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A vigour test for quality assessment of stored beech (*Fagus sylvatica* L.) nuts

Abstract: Many seed lots of beech (*Fagus sylvatica* L.) nuts are characterized by various levels of infection with pathogenic fungi, such as *Fusarium*, *Rhizoctonia* and *Cylindrocarpon* spp. Favourable conditions for fungal infection of beechnuts are created especially during their long-term provisional storage without earlier drying to the moisture content of 8–10%. Strongly infected seed lots are not suitable for long-term storage. During the pre-sowing treatment of seeds by stratification without medium, fungi develop under the moist conditions and infect many healthy seeds.
To assess the quality of beechnuts, a vigour test has been developed. Seeds removed from the pericarp (4 replications of 50 nuts each) are placed in boxes between two layers of moist tissue paper and incubated at 25°C for 120 hours. After this period decayed seeds (mouldy seeds) are excluded, and the remained are subjected to embryo viability by the tetrazolium test. When the percentage of decaying seeds (including those with necroses) does not exceed 10–15%, such seed lot can be used for long-term storage. If the percentage exceeds 50%, the seed lot should be considered unsuitable both for storage and for pre-sowing treatment, because only a very small proportion of seeds will emerge in the nursery.

Additional key words: *Fagus sylvatica*; seed quality, pathogenic fungi; seedling emergence

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Introduction

High yields of beechnuts recur in Poland every 6–8 years. Methods of storage and pre-treatment of beechnuts for sowing, developed by Suszka and Kluczyńska (1980), influenced the decision of the Polish State Forest Holding for building cold stores with regulated temperature. This enables the use of stored seeds for seedling production in years of crop failure.

At the end of the 20th century some cold stores for beechnuts were built in Poland: in Dukla in the Krosno Forest District (FD) for the Carpathian beech stands, and in the Białogard and Gryfino FDs for the Pomeranian beech stands. Each of them can store 30 tons of nuts. Moreover, in many FDs (e.g. in Jarocin and Siedlisko) smaller stores with regulated temperature have been built, where smaller quantities of beech nuts are stored for local use.

The quality of the stored seed lots varies considerably. Seeds supplied to stores are usually cleaned there, sometimes dried to 8–10% of moisture content, and stored in sealed containers at –10°C. Before storage the quality of every seed lot is defined. On the basis of a germination test during stratification in a moist medium at 3°C, the duration of the period needed for seed pre-treatment (cool stratification without medium) is fixed for each seed lot separately (Suszka et al. 2000).

During storage some seed lots quickly lose their initial high quality. The biochemical test for seed viability recommended by the ISTA (1999), gives unreliable results in this case. Basing on these rules we can

conclude only about the potential germinability of seeds. True values of seed germinability can be assessed only after a full stratification and germination or emergence tests. These tests usually last longer than 10 weeks. The data on seed quality can be obtained too late to be able to pre-treat the seeds for sowing in spring, while cold storage until the following year is associated with a gradual decline in seed quality.

The aim of this study was to develop a vigour test based on the seed health test. The results of this test should permit to define the quality of seeds in the examined lots and to make it easier to decide about their further destiny – could such seeds be stored longer at an unchanged viability level or should they be pre-treated for sowing in the nearest spring.

Material and methods

Test I – Accelerated ageing of seeds in the stratification medium.

Beechnuts of two seed lots (No. 1240 and 1261), in 4 replicates of 50 seeds each, were used for the test of accelerated ageing lasting 48, 72 and 96 hours in a moist mixture of peat and sand (1:1, by vol.) at 25°C, 30°C and 35°C. When the tests were finished the embryos extracted from nuts were subjected to the Topographical Tetrazolium Test (TTT) after 20 hours at 30°C (ISTA, 1999).

Test II – Accelerated ageing of seeds placed between two layers of tissue paper.

