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CURRENT RESEARCH WITH CARBON DIOXIDE IN CROATIA

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ABSTRACT

Seeds of different species were stored in different packages and under different storage conditions, including CO_2 enriched atmospheres for varying periods, and examined for germination. Germination decreased especially in seeds stored in original packages and in jute bags in a CO_2 enriched atmosphere. However, the seeds packed in polyamide/polyethelene (PA/PE) laminated bags frequently maintained good germination levels.

Several research projects using carbon dioxide (CO_2) as a potential insect control method in grain have been conducted in Croatia from 1986 to the present day. The efficacy of CO_2 on insects has been studied both in silos and in the laboratory. The field studies in silos did not give satisfactory results even though more than 5 kg of CO_2 was used per tonne of wheat. Heavy losses of CO_2 were evidenced when CO_2 was applied in old bins. However, in bins constructed in the early eighties higher concentrations could be held more easily. Commodity temperatures were between 18 and 24^0C. Laboratory results have shown that a concentration range of 55 - 65 % CO_2 effectively controlled adults moths *Corcyra cephalonica* and *Sitotroga cerealella*, whereas immature stages of these species developed within 3 weeks after treatment.

INTRODUCTION

Protection of grains and seeds in storage includes control of insects and preservation of germination. Although some studies had been conducted with carbon dioxide (CO_2) before the early seventies (e.g., Oosthuizen and Schmidt, 1942; Press and Harein, 1967), it was Jay (1971) who revolutionized the use of CO_2. His publication prompted many scientists world-wide to seek the best dosages, conditions, and ways of applying CO_2.

Carbon dioxide can be applied in all storages where conventional fumigants are used, but it is necessary to provide the acceptable or even better sealing. After the initial purge by CO_2 it is necessary to follow-up by the periodic addition of some CO_2 above the top of the grain bulk to maintain the concentration (Jay *et al.*, 1970). According to some studies it suffices to use

1-2 kg CO_2/tonne of grains (Banks *et al.*, 1980) whereas Jay and Pearman (1973) used the considerably higher dose of 4,130 kg CO_2 for 958 m^3 of stored corn (about 5 kg/tonne). There have been some studies on the effect of CO_2 on seeds. Results obtained by Marzke *et al.* (1976), showed that CO_2 atmospheres have no significant effect on the germination of unshelled peanuts, whereas shelled peanuts lost their viability after 6 months.

The leading countries in the application of CO_2 treatments are the USA and Australia, but Israel, Canada, Italy, Germany, and other countries are progressively developing this technology as appropriate for their purposes.

In Croatia, the efficacy of CO_2 was tested on insects in the laboratory and in silo bins. The complete results of the effect of CO_2 under different storage conditions on the germination of 13 types of seed, after 6, 12, 18, and 24 months, are presented here. The experiments with CO_2 were undertaken within the framework of several projects in Croatia since 1986.

SEED GERMINATION STUDIES

Materials and methods

Thirteen cultivars of seeds were collected for the long-term seed germination trials, as per Hamel (1990). All of the seeds were put in laminated plastic bags made of PA/PE (polyamide/polyethelene - 30/60 micrometers), and in jute bags. The green peas and grass seeds were put in the original package (paper bags), whereas other seeds were put in kraft paper. The PA/PE bags were flushed with CO_2 and sealed. All of the bagged seeds were placed in three different types of storages under the following conditions: normal warehouse storage, storage in an air-conditioned chamber at 9°C, and 65± 10% relative humidity (r.h.); and storage under CO_2 (35± 5%) in a storage chamber.

Germination of the seeds was tested before packing and after 6, 12, 18, and 24 months of storage. Two hundred grains were used for tests according to Croatian standard methods (Hamel, 1990). The seeds packed in the hermetically sealed PA/PE bags were used for tests only if they were under partial vacuum. Temperature and humidity were monitored in the warehouse during the storage period. The mean ambient temperatures were recorded by the near-by meteorological station.

