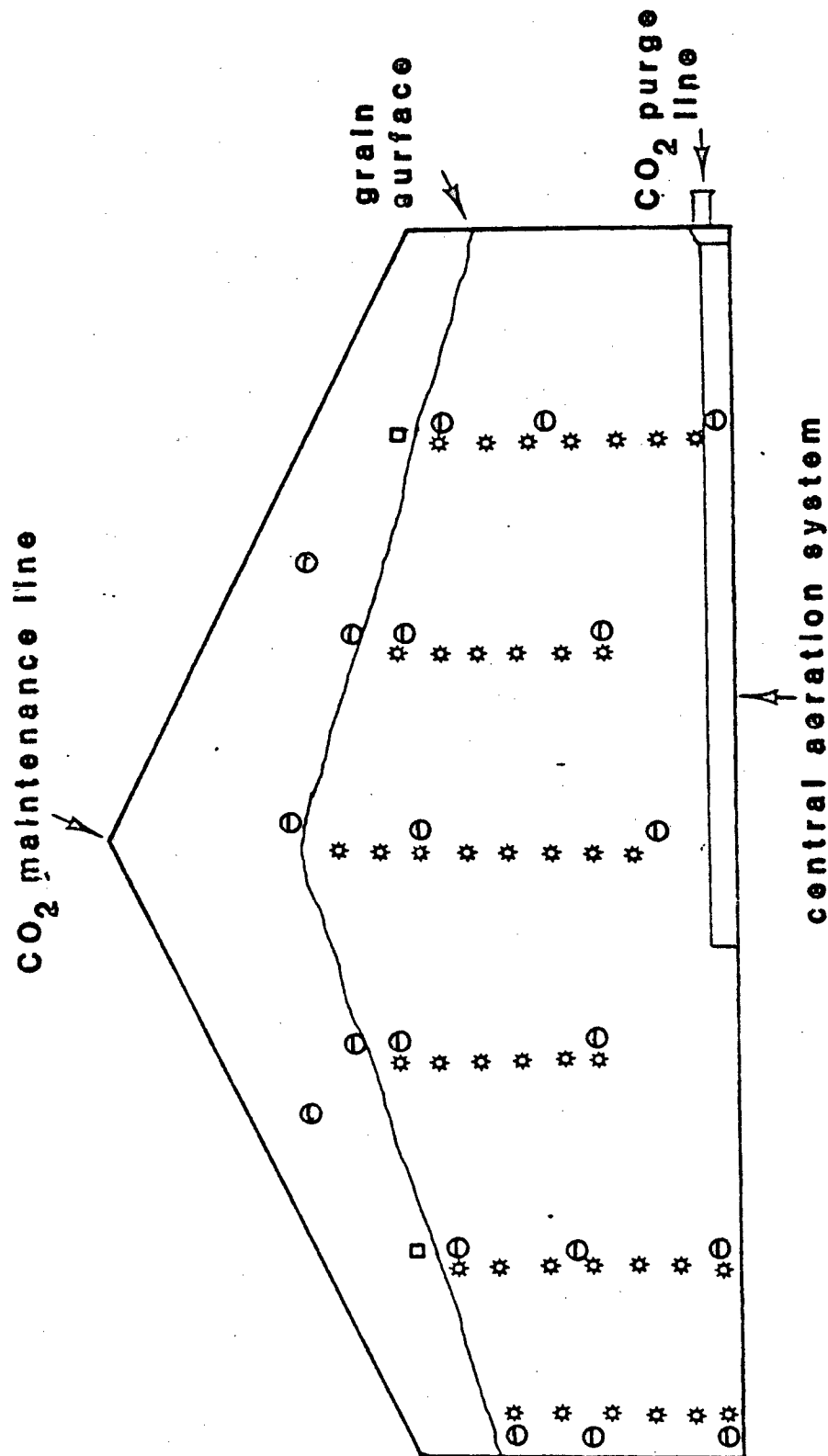


Figure 2. Cross sectional view of grain showing the location central aeration system; * probe samples, ⊙ ropes containing caged insects and gas sampling lines; and □ daily pull cages.

