ARBORETUM KÓRNICKIE
Rocznik XXII — 1977

TADEUSZ TYLKOWSKI

Cold storage of *Quercus robur* L. acorns in an atmosphere of increased content of CO_2 and a reduced O_2 level*

INTRODUCTION

The use of methods of storing seeds in low temperatures in sealed containers is satisfactory in the case of seeds that have a low water content. In cases where the seeds cannot be dried too much because of the danger of loosing their viability, as is the situation with acorns, the use of sealed containers is inadequate and leads to a quick deterioration and decaying of the seeds.

Holmes and Buszewicz (1956) and Suszka (1975) have shown that the seeds of *Quercus robur* can be stored over 4 winter seasons. The best results were obtained after storing seeds at a temperature of −1°C in closed but not sealed containers with peat or sawdust (Suszka, 1975).

Studies on the storage of seeds of cotton, rice, maize or poplars in containers filled with air, oxygen, carbon dioxide or with nitrogen at various temperatures were conducted among others by Kondo and Okamura (1934), Sampietro (1931), Busse (1935), Sayre (1940), Simpson (1953) and Barton (1939). Ančák (1972) studied the storage of acorns of *Quercus robur* in bottles filled with nitrogen. Viability of so stored seed fell to zero already after 10 months.

Recently increasingly frequently methods of storing fruits in containers with a strict composition of oxygen, carbon dioxide and nitrogen are being employed (Lange, 1969, 1970; Smock and Blanpied, 1963).

On the basis of results on the use of these methods, in the present study an attempt was made to store *Q. robur* acorns in an atmosphere with controlled levels of O_2, CO_2 and nitrogen.

It was the purpose of these studies to determine the optimal con-

* This work has been partially supported by grant no. FG-Po-253 from the U.S. Department of Agriculture under U. S. Public Law-480.

centrations of O_2 and CO_2 in the atmosphere of the containers with stored acorns and to determine the effect of these concentrations on the laboratory germinative capacity of the stored acorns.

METHODS

For the study acorns of *Q. robur* collected on September 30th 1975 in Forest Range Zwierzyniec near Kórnik were used.

The year 1975 was characterized by a great oak mast. The acorns at the time of collection have had a low water content of 39.7% in fresh weight. Such a low water content was caused by the fact that 1975 was a very dry year. Immediatelly after collection, the acorns were placed in a loft, segregated and covered with sacks.

On the 22nd of October 1975 the acorns having still 39.7% of water content and a viability of 98.5% (cutting test) were placed in six 20 liter milk cans at a temperature of −1°C. Into each can 1500 acorns were placed and then the cans were closed and sealed. The acorns occupied about 1/3 of the can.

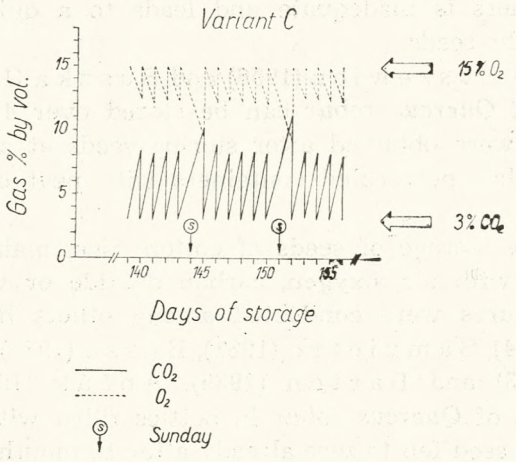

Fig. 1. An example of the course of CO_2 and O_2 concentrations regulation to a level of 3% and 15% respectively during 17 days of the experiment in treatment C. On Sundays (marked by „S") the gas concentration was not regulated

The experiment was conducted in three variants of CO_2 and O_2 concentrations using two cans per variant:

B 1.0% CO_2 and 2.5% O_2 the rest nitrogen,
C 3.0% CO_2 and 15.0% O_2, the rest nitrogen,
D 5.0% CO_2 and 10.0% O_2, the rest nitrogen

and there was one control variant A where the acorns were stored in a container with air access. The gas mixtures circulated from the can

to the analyzer and back to the can, and apart from that each can was supplied with an individual pump for the mixing of the gases. In the control variant forced movement of air was not employed. The proportion of the various gases was regulated daily (6 times during a week, excluding Sunday) using a Infralyt apparatus (an infra red analyzer for CO_2) and Permolyte, a magnetic device for the measurement of oxygen (Fig. 1). Each container was connected with the analyzers by polyvinyl chloride tubes of 5 mm inner diameter.