Results

Germination of seeds

A wide range of results was obtained when the thirteen different types of seeds were tested for germination after storage in different packages and under different storage conditions for 24 months.

Normal warehouse storage

Germination decreased considerably in paprika, kale, parsley, and grass seeds during the first 12 months of storage (Table 1). Germination of winter wheat (varieties: "Sivka" and "Baranjka") and rape seed declined slightly, as compared with levels at the beginning, but were still above the acceptable standard. Corn and green pea maintained the highest germination while the other seeds were close to regulatory standards.

After 24 months, a decrease in germination had occurred in the all of the seeds except green pea, whereas germination of lettuce and kale actually increased. Especially low results were recorded for soybeans and paprika where germination reached zero. The germination of the corn and grass seeds was slightly below the required regulatory standard of germination. The onion, kale, lettuce and green pea germination were above regulatory standard. The germination of wheat was sub-standard. The poorest result was obtained for untreated winter wheat (var. "Sivka").

A comparison of germination in various packages revealed that it decreased in wheat, soybean, parsley and lettuce in the PA/PE bags flushed with CO_2. Germination of other seeds in PA/PE bags was was not different from that in other packages. The differences in germination among seeds held in the original packages and those placed in jute bags were less marked after 12 months than after 24 months.

The humidity measured in the warehouse was lowest at about 50% in the second part of July and in August. It then increased to 65% until the end of the year. The humidity remained at about 65% during the first 6 months of the following year.

The temperature was above 0°C in the warehouse in January and increased to 10°C in early May. It was approximately 15°C at the end of June and increased to 20°C in July and August. A decrease in temperature started in September, reaching 16°C in the second half of the month. A temperature of 10°C was measured in the second half of October, decreasing to 5°C at the beginning of November and to almost 0°C in December.

Mean monthly ambient temperatures taken from the near-by meteorological station, - about 2 km away - are presented together with maximum temperatures in the warehouse in Fig. 1.

From a comparison of temperatures in the warehouse with those of the meteorological station, it can be seen that until August the maximum temperatures in the warehouse were lower than the mean ambient temperatures, whereas from August until the end of the year, they were higher. This clearly shows the seasonal delay in both warming and cooling cycles within the warehouse.

Air-conditioned storage

The results obtained for seeds stored in air-conditioned storage were better than those under other storage conditions. Germination was the same, or almost so, from the beginning of storage until 12 months, except for parsley, paprika, kale, and grass. When the results were compared with the

Table 1a: Viability of seeds (%) stored for up to 24 months in a warehouse, an air-conditioned chamber, or in a carbon dioxide atmosphere.

	Testing time (months)		Normal warehouse storage			Air-conditioned storage (9¤C)			CO_2 (35%) storage		
			OP	JU	PA/PE	OP	JU	PA/PE	OP	JU	PA/PE
Baranjka (wheat)	SR	88									
	0	98									
	6		91	93	83	92	85	93	55	73	81
	12		87	85	89	86	90	92	25	69	79
	18		77	80	72	82	80	90	4	18	24
	24		79	81	68	82	79	90	19	65	45
Sivka (wheat)	0	93									
	6		92	91	79	91	91	89	47	25	83
	12		85	86	89	96	86	86	24	65	71
	18		86	47	54	84	70	91	1	5	8
	24		77	56	60	90	77	93	2	7	2
Anka (wheat)	0	73									
	6		83	81	78	76	83	80	-	-	83
	12		77	72	76	76	79	85	-	-	73
	18		80	63	74	85	75	83	75	-	62
	24		80	72	76	83	-	82	-	-	71
Oil rape	SR	75									
	0	90									
	6		75	68	66	43	60	69	32	37	53
	12		83	79	86	63	85	84	44	21	78
	18		54	60	79	77	70	87	19	12	72
	24		73	57	93	27	30	87	16	16	54
Corn	SR	90									
	0	97									
	6		94	92	95	94	94	92	95	93	95
	12		96	96	95	96	97	96	94	96	96
	18		90	90	89	84	88	92	87	86	87
	24		84	-	89	94	90	92	80	-	-
Soybean	SR	75									
	0	76									
	6		67	76	70	82	75	75	56	54	60
	12		80	77	70	78	85	80	25	40	66
	18		73	91	47	91	83	84	0	6	3
	24		0	0	0	66	53	77	0	-	2
Green pea	SR	75									
	0	93									
	6		93	91	92	94	95	92	90	89	91
	12		86	94	93	87	92	92	90	87	86
	18		83	83	87	93	88	86	93	90	92
	24		92	-	93	82	93	91	-	93	85