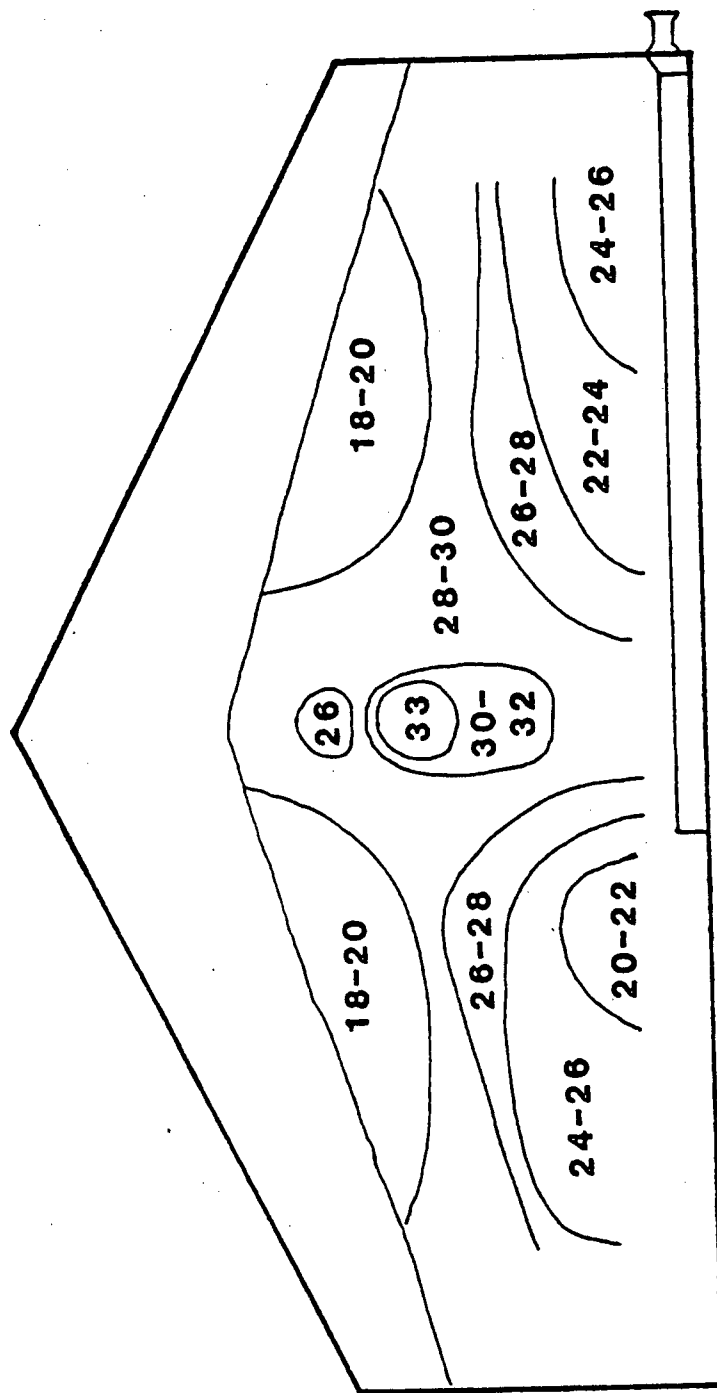


Figure 3. Temperature isotherms in the bulk during treatment with carbon dioxide.