The methods of checking CO_2 and O_2 concentrations against the initial values depended on the leading off of the excess CO_2 and supply of appropriate levels of O_2 from the atmosphere. If necessary the gases were supplemented with N_2.

On April the 9th 1976 that is after 170 days of storage from one of the cans in each variant the seeds were taken out and subjected to an analysis of viability by the cutting test, an analysis of water content in the fresh weight (48 hrs of drying at 105°C) and a germination test at 20°C during stratification in a mixture of sand and peat (1 : 1 by vol) in four replicates with 50 acorns in each.

RESULTS

The oxygen uptake and the emission of CO_2 by the stored acorns was initially very intense. The results are presented in Table 1. After about 5 - 6 weeks a certain stabilisation in the O_2 uptake and CO_2 evolution was observed which was maintained till the 170th day of storage, that is to the time when the acorns were taken out for testing.

Table 1

The course of fluctuations in CO_2 and O_2 concentrations during storage of *Quercus robur* acorns at −1°C in various gas mixtures over 24 and 48 hour periods from the moment of regulation of gas concentrations

Days of Storage	Treatment	Adopted conc. in %		Concentration in % after			
				1 day (Friday-Sat.)		2 days (Sat.-Mon.)	
		CO_2 min.	O_2 max.	CO_2	O_2	CO_2	O_2
3 - 5	B	1.0	2.5	3.8	1.0	8.2	0.7
	C	3.0	15.0	5.8	10.6	9.2	9.2
	D	5.0	10.0	6.0	9.8	9.3	7.2
17 - 19	B	1.0	2.5	3.8	1.2	5.4	0.8
	C	3.0	15.0	7.7	11.8	10.6	9.4
	D	5.0	10.0	8.4	8.7	11.8	5.9
38 - 40	B	1.0	2.5	3.7	1.6	4.4	1.0
	C	3.0	15.0	7.7	12.0	9.5	9.6
	D	5.0	10.0	8.4	8.8	9.9	6.4
164 - 166	B	1.0	2.5	3.2	1.7	4.5	0.9
	C	3.0	15.0	7.4	12.0	9.9	9.5
	D	5.0	10.0	8.4	8.4	10.9	5.7

Table 2

Mean daily increase in CO_2 concentration and decline in O_2 level during storage of *Quercus robur* acorns at a temperature of −1°C in various gas mixtures

Treatment	Adoptet conc. in %		Changes in conc. in % after 24 hrs.	
	CO_2 (min.)	O_2 (max.)	CO_2 (increase)	O_2 (decline)
B	1.0	2.5	+2.5	−0.8
C	3.0	15.0	+5.0	−3.0
D	5.0	10.0	+4.3	−1.5

When taken out of the cans the acorns did not show any external symptoms of decay, and the general appearence suggested good storage conditions. The water content in the fresh weight did not change substantially, only in the control variant the water content was reduced to 38.5% of fresh weight that is by 1.2%. In the germination tests the emergence of the first roots took place already after the 10th day while