SR = Minimum germination capacity requirement according to Croatian government standard regulations.
OP = original package; JU = jute bags; PA/PE = polyamide/polyethelene laminate.

Table 1b: Viability of seeds (%) stored for up to 24 months in a warehouse, an air-conditioned chamber, or in a carbon dioxide atmosphere.

	Testing time (months)		Normal warehouse storage			Air-conditioned storage (9¤C)			CO_2 (35%) storage		
			OP	JU	PA/PE	OP	JU	PA/PE	OP	JU	PA/PE
Parsley	SR	60									
	0	79									
	6		66	68	51	69	71	63	34	34	62
	12		27	44	41	25	14	17	6	15	5
	18		61	35	74	55	41	74	1	1	0
	24		48	41	55	35	27	57	0	2	4
Paprika	SR	65									
	0	85									
	6		33	46	51	68	78	71	1	6	16
	12		2	2	-	18	18	66	0	0	0
	18		5	8	5	12	18	37	0	1	1
	24		6	13	0	0	0	0	0	0	0
Lettuce	SR	70									
	0	78									
	6		76	50	38	75	70	79	20	86	37
	12		76	73	65	75	78	79	0	89	53
	18		31	75	45	55	51	31	1	41	36
	24		88	96	-	98	97	-	23	100	100
Kale	SR	75									
	0	90									
	6		88	97	97	95	96	94	95	95	94
	12		64	57	48	73	70	71	58	90	54
	18		94	94	95	92	95	97	85	91	95
	24		93	93	97	92	96	-	98	90	86
Onion	SR	65									
	0	91									
	6		89	77	95	88	91	89	69	85	95
	12		91	93	82	99	83	91	67	66	98
	18		78	85	88	91	91	95	0	0	51
	24		87	79	86	87	48	95	1	21	45
Grass	SR	70									
	0	82									
	6		67	76	70	67	55	51	35	64	54
	12		52	32	15	27	56	59	38	-	-
	18		70	70	67	68	34	69	27	-	20
	24		67	81	67	65	43	-	6	-	16

SR = Minimum germination capacity requirement according to Croatian government standard regulations.
OP = original package; JU = jute bags; PA/PE = polyamide/polyethelene laminate.

regulatory standards, the germination of corn, green pea, lettuce, soybean and onion was above the required level. The highest germination among the wheat varieties was achieved for the treated winter wheat, followed by the untreated winter wheat, and the untreated spring wheat that showed poorest germination.

Germination increased for lettuce and kale and was far above the required standard, as was germination of green pea and onion after 24 months. At this time, germination of corn and the grass was around the permissible level. The poorest results were obtained for parsley and paprika. Germination of the wheats, rape seed, and soybean were lower than regulatory requirements, in at least one type of package. Germination of the seeds packed in PA/PE bags was better or as good as that from other types of packages. The germination of seeds stored in original packages was better than that in jute bags.