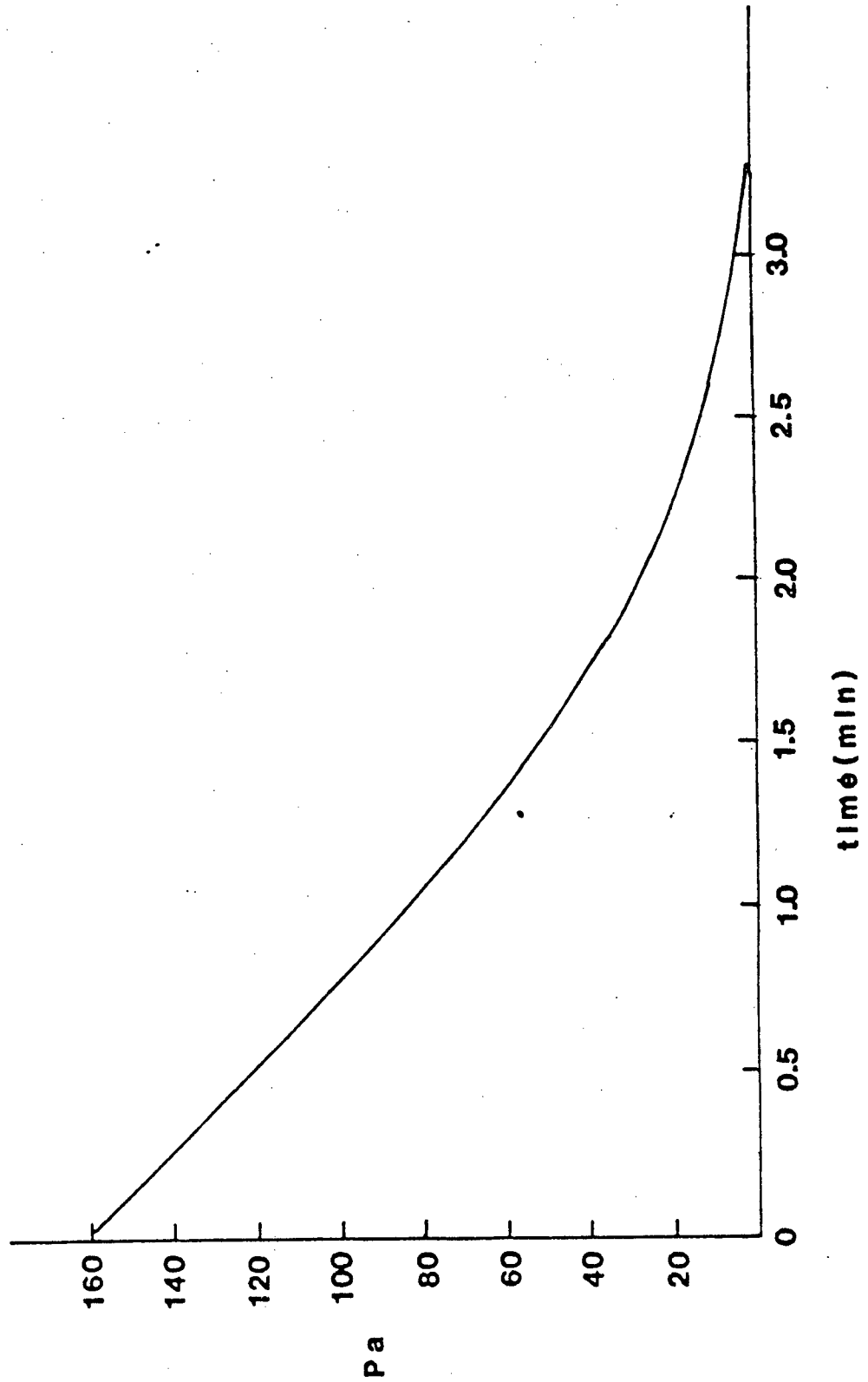


Figure 4A. Pressure decay observed in evaluating the gastightness of the bin.