Seeds of the same seed lots as above, extracted from nuts (4 replicates of 50 seeds each), were placed in plastic boxes on a lignin layer moistened with distilled water. Seeds were covered with a damp tissue paper and the boxes were covered with a lid. Seeds were incubated at the same temperature, as in Test I, for 48, 72 i 96 or 120 hours. After each incubation the healthy seeds were separated from those affected by harmful necroses and infested by insect. The healthy seeds were additionally tested by TTT.

Seedling emergence

Beechnuts of 7 lots from SW Poland (Zielona Góra I and II, Cybinka, Nowa Sól, Wymiarki, Lubsko and Brzózka) were pretreated before sowing by a mediumless stratification at 3°C in Siedlisko (Nowa Sól FD). After that, all seed lots (each represented by 4 replicates of 50 seeds) were sown in spring in the nursery. In early summer the seedlings were counted and mean seedling emergence was calculated.

Results

After the warm stratification in a peat/sand medium at 25°, 30° and 35°C lasting 48, 72 and 96 hours, the rate of decline in seed viability of the two seed lots was similar (Fig. 1). The greatest decline was observed at 35°C, and it differed considerably from the viability decline of seeds at 25°C and 30°C. Seed viability after 48 hours at 35°C was lower (45%) than after the same time at 25°C and 30°C (58.5% and 59% for seed lot 1240 and 66.5% and 74.5% for seed lot 1261, respectively). The decline in seed viability observed between 48 and 72 hours of the test was lower than between 72 and 96 hours (Fig. 1).

Seeds extracted from beechnuts (lot 1240) decayed faster when tested on tissue paper (Fig. 2) than in the medium (Fig. 1). Between two tissue paper layers, only 30.5% of seeds remained viable after 48 hours at 35°C, and 13% and 8% after 72 and 96 hours respec-

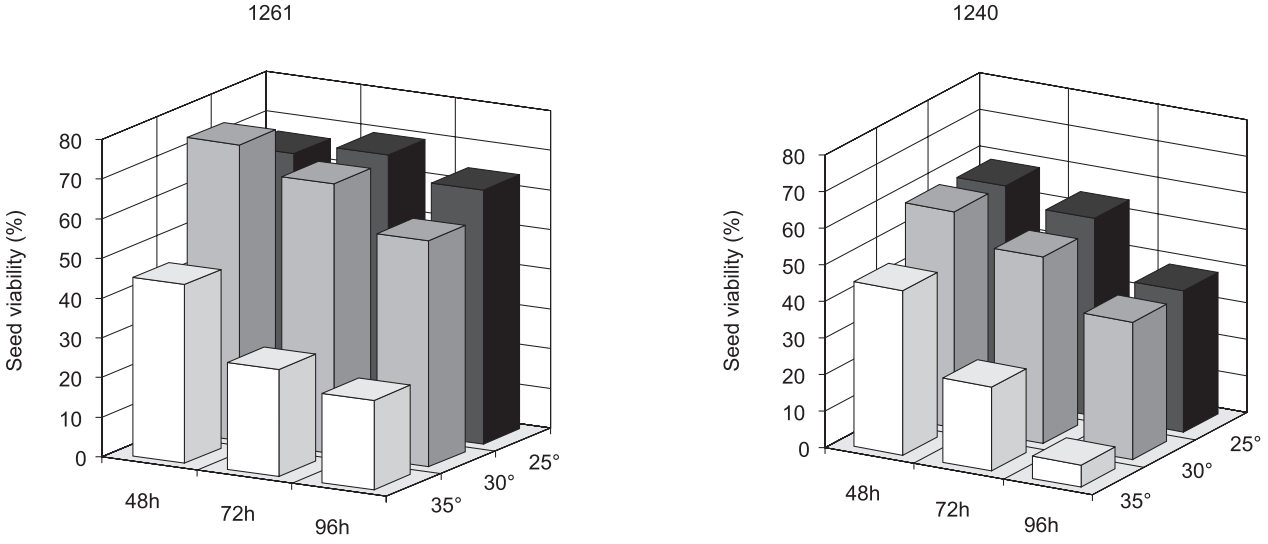

Fig. 1. Influence of incubation of nuts of two lots (1240 and 1261) in a stratification medium at 25°, 30° and 35°C for 48, 72 and 96 hours on the viability of their embryos. Seed lot 1240, in contrast to seed lot 1261, was characterized by a low percentage of seedling emergence in the nursery