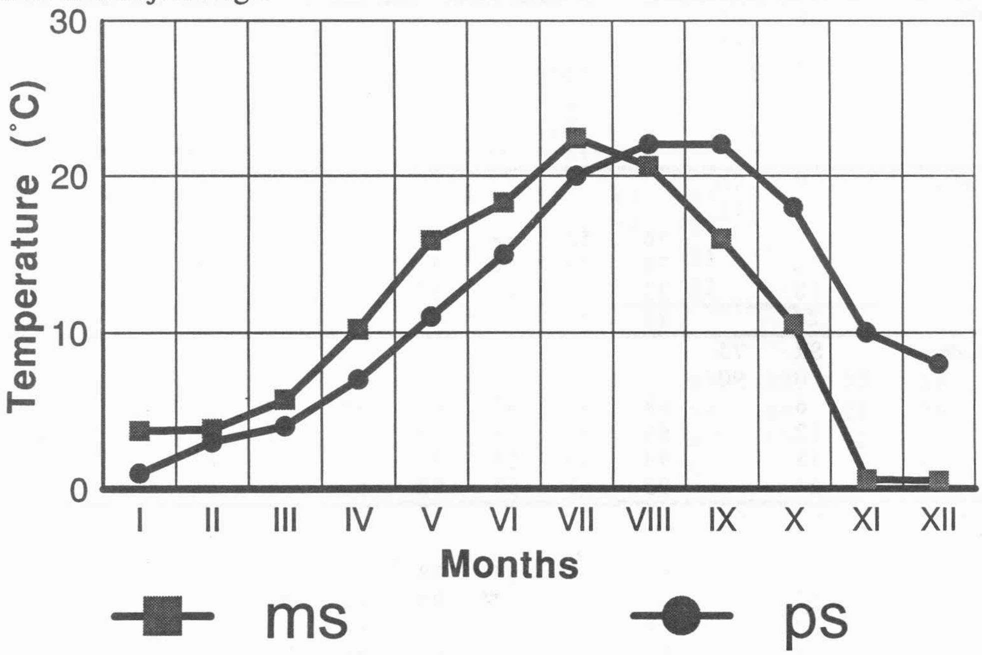

Fig. 1: Mean ambient monthly temperatures at meteorological station (**ms**) and maximum monthly temperatures in warehouse (**ps**).

Carbon dioxide chamber storage

Seeds stored in the presence of a constant CO_2 atmosphere (35±5%) showed, in general, a decrease in germination as compared with the initial level. The exception was kale and green pea to a certain degree. The poorest results obtained were, as with the other storage methods, for parsley and the paprika and to a lesser extent, grass. The germination of corn was above the regulatory standard after 12 months, and then started to decline. Soybean germination was always below regulatory standard but the decline was most marked after 12 months. Among the wheats, the untreated winter wheat showed the poorest germination. Results with the spring wheat were not complete because not enough seeds were available, but the germination obtained in the PA/PE bags was very close to the initial level. The best result was obtained for lettuce after 24 months. Kale and green pea germination did not differ very much during storage.

Comparing the values of germination, it is obvious that some improved results were obtained with seeds in the PA/PE bags, whereas differences between jute and the original packages were not great.

Discussion

Germination changed with time and differed depending on the storage conditions, the type of storage, and the kind of package. The best results were obtained with seeds stored under air-conditioned storage. From the results it would be preferable for some of the seeds to be stored under such conditions. Germination of seeds stored in the warehouse was changeable. Since temperatures fluctuated greatly, from 0°C - 20°C, it can be assumed that in addition to other factors, temperature has a very important influence on the maintenance of germination quality.

Germination of some seeds increased and of others decreased during storage. The best germination was achieved by green peas and kale during the whole storage period. It is interesting to note that the germination of lettuce increased during storage, especially in the CO_2 atmosphere. Germination decreased especially for those seeds stored in original packages and in jute bags in the CO_2 atmosphere. However, the seeds packed in the PA/PE bags very often maintained good germination, even better than that stipulated by regulatory standards.

The CO_2 that caused reduced pressure in the PA/PE bags had less influence on the decline of seed germination.

All insects would be controlled in PA/PE bags during the storage period, unless the bags were punctured or damaged from the outside. Complete control of insects would also occur in the chamber under constant CO_2 atmosphere.