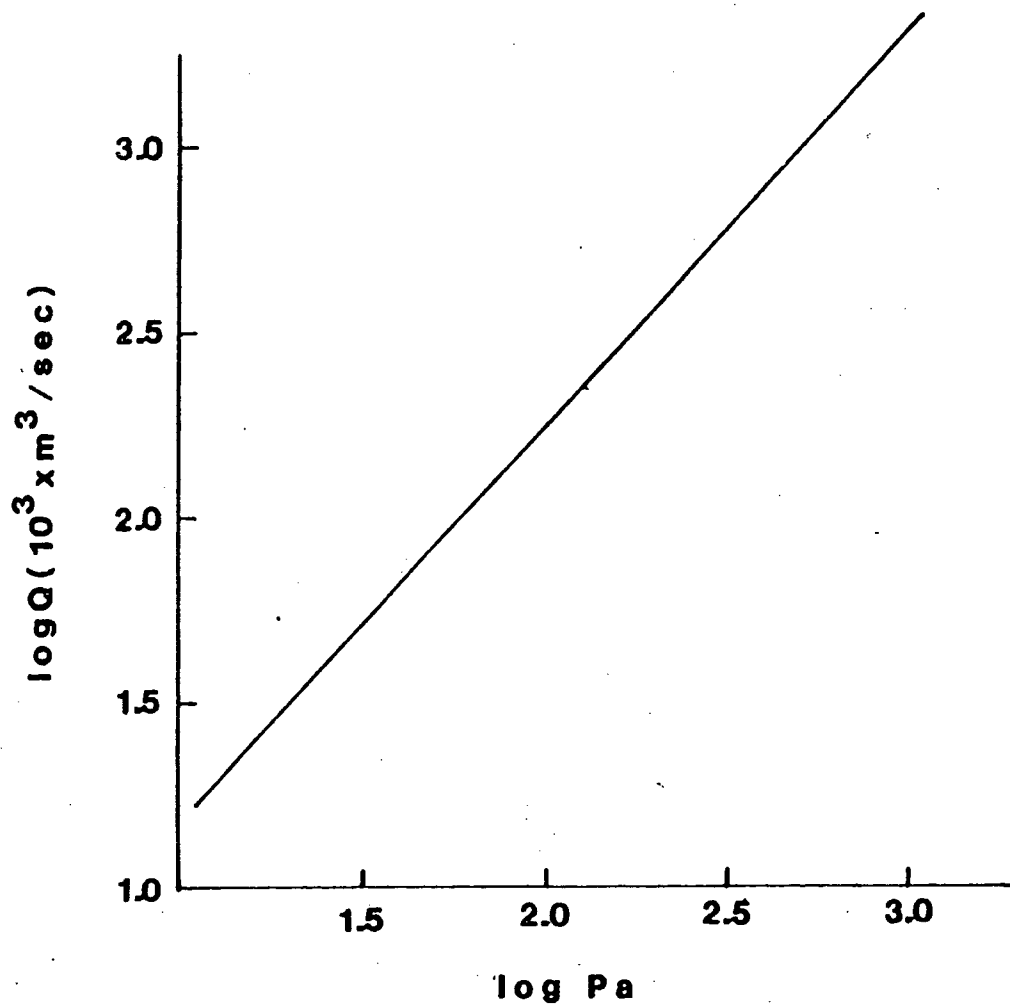


Figure 4B. Constant pressure test which shows the volumetric flow (Q) required to maintain a pressure differential (P) which was used to evaluate the gastightness of the bin.