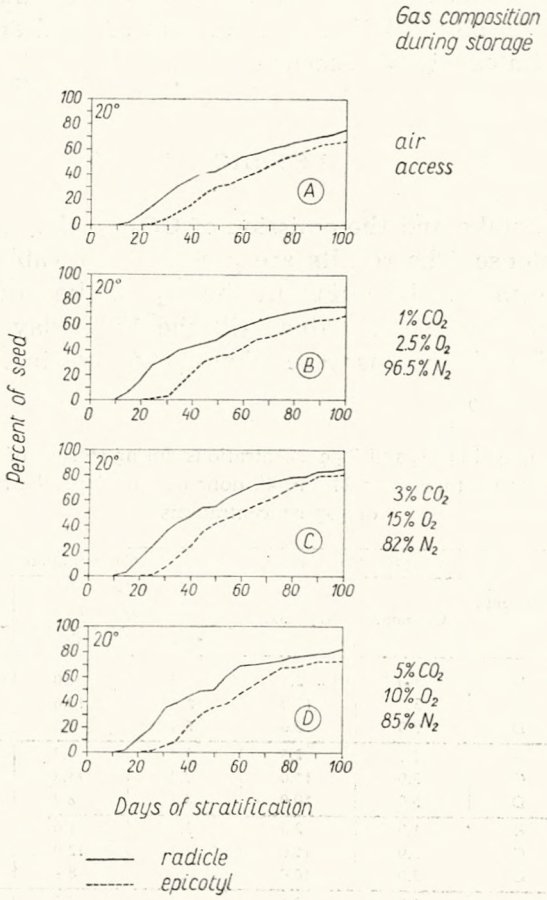

Fig. 2. Course of laboratory germination (radicle and epicotyl) of *Quercus robur* acorns stored over one winter (170 days) at a temperature −1°C in various gas mixtures

Table 3

The course of *Quercus robur* acorns germination after storage at a temperature −1°C over one winter (170 days) in various gas mixtures

Day of germination test	Treatment	Radicles [%]	Epicotyls [%]
20	A (control)	8.0	0.0
	B	16.0	0.0
	C	12.0	0.0
	D	11.0	0.0
40	A (control)	37.0	16.0
	B	42.0	21.0
	C	47.0	24.0
	D	44.5	22.0
60	A (control)	55.0	37.0
	B	58.0	41.0
	C	67.0	50.0
	D	68.0	47.0
80	A (control)	65.0	55.0
	B	70.0	57.5
	C	79.0	71.5
	D	75.0	68.0
100	A (control)	75.0	66.5
	B	74.0	68.5
	C	85.0	80.5
	D	82.5	76.0

the shoots began to appear between the 20th and 25th day of the test regardless of the experimental variant.

The course of germination is shown in Fig. 2 and in Table 3.

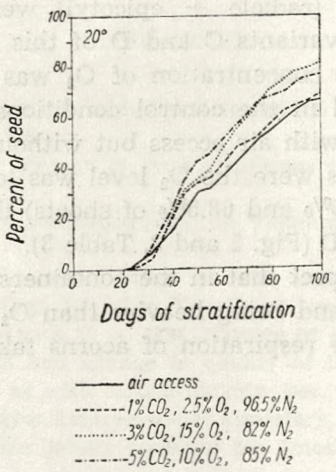

Fig. 3. Comparison of the laboratory germination (radicle+epicotyl) of *Quercus robur* acorns stored over one winter (170 days) at a temperature of −1°C in various gas mixtures

DISCUSSION OF RESULTS

The initially very intense absorption of oxygen and emission of carbon dioxide was caused among other things by the fact that the acorns after collection remained for a certain period of time on a loft at

a relative high, 10°C temperature. After placing in containers into a temperature regime of −1°C, as the acorns cooled, their rate of respiration declined. After about 5 weeks there resulted a stabilisation of O_2 uptake and CO_2 emission (Table 1).

It is notable that a differentiation in the uptake of O_2 and emission of CO_2 resulted, depending on the levels of these gases adopted in the various treatments (Table 2). Thus in variant B the mean decline in O_2 concentration during a day was 0.8% while the increase in CO_2 level was at the same time about 2.5%. In variant C the mean decline in O_2 concentration was 3% and the corresponding increase in CO_2 was about 5% and in variant D the decline in O_2 concentration amounted to 1.5% while the increase in CO_2 level was about 4.3% over a 24 hr period. As an example the course of changes during 17 days is shown for the experimental variant C. On Sundays the gas concentrations were not regulated and therefore during two days (Saturday to Monday) the changes were somewhat greater (Fig. 1).