TRIALS IN SILO BINS

Materials and methods

The trials were set up in 150-tonne capacity concrete bins containing wheat. A different bin was used for each trial. Sealing was achieved with special adhesive paper as is used for conventional fumigations. The CO_2 was applied from cylinders and a GOW MAC gas analyser was used for measuring CO_2 concentrations. Cages with various stored product pests (25 adults each of *Sitophilus oryzae* L., *S. granarius* L., *Rhizopertha dominica* F., *Cryptolestes* sp., *Tribolium confusum* Du Val., *T. confusum* var. Yugoslavian black Korunic and Sokoloff, *Sitotroga cerealella* Oliv., and

Tribolium larvae) were placed at different depths beneath the grain surface. Wheat samples were also taken before and after the treatment. Plastic tubing was used for gas sampling. The samples removed from the silo were placed in a rearing room for incubation. Analysis for verification of fungal presence was also undertaken.

Results

Trial 1

After the application of 171.6 kg CO_2, concentrations were measured and these are presented in Table 2.

Infestation results obtained from examination of the cages from 42, 112 cm depth, from bottom and from the conveyor showed that all test insects survived except for adults and larvae *Cryptolestes* spp. and *S. cerealella.*

One of two wheat samples taken at the bottom of the silo revealed 2 live *R. dominica* adults 45 days after treatment. The high insect survival was not surprising in view of the rapid decrease in the CO_2 concentrations after treatment (Table 2).

Trial 2

A total of 570 kg CO_2 was applied (300 kg on the first day, 210 kg on the second day, and 60 kg on the third day). The CO_2 concentrations during the trial are shown in Table 3.

The insect cages examined after the treatment revealed many dead insects but also some live ones. The exception was *O. surinamensis* adults which were all dead. The grain samples taken before and after treatment did not contain insects.

Trial 3

A dosage of 600 kg CO_2 (300 kg on the first day, 240 kg on the second day, and 60 kg on the third day) was applied. The CO_2 concentrations measured during the treatment are shown in Table 4.

Very similar results to those recorded above were obtained in this trial. The most susceptible species was *O. surinamensis*, whereas *R. dominica, S. granarius, S. oryzae* and *T. castaneum* were less susceptible.

The samples of wheat taken before and after treatment did not contain insects except for the sample taken from the middle. The samples taken before treatment contained 3 *S. oryzae* adults, whereas the sample taken after treatment and incubated for 30 days contained 35 *S. oryzae* adults.

Trial 4

A dosage of 700 kg CO_2 was applied to stored corn (600kg initially and 100 kg after 24 hr). Concentrations of CO_2 measured 3 hr after purge, were 65 and 85%, and after 48 hr they were 3 and 24% at top and bottom of the bin, respectively.

Examination of samples taken before treatment revealed infestation with Psocoptera, and the molds *Aspergillus* sp. and *Penicillium* sp. The treated samples did not contain any pests.

Table 2: Carbon dioxide concentrations measured in the silo (Trial 1).

Time after beginning of trial (hr)	Carbon dioxide concentrations (%) at different levels in the silo				
	42 cm	112 cm	193 cm	Under cover	Bottom
2	16	14	12	-	-
3	19	15.5	15	66	-
4	12	12	15	-	-
5	74	27	14	80-86	0
6	88	72	62	90	-
7	78	62	68	82	-
24	56	52	57	52	37
25.5	58	44	48	58	-
32	18	26	30	5	20
48	3	4.8	4.6	4.8	0
57	2.2	3	3.8	1.2	0
75	2.5	3	3.4	1	0
100	0.2	0.3	0.2	0.2	0

Table 3: Carbon dioxide concentrations measured in the silo (Trial 2).