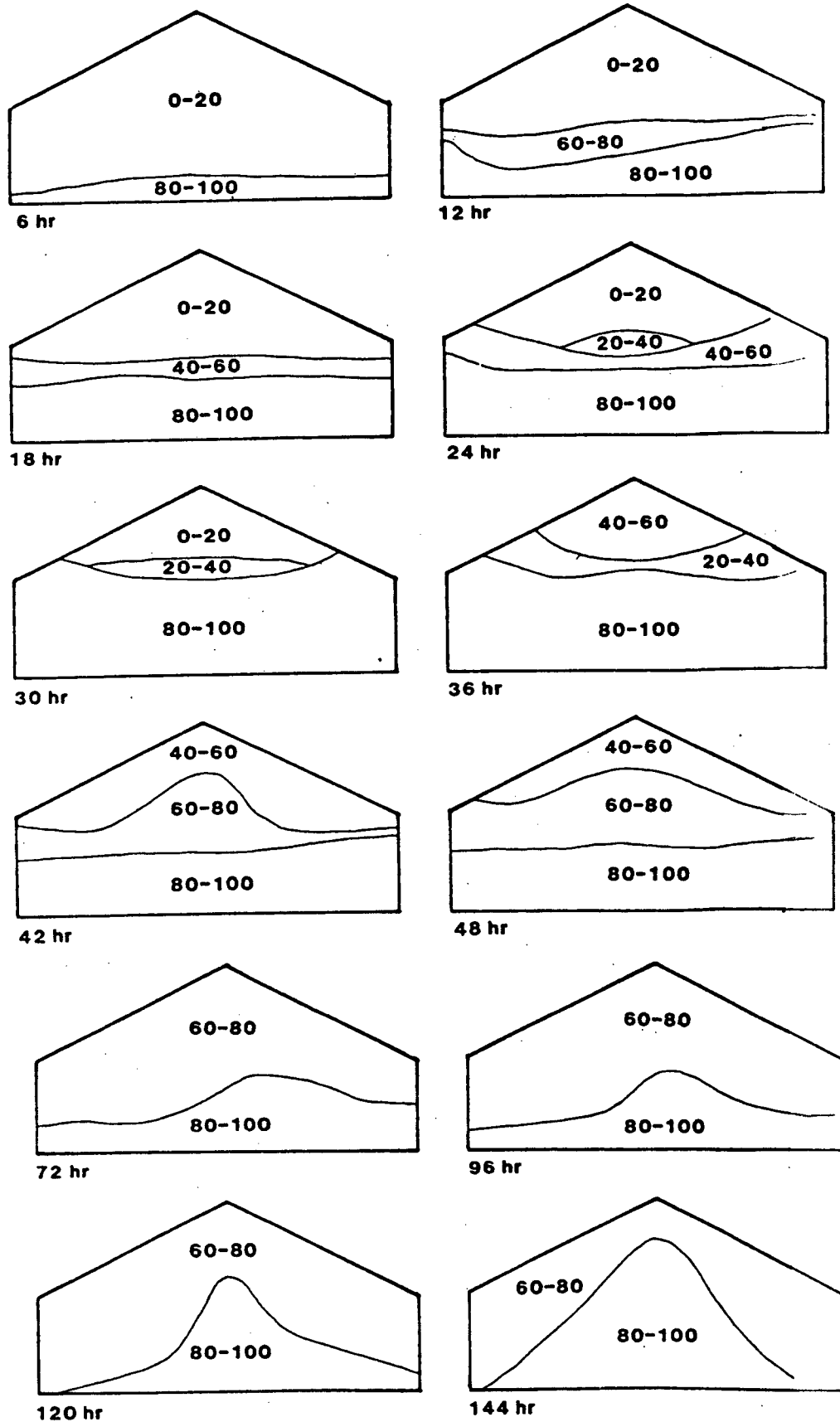


Figure 5. Isoconcentration lines for CO₂ distribution throughout the treatment.