These values indicate that the acorns of *Q. robur* which are in environments with higher O_2 concentrations absorb much more of this gas (Table 2) than in environments poor in oxygen, similarily as was the case with acorns of *Q. borealis* (T y l k o w s k i, 1976).

Between acorns stored in this study in atmospheres with a controlled gaseous composition no significant differences were observed during the first 35 days of the germination tests. After that time the best results in germination (radicle + epicotyl) were observed when the acorns were stored in variants C and D of this study (80.5% of epicotyls), that is when the concentration of O_2 was highest. On the other hand the acorns stored in the control conditions, that is in containers closed but not tightly, with air access but without its forced circulation, and in variant B that is were the O_2 level was low, the percentage germination was akin (66.5% and 68.5% of shoots) that is about 11% lower than in variants C and D (Fig. 2 and 3, Table 3).

This leads us to suspect that in the containers with air access during storage CO_2 builds up and being heavier than O_2 it blocks the access of O_2 which results in the respiration of acorns taking place in conditions poor in oxygen supply.

CONCLUSIONS

For the storage of *Quercus robur* acorns over one winter (170 days) at −1°C the gaseous compositions 3% of CO_2 and 15% of O_2 in nitrogen and 5% of CO_2 and 10% of O_2 are more satisfactory than a mixture of 1% CO_2 and 2.5% of O_2 or a free but not forced access of air through an unsealed lock on the storage cans.

SUMMARY

In the study the effect various gas mixtures of O_2, CO_2 and N_2 on the germinability of freshly collected *Quercus robur* L. acorns stored at −1°C was investigated. The control variant A consisted of acorns stored in a closed but not sealed container without a forced circulation of gases. In the other experimental variants (B, C and D) sealed containers were used with a forced circulation of gas mixtures of the following composition:

B	1.0% CO_2	2.5% O_2	96.5% N_2,
C	3.0% CO_2	15.0% O_2	82.0% N_2,
D	5.0% CO_2	10.0% O_2	85.0% N_2.

The composition of the gas mixtures was regulated daily except on Sundays. After 170 days of storage, the acorns were subjected to a germination test at a temperature of 20°C.

Gas mixtures C and D (80.5% and 76.0% of roots and shoots appeared respectively in a germination test lasting 100 days) proved more satisfactory for the storing of acorns at −1°C than mixture B or air in variant A (68.5% and 66.5% of roots and shoots appeared respectively).

Institute of Dendrology
Kórnik nr. Poznań

LITERATURE

1. Ančák J. — 1972. Biológia a uskladňovanie semien lesných drevin. Vyd. Slov. Akad. Vied. Bratislava, 339 pp.
2. Barton L. V. — 1935. Storage of some coniferous seeds. Contr. Boyce Thompson Inst., 7, 379 - 404.
3. Busse I. — 1935. Samenaufbewahrung im Vakuum. Z. Forst- u. Jagds., 67, 321 - 6.
4. Holmes D. G. and Buszewicz G. — 1956. Longevity of acorns with several storage methods. Rep. For. Res. For. Com. London 1954/55, 88 - 94.
5. Kondo M. and Okamura T. — 1934. Storage of rice. IX: Relation between varying moisture content and change in quality of hulled rice stored in containers air-tight as well as with carbon-dioxide. Ber. Ohara Inst., 6, 149 - 74.
6. Lange E. — 1969. Wpływ kontrolowanej atmosfery, terminu zbioru i temperatury na przechowywanie jabłek odmiany Mc Intosh. Pr. Inst. Sad., PWRiL, Warszawa, 13, 167 - 175.
7. Lange E. — 1970. Wpływ zmodyfikowanej atmosfery i temperatury na przechowywanie jabłek odmiany Mc Intosh, Spartan i Macoun. Pr. Inst. Sad., PWRiL, Warszawa, 14, 295 - 305.
8. Sampietro G. — 1931. Prolonging the longevity of rice seed. G. Risic., 21, 1 - 5. (Chem. Abstr., 25, 2172).
9. Sayre J. D. — 1940. Storage tests with seed corn. Ohio. J. Sci., 40, 181 - 5.
10. Simpson D. M. — 1953. Cottonseed storage in various gases under controlled temperature and moisture. Bull. Univ. Tenn. Agric. Exp. Sta., No. 228, pp.

