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Original article

Physiological and pathological aspects of long-term storage of acorns

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Summary — The development of a seedlot of oak has been followed over a period of 32 months. During the observation period, the absolute starch content of acorns and the exploitation of starch reserves at germination decreased with increasing duration of storage. Ageing processes are probably impairing the availability of food reserves. After 12 months, a marked decrease in germination capacity appeared, caused by a fungal disease. However, the low storage temperature and high CO_2 content seem to have stressed the acorns which has probably predisposed them to infection with the weak pathogen, *Cylindrocarpon didymum*. Under natural conditions, the acorns developed typical frost hardiness.

acorn storage / germination / food reserves / frost hardiness

Résumé — Aspects physiologiques et pathologiques de la conservation des glands à long terme. *Le développement d'un lot de glands a été observé pendant une période de 32 mois. Pendant la période observée, la teneur absolue en amidon et l'utilisation des réserves d'amidon des glands ont diminué avec l'augmentation de la durée de conservation. Il est possible que le processus de vieillissement réduise la disponibilité des réserves nutritives. Après 12 mois, une diminution nette de la faculté germinative s'est manifestée, causée par une infection fongique. Il semble que la température basse et les teneurs en* CO_2 *élevées constituent des facteurs de stress sur les glands, qui pourraient favoriser l'infection par le champignon* Cylindrocarpon didymum. *Dans les conditions naturelles, les glands manifestent une résistance au gel.*

conservation des glands / germination / réserves nutritives / résistance au gel

INTRODUCTION

Knowledge of physiological processes in seeds is a prerequisite for the development of seed-storage methods. The current rule of thumb is that a water content of 40% and a temperature of –4 °C are the minima acorns require to survive (Holmes and Buszewicz, 1956; von Schönborn, 1964; Bonnet-Masimbert *et al*, 1977; Suszka and Tylkowski, 1980; Muller and Bonnet-Masimbert, 1984).

Nevertheless, traditional but also current methods are not up to the high standards required for seed storage today and in the future. Above all, for gene conservation, improved methods will be necessary.

In the context of a long-term storage project at the University of Hannover, the physiological development during storage and germination of different oak seedlots were observed from November 1988. Additional investigations were concerned with the ecophysiological behavior during the development of winter-hardiness (Guthke and Spethmann, 1991a,b).

MATERIALS AND METHODS

The long-term storage trial started in November 1988 with 250 kg of *Quercus petraea* acorns from Lüß. After thermotherapy (2 h in 42 °C hot water; Delatour, 1978) and surface-drying, the acorns were placed in boxes with dry peat and put into a jacket cooling at –2 °C. At this stage, the acorns had a water content of 54%. Different CO_2 contents (0.5, 1, 3 and 5%) were applied.

Every 4 months, a sample was taken. Fresh weight, germination and fungal infection were determined for 700–1200 acorns/CO_2-treatment. 200–300 obviously sound acorns were taken from each group for a cutting-test. The fresh matter of the really sound acorns was instantly deep frozen and later dried to constant weight over about 1 week at 70 °C. The cotyledon dry matter was ground for analysis to < 0.25 mm. Another 200 acorns were sown for a germination test at each sampling date. When the first leaves on the epicotyl began to appear, a sample of 50 seedlings/treatment was taken to determine the consumption of food reserves and build-up of shoot and root dry matter.

To investigate the development of winter-hardiness, acorns were sown in a forest and a nursery seedbed. Every 30 days, samples were taken for analyses (as above) and laboratory frost-hardiness test were run for about 20 days at specific temperatures.

The great variability within the acorn population contrasted with a variance-analytical interpretation of the results. The observed pathological disintegration of the population also impeded statistical evaluations.

RESULTS

The temperature of –2 °C proved not to be low enough to prevent germination in storage. Within 12 months, epicotyls began to appear.

A specific aim of the study was to determine the influence of CO_2 on stored acorns (fig 1). This aim failed because of fungal invasion but, nevertheless, it was possible to assess the physiological and pathological aspects of acorn ageing.

Although thermotherapy was reasonably effective against attack by *Ciboria batschiana,* it failed to protect against other microorganisms. After 12 months, there was a distinct-wide decrease in germination capacity caused by another fungal disease, possibly *Cylindrocarpon didymum.* At higher CO_2 concentrations the fungal infection was even worse.

The dry matter of sound acorns (after the cutting test) fluctuated and a relationship could not always be shown with the dry matter residue after the germination test. Therefore, different dry weights were calculated in comparison to the original fresh weight of the whole samples and to the acorn residues after germination. It turned out that the sound acorns analyzed were heavier than the mean of the whole population and the germinated acorns were even heavier than the sound acorns, especially in the last year.

Another feature observed was the development of starch (%) during storage (fig 2). While the starch weight decreased continuously, the percentage did not change but fluctuated around 35%. Since only sound acorns were analyzed, it seems probable that not only heavier acorns but also acorns with a higher percentage of starch were more viable.

After prolonged acorn storage, seedlings showed gradual reductions in shoot and root dry matter. It is not yet clear if the decrease in shoot and root dry matter was only associated with decreased acorn dry matter before the germination test. A distinct decrease also appeared in the percentage of starch consumed at germination, so that exploitation of food reserves could be impaired.