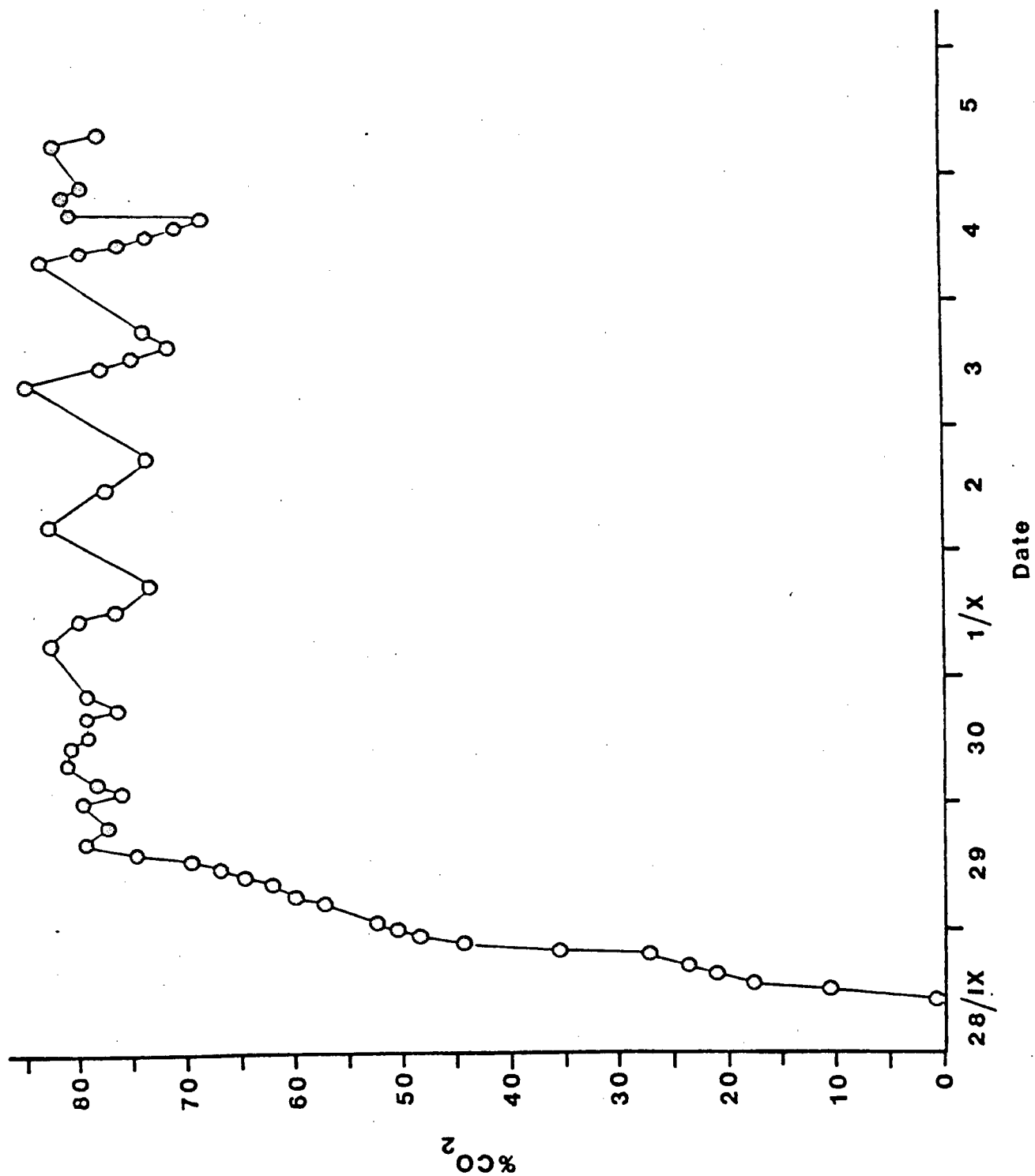


Figure 6. Average CO₂ concentrations observed in the bin during treatment.

F. Description of cooperation

Cooperation was characterized by the visits of Dr. Jay to Israel in planning and actively contributing to the project. The close cooperation of the investigators from both countries had a significant impact during both the planning stages and the operation of the project. During the operation of the project the investigators were in constant correspondence and exchange of ideas. The bioassays related to the project were carried out in both countries and they constitute a significant portion of the input of both laboratories. The MA application techniques and a field trial were carried out while the principal investigator was on a sabbatical leave in the US. During this time, Dr. E. Donahaye served as acting principal investigator. The other field trials were carried out in Israel while the cooperating investigator took an active part in the realization of these tests.