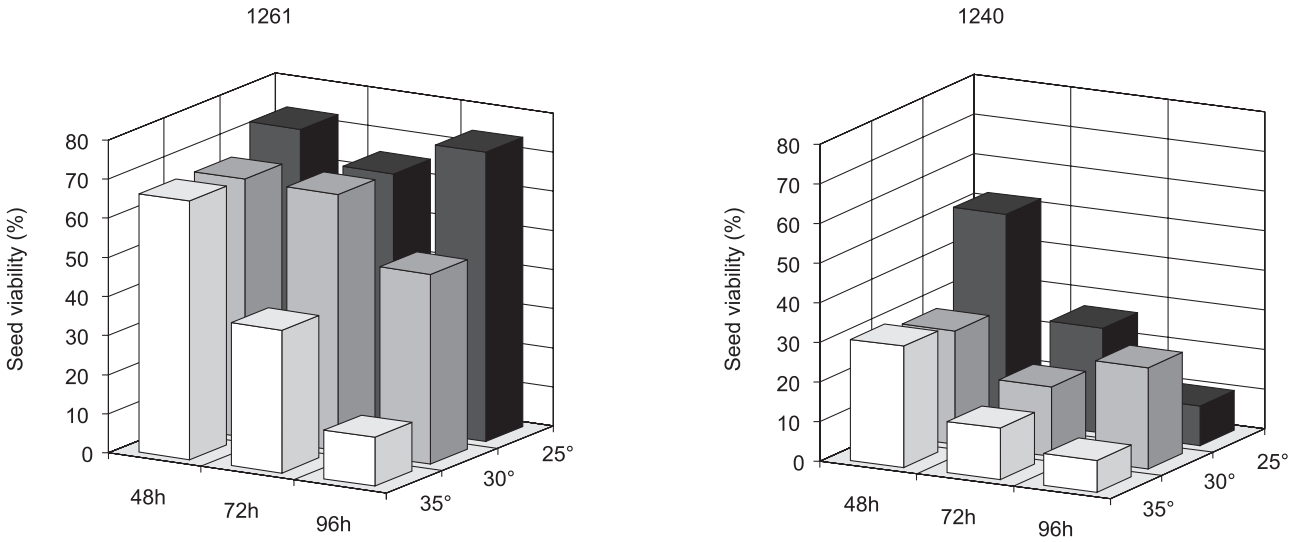

Fig. 2. Influence of incubation of nuts of two lots (1240 and 1261) between layers of wet tissue paper at 25°, 30° and 35°C for 48, 72 and 96 hours on the viability of their embryos. Seed lot 1240, in contrast to seed lot 1261, was characterized by a low percentage of seedling emergence in the nursery

tively. At 25°C the viability of the seeds declined rapidly but the percentage of healthy seeds was somewhat higher (52%, 26.5% and 10%).

The seeds (lot 1261) were more viable after testing between tissue paper. At 25°C their viability did not change during 96 hours and was 74%. Only at 30°C and 35°C a larger decrease in the number of healthy seeds was observed after 96 hours, to the level of 48.5% and 12.5%, respectively. At 25°C viability of seeds changed gradually but more gentle than at 30°C, therefore the temperature of 25°C was chosen as more proper for estimation of the vigour of beech seeds.

After sowing in the nursery, only in 4 seed lots (Zielona Góra I, Cybinka, Zielona Góra II, and Nowa Sól) more than 40% of seedlings emerged. The same seed lots were defined as healthy at a level higher than 50% by the vigour test and as viable at a level higher than 80% by TTT (Fig. 3). In the other 3 seed lots (Wymiarki, Lubsko and Brzózka) only at 12, 33 and 7%, respectively, emerged in the nursery and the seeds were defined in the vigour test as viable at the level of 48-50%. However, in the case of seed lot Wymiarki and Lubsko the TTT gave values about 60%, while seed lot Brzózka had the highest level (90%) of viable seeds.