11. Smock R. M. and Blanpied G. D. — 1963. Some effects of temperature and rate of oxygen reduction on the quality of controlled atmosphere stored Mc Intosh apples. Proc. Amer. Soc. Hort. Sci., 83, 135 - 138.
12. Suszka B. — 1971, 1972, 1973, 1974, 1975, 1976. Studies on the long-term storage of acorns. Polish Acad. of Sciences, Inst. of Dendrology and Kórnik Arboretum, FG-Po-253, E21-FS-44.
13. Tylkowski T. — 1976. Respiration of northern red oak (*Quercus borealis* Michx.) acorns. Arboretum Kórnickie 21 (in press).

TADEUSZ TYLKOWSKI

Przechowywanie żołędzi Quercus robur L. w obniżonej temperaturze, w atmosferze o podwyższonej zawartości CO_2 i obniżonej zawartości O_2

Streszczenie

W pracy badano wpływ mieszanin gazowych o różnym składzie O_2, CO_2 i N_2 na zdolność kiełkowania świeżo zebranych żołędzi dębu szypułkowego (*Quercus robur* L.), przechowywanych po zbiorze w temperaturze −1°C. Wariantem kontrolnym A były żołędzie przechowywane w pojemniku nieszczelnie zamkniętym bez zastosowania wymuszonego obiegu powietrza. W pozostałych wariantach eksperymentalnych (B, C i D) zastosowano szczelnie zamknięte pojemniki z wymuszonym obiegiem mieszanin gazowych o następującym składzie:

B 1,0% CO_2 2,5% O_2 96,5% N_2,
C 3,0% CO_2 15,0% O_2 82,0% N_2,
D 5,0% CO_2 10.0% O_2 85,0% N_2.

Skład gazowy mieszanin w pojemnikach regulowany był codziennie z wyjątkiem niedziel.

Po 170 dniach przechowywania, żołędzie poddano próbie kiełkowania w temperaturze 20°C. Mieszaniny gazowe C i D (80,5% i 76,0% korzeni i pędów w próbie kiełkowania trwającej 100 dni) okazały się bardziej korzystne dla żołędzi przechowywanych w temperaturze −1°C niż mieszanina B lub powietrze A (odpowiednio 68,5% i 66,5% korzeni i pędów).

ТАДЕУШ ТЫЛЬКОВСКИ

Хранение желудей Quercus robur L. при пониженной температуре, в атмосфере с повышенной концентрацией CO_2 и пониженной O_2

Резюме

В работе исследовалось влияние газовых смеси с различным содержанием O_2, CO_2 и N_2 на способность прорастания желудей (*Quercus robur* L.) хранимых после сбора при температуре −1°C. Контролем А служили желуди хранимые в неплотно закрытых контейнерах без применения принудительной циркуляции воздуха. В остальных вариантах опыта (B, C и D) были применены плотно закрытые контейнеры с принудительной циркуляцией газовых смеси.

B 1,0% CO_2 2,5% O_2 96,5% N_2,
C 3,0% CO_2 15,0% O_2 82,0% N_2,
D 5,0% CO_2 10.0% O_2 85,0% N_2.

Состав газовых смеси в контейнерах регулировался ежедневно кроме воскресений.

После 170 дней хранения, желуди проращивались при температуре 20°C. Газовые смеси C и D (при проращивании в течение 100 дней дали соответсвенно 80,5% и 76,0% побегов) оказались более выгодными для желудей хранимых при температуре −1°C, нежели смесь B или воздух A (соответственно 68,5% и 66,5% побегов).