G. Evaluation of the research achievements with respect to the original research proposal

The major portion of the project objectives were achieved. The amassment of bioassay data for determination of the effect of MAs on insect survival was extensive and quantitatively sufficient, but full analysis of all details was not possible within the time allocated for the completion of the present report. Except for Rhyzopertha dominica, all other proposed insects included in the research project, were investigated in full. However, since both investigators had the opportunity to investigate the above mentioned species using funds supplied from other sources, this deviation from the original project should not be viewed as a major disadvantage. On the other hand, the effect of MAs on three Trogoderma species were included in the investigation in view of the increasing interest in these insects especially by the US grain industry. The tested MAs included a range of high CO₂ concentrations in air, a 1% O₂ in N₂ and a mixture obtainable by exothermic gas generator (gas burners) namely 1% O₂, 14% CO₂ and 85% N₂. The range of temperatures tested was also characteristic of that encountered in field conditions in US or Israel. This was 15° to 32°C for the most common insect species, whereas for thermophilic species such as Trogoderma spp. higher temperatures, of up to 38°C were tested.

Although the MA principle has been considered as a control method devoid of the disadvantages of conventional chemical insecticides, a series of tests were conducted to reveal the potential of insects to develop tolerance to MAs. This was carried out on the recommendation made by one of the reviewers of the project. Two species were selected for this purpose; Sitophilus oryzae and Tribolium castaneum. This aspect was investigated to a level that showed that both species have the genetic potential to develop resistance.

Application techniques related to the gastightness of silos were investigated in detail. In view of the increased use of CO₂ as a component in MA composition detailed studies were undertaken to investigate the dynamics of sorption of the gas and its recirculation in the silo.

A detailed study on the application of CO₂ under field conditions was carried out. However, due to technical difficulties, and lack of

sufficient coordination, but mainly due to limited funds, experiments with exothermic gas generators (gas burners) were not carried out. Although the researchers have experience on the application of MAs using gas burners, an investigation of the feasibility of the method under varying leak rates and climatic conditions should be an objective for further study.

H. Benefit to agriculture

Under the current control strategy losses of field crops due to insects in post harvest marketing channels in the U.S.- amount to an estimated 3.9 percent or 1,748.8 million dollars (including cost of control). In Israel, a grain importing country, annual losses of stored grain have not been documented yet. However, preliminary observations indicate that these losses are around 1% (not including cost of control). These losses occur even though a significant effort is put into controlling these pests. Currently used methods are often a hazard to personnel applying them. Many of these hazardous chemicals are in a questionable status in the U.S. In addition, fumigants such as methyl bromide and phosphine leave chemical residues on the food.

Data collected within the framework of the present project contributes directly towards solution of the problem. The proposed technique for insect control is based on the application of MAs which involves changing the proportions of the normal atmospheric constituents of the storage environment. Information gathered on the effect of MAs on insect survival enables the determination of necessary exposure time in relation to temperature of the commodity, the chosen MA and the insect species.

Although the use of MAs in effecting control of storage insects has been shown to be slow in action relative to methyl bromide (fumigant) they are comparable on a time basis with the action of phosphine gas (fumigant) for which the recommended exposure time should be extended to up to 10 days. On the other hand one of the principal advantages of MAs as shown in the present report, is the effective control achieved for the egg stage of the tested insects.

The application of MAs has been considered as a supplementary or alternative control method that the grain industry will take advantages of over a long time period. Although the potential of laboratory selected insects to develop resistance to MAs has been demonstrated, the low levels of resistance exhibited under laboratory conditions indicate that the risks of resistance endangering the practical application of MAs are relatively small.

Information gathered on the application techniques of MAs may be viewed as a significant contribution to the design requirements of grain storages. The basic characteristics of atmospheric conditions

and their influence on gas losses from silo bins treated with MAs under different leak rates were studied. The role of barometric pressure changes and the influence of daily temperature fluctuations on air infiltration into the treated bins were demonstrated. In addition the recirculation rate requirement for adequate distribution of the desired MA composition in silos was determined. This information together with data on the sorption capacity of CO_2 may be used as basic guidelines for the planning stages of the application of MA on a commercial scale. The application techniques studied in the present project and the field trials should serve as basic technical information for evaluation of the economics involved in the application of MAs in grain storages. The cost of obtaining the desired MA, under the tested commercial application, seems to be very competitive in comparison with that of conventional fumigation. The essential importance of obtaining a well sealed, gastight structure has been demonstrated for the successful application of MAs. This engineering aspect of the MA technology will require further attention in the future.