An additional investigation was explored as to how acorns survive the winter under natural conditions. Even during the quite warm winter of 1989-1990, acorns showed typical development of frost hardiness (fig 3). About 50% of the acorns survived at laboratory tests −8 °C in January.

Unexpectedly, water content increased from 49% in October to 62% in March. Sugar content increased with the development of frost hardiness. Sugars exhibited a distinct reaction to cold, especially in the cell sap. Cutting tests showed, by the natural browning reaction of frost damaged tissues, that frost hardiness of the acorn was higher around the embryo axis and in inner

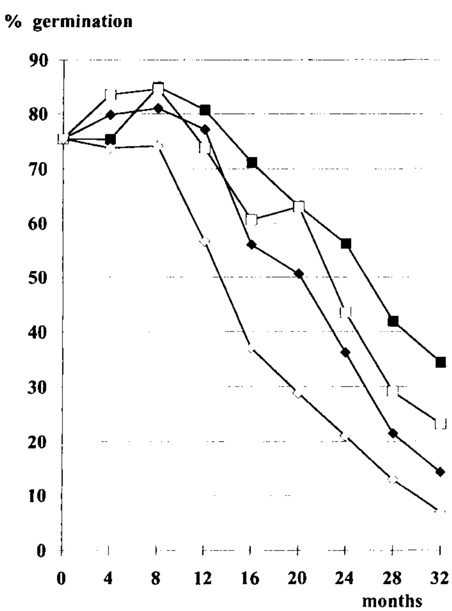

Fig 1. Germination capacity of acorns of *Quercus petraea* from Lüß, after prolonged storage under different CO_2-treatments. —■— : 0.5% CO_2; —□—: 1% CO_2; —◆—: 3% CO_2; —◊—: 5% CO_2.

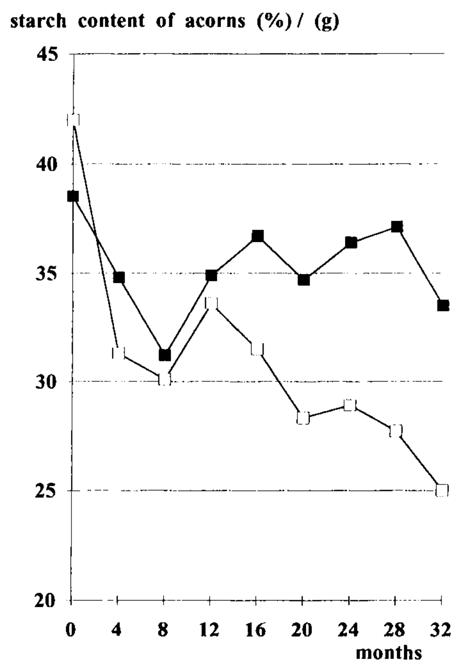

Fig 2. Development of the starch percentage and starch weight (g/100 acorns) during storage, *Quercus petraea* from Lüß, mean of all CO_2-treatments. —■— starch (%); —□— starch (g).

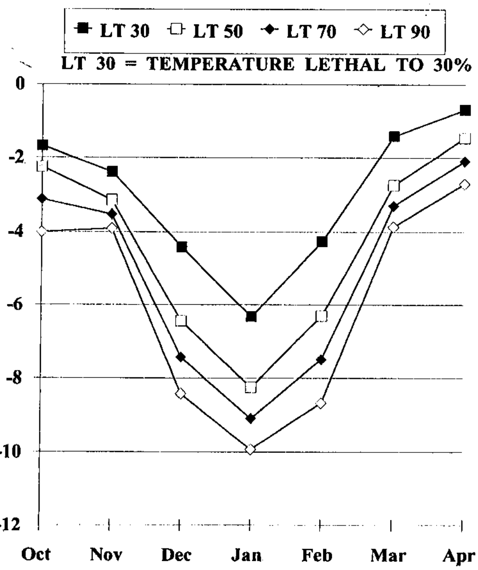

Fig 3. Temperatures lethal to acorns hardened under natural conditions (1989/1990).

areas than in outer ones (Guthke and Spethmnn, 1991a, b).

DISCUSSION

One aim of this storage trial was to investigate the influence of elevated CO_2 content which seemed to be a suitable way of reducing the metabolism of acorns. This effect cannot be excluded, but it was impossible to prove because, simultaneously, infection with *Cylindrocarpon* increased with the CO_2 concentration. It is possible that *Cylindrocarpon* infection is stimulated by elevated CO_2, but this was not investigated. Apart from this obvious pathological development, some symptoms of physiological ageing of the acorns were also evident. In addition to the gradual reduction of shoot and root dry matter development at germination, the exploitation of starch at germination was reduced and may have resulted from a reduced availability of food reserves. In respect to these ageing processes and the more obvious physiological and pathological disintegration of the populations, it also seems to be necessary to investigate the genetic development of further storage trials.

New methods must be found to solve the old problem of acorn storage. A promising way may be a controlled hardening treatment of acorns.

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