Time after beginning of trial (hr)	Carbon dioxide concentrations (%) at different levels in silo				
	2 m	5 m	10 m	15 m	Bottom
4	40	48	42	-	-
5	50	94	60	-	-
7	20	46	82	44	-
11	16	18	20	48	42
*24	6	8	9	16	19
30	22	28	40	10	12
34	32	30	28	22	18
*48	3.6	5.6	16	18	12
58	20	20	26	28	14
75	2	3	7.8	14	14
107	0.2	0.4	1.8	4.0	5.4

* CO_2 added

Table 4: Carbon dioxide concentrations measured in the silo (Trial 3).

Time after the beginning of trial (hr)	Carbon dioxide concentrations (%) at different levels in silo				
	4 m	7 m	10 m	15 m	bottom
2	100	54	50	-	-
3	80	40	78	64	-
5	82	52	64	66	-
9	68	38	40	36	34
*22	0	2	2	6	12
28	70	34	44	26	16
33	64	32	26	22	28
*46	1.2	3.6	4	6.8	10
56	66	38	42	38	28
73	1.2	3	4	7	0.6
105	0.4	0	1.8	1.6	1.4

* CO_2 added

Discussion

The trials performed in silo bins did not give satisfactory results. The problem was attributed to incomplete sealing, or more specifically, to leaks in the silos. Actual losses of CO_2 could be measured only at the bottom or top of the bin though there were some losses when CO_2 was applied at the top. The concentrations outside the bin at the bottom were from 0.2 - 0.8% . An additional indication of leaks was obtained from CO_2 concentrations measured in adjacent bins (Trial 1). However, the trials showed that if about 1 kg of CO_2/tonne of grain is applied especially in old bins, losses are very high and it would be necessary to add a considerable amount of CO_2 (Trial 1). When CO_2 was applied in new bins (Trials 2, 3, 4), that were constructed in the early eighties, it was easier to maintain high concentrations. The best results would be possible in newly-constructed bins. Probably the application of CO_2 from cylinders also caused some decrease in concentration because small quantities of CO_2 were applied at the same time and losses were comparatively higher. The temperature of the commodity was not very high, (18-24°C), and this also had a negative effect on the results.

According to the results obtained it would be necessary to improve sealing of these silo bins.

LABORATORY EXPERIMENTS WITH MOTH SPECIES

Methods

Different developmental stages of *Sitotroga cerealella* Oliv. and *Corcyra cephalonica* Staint. were used in three experiments. The original rearing jars were placed in a CO_2 atmosphere. In one experiment only the larvae of *C. cephalonica* were used. The larvae were transferred from rearing jars to the experimental jars with the same kind of food. The concentrations of CO_2 were from 50% - 70%. Exposure was 1-7 days. Experimental conditions were 22±2°C and 60±5% r.h.

Results and discussion

The treatments (55± 5% CO_2 for 6 days, 65±5% CO_2 for 3 days, and 60±10% CO_2 for 7 days) caused mortality of adult *S. cerealella,* whereas immature stages survived and moths developed. Similar results were obtained for *C. cephalonica* adults at 60±10% CO_2 during the 7-day exposure. However, all of the larvae of *C. cephalonica* were dead when held at 55±5% CO_2 for 1, 2 or 5 days, whereas moths developed from untreated larvae.

CONCLUSIONS

From the results obtained, it can be concluded that the presence of a relatively low CO_2 concentration (35±5%) in air, for a long period, has a negative effect on the germination of seeds in jute and original packages. Such a concentration affects some processes and reduces germination in some seeds. The temperature also has a very important effect in the maintenance of germination levels. To preserve good germination quality, it is recommended that facilities that store seeds be equipped with air-conditioning.

The sealing procedure for concrete bins did not give satisfactory results. Although a considerable amount of CO_2 was added to the bins, the failure was due to high losses of gas. These trials showed that the usual sealing technique for conventional fumigants is not applicable to CO_2 treatments. Accordingly, it would be much better to apply CO_2 in properly-sealed structures with the necessary precautionary measures (Banks and Annis, 1977).

Studies performed in the laboratory showed that insects (larvae) are more susceptible when changed from their original rearing medium. Consequently, the recommendation for CO_2 application should not be based on laboratory results alone.

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