Discussion

In the beech cold stores in Białogard, Dukla, Siedlisko and Jarocin some seed lots went bad during the presowing treatment. The main reason of seed decay was the growth of mould fungi on the surface of seeds and nuts. This fungi are not visible at the time of collection, but when the relatively moist nuts are temporarily stored after collection (before drying) fungi grow intensively.

Defining viability of beech seeds by the TTT method is unreliable, when seeds are infected by fungi. The quality of seeds gradually declines as a result of fungal activity. Some investigators suspect the possibility of falsifying the results of the test by the seed microflora. Accelerated ageing which is applied in the seed vigour test, stimulates the seed microflora to intensive activity and growth. At a higher temperature and in a humid environment these fungi and bacteria grow on the surface of the infected seeds. On the basis of their destructiveness one can conclude about the usefulness and quality of the tested seed lot.

We have sometimes observed that only a low percentage of seedlings emerged from beechnuts containing seeds defined as high viability by the TTT and sown after the pre-treatment in the forest nursery (Fig. 3). It seems that one of the true reasons may be

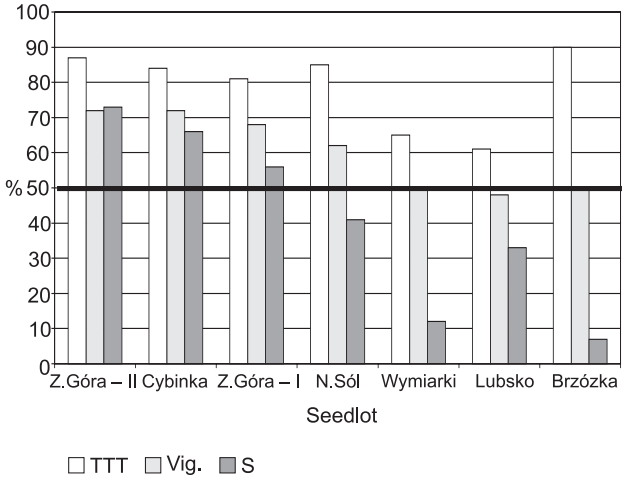

Fig. 3. Results of viability test (TTT) and the vigour test (Vig.) of 7 seed lots before stratification and seedling emergence (S) in the nursery

the poor health of the seeds and their lower vigour resulting from the activity of fungi growing on their surface during the pre-sowing treatment.

A low quality of seeds can be detected during the vigour test carried out between moist tissue paper layers at 25°C. The test is relatively simple and enable us to make a decision about the seeds without the long-lasting emergence tests. At the same time the vigour test allows to save seeds which could be wasted if they were stored one year longer.

The testing of seeds for 120 hours by accelerated ageing at 25°C, according to the method described above, reflects their condition of health and quality in a way similar to the seedling emergence test. The rate of seed deterioration is a result of the activity of the microflora. The surface of the infected embryos becomes soft and slippery, and such embryos can be easy distinguished without TTT.

About 30 species of fungi occur on beechnuts, and the species responsible for the fast seed deterioration during cold stratification include *Fusarium* spp., *Cylindrocarpon* spp., *Rhizoctonia solani* Kühn. and *Phytophtora cactorum* (Leb. et Cohn) Schroet (Procházková and Sikorová 1997).

Some seed lots can be infected to a large extent and their quality can be estimated only when the health or vigour test is applied. The TTT is useless in this context (see results of Brzózka, Fig. 3).

If during the vigour test the percentage of viable seeds in a seed lot was below 50%, then less than 30% of seedlings emerged from this seed lot (Fig. 3).

Research concerning this problem is continued.

Conclusions

1. Results of the vigour test reflect the quality of beech seed lots.
2. Seed lots that after the vigour test (120 h at 25°C) remain viable at the same level as before the test can be recognised as high quality lots suitable for long-term storage.
3. Seed lots with viability decreasing during the vigour test by more than 20% should be considered as low quality lots. Such seeds cannot be stored for longer periods.

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