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Practical implications of inconsistent germination and viability results in testing stored *Fagus sylvatica* seeds

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Abstract: Germination and viability of stored European beech (*Fagus sylvatica*) seeds can vary depending upon the time when the tests are done during seed storage. To determine the possible sources of such variation the germination (GERM), germination rate expressed as mean germination time (MGT) and viability (VIAB) of six beechnut lots (three lots from two crop years) were determined monthly for one year using controlled laboratory conditions and standard tests. Higher GERM of some lots occurred when tests for stored seeds were carried out in spring and early autumn while other lots germinated better during summer tests. Similarly, different germination speed (dormancy release) and VIAB were observed in different months for different lots. However, no consistent seasonal fluctuation in GERM, MGT or VIAB of the beechnuts was observed in the tests. The reason for this fluctuation seems to be initial quality (germination and dormancy) of beechnut lots rather than any endogenous factors.

Additional key words: beechnuts, fluctuation in viability, germination

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Introduction

The object of a germination test is to determine the germination potential of a seed lot, which can then in turn be used to compare the quality of different lots and also estimate field (nursery) planting value (International Seed Testing Association 2012). Both the international (International Seed Testing Association 2012) and the Czech national (ČSN 48 1211 2006) rules for seed quality testing give specified methods and test conditions that, together with trained staff, should ensure the repeatability of the test results obtained anytime throughout the year.

European beech (*Fagus sylvatica* L.) is the most important broadleaf tree species grown in Czech forests. Historically, in natural forests beech accounted for 40.2% of the total area while the current area of 7.2% (Anonym 2011) is destined to increase to 18% (Vokoun 1996; Šindelář 1998). Beechnuts, seeds of European beech, are suborthodox seeds (Palátová 2008) having intermediate physiological dormancy (Baskin and Baskin 2001). Due to irregular full masts (beechnut crops) every 5 to 9 years the seeds must be stored for a period of 3-4 years (Palátová 2008) to ensure sufficient amounts of seedlings for reforestation. Annual demand amounts to 45–50 tons of beechnuts and approximately half of this could be covered by long-term stored beechnuts in years following crop failure. A critical factor which has a major economic impact is beechnut quality during seed storage, e.g. if a dramatic decline occurs in germination from year to year. Thus, reliable up-to date information about the quality of stored beechnuts is needed to determine when to deliver seeds from the warehouse to nurseries.

The viability by tetrazolium test and germination (ČSN 48 1211 2006) of stored beechnuts is determined throughout each year, i.e. first after collection and processing when they are stored (with approx. 8-10% mc) and then annually, again in different months. Ageing of beechnuts even under "optimum" storage conditions results in a gradual decrease in their germination. However, sometimes both the viability and germination of beechnut lots stored for several years varies (e.g. an unexpected increase) significantly compared to the initial values for these lots as does the values obtained in previous years of testing (Procházková et al. unpublished data). There are several explanations for this phenomenon such as high lot heterogeneity, improper sampling, and variation in testing procedures or the effects of the so called "biorhythm".

Several authors have reported more or less significant fluctuations in germination when the same seed lots were tested several times during a year (Schmidt 1930; Baldwin 1935; Řeháčková 1954; Mamonov et al. 1986; Barnett and Mamonov 1989; Procházková 2002; Debnárová and Šmelková 2008, 2008a). Most of these studies dealt with seeds of non-dormant conifers, e.g. *Pinus sylvestris* L., *P. palustris* Mill., *P. elliottii* Engelm., *Picea abies* (L.) Karst., *P. rubens* Sarg., *Larix decidua* Mill., *L. sibirica* Ledeb, and showed either late winter-early spring or early autumn increases and decreases in summer. However, the significance of such deviations has been assessed differently by different authors. Barnett and Mamonov (1989) attributed this phenomenon to seasonal periodicity, or biorhythms. They concluded that biorhythms are more likely to occur in stored seeds of non-dormant species with germination peaks occurring in early spring and decreasing in late summer while differing yearly. The occurrence of significant variation in germination of stored Scots pine and Norway spruce seeds was also attributed to seasonal fluctuation by Debnárová and Šmelková (2008, 2008a). Řeháčková (1954) reported two peaks of germination energy and germination capacity for Scots pine in spring (March, April), early autumn (September) but also in July, and for European larch seeds maximum germination in April and June, but such changes were not considered to be a reliable indicator of periodicity. Similarly, a statistically significant decline was observed in germination energy during summer (namely in July and August) for both, chilled and non-chilled, Scots pine and Norway spruce stored seeds, but no change was detected in germination capacity by Procházková (2002). However, she concluded that over time fluctuations and the effect of tree species (or a provenance) accounted for less than 50% of the total variance. This might indicate the effects of inherent germination variability and random fluctuations. As well, Wang (2003) found no consistent trend of endogenous control of germination periodicity in *Picea glauca* (Moench) Voss, *Pinus banksiana* Lamb. and *P. contorta* var. *latifolia* Engelm. seeds. Earlier Baldwin (1935) or Rostovcev et al. (1975) also failed to find significant seasonal variations in germination of red spruce or Scots pine seeds, respectively.

The purpose of the present study was to determine the significance of germination and viability fluctuations in stored dormant European beech seeds that were repeatedly tested each month for one year under controlled laboratory conditions using standard testing procedures and to determine the potential sources of such variation with the goal of providing more reliable results to seed owners.

Materials and Methods

Seeds

Six seed lots were used in the experiments, three of which were harvested from forest stands in 2009 in the Czech Republic and three were collected in 2010 from forests in Poland (Table 1). The beechnuts were collected from the forest floor and then processed at the Tree Seed Centre in Tyniste nad Orlici (crop 2009) or at the forest company ATRO Rymarov (crop 2010), both in the Czech Republic. Afterwards the beechnuts were dried at 20°C to a moisture content of 8–10% and then stored in sealed, 0.11 mm thick, polyethylene bags (CZECHOBAL s.r.o., Hradec Kralove, Czech Republic) at –7°C. To prevent heat damage the beechnuts were transported in insulated boxes to the Seed Testing Laboratory (FGMRI, Research Station Kunovice) and stored at –5°C until used.

Moisture content, germination and viability determination

At the beginning of the experiment, the moisture content (mc), fresh weight basis, was determined using two replicates per seed lot of cut beechnuts (10 g each replicate) which were then dried at 103±2°C for 17±1 hours (ČSN 48 1211 2006). To determine the possible effects of time of year when germination and viability tests are done we assessed seed germination and viability of all lots every month from April 2010 to April 2011 (crop 2009) and from January 2011 to

Table 1. Information about the European beech (*Fagus sylvatica*) seed lots used in the experiments

Seed lot number	Date of harvest	Provenance	Stand identification	Altitude (m above sea level)
5102	October 2009	NFR 21 Jizera Mountains and Ještěd Czech Republic	CZ-1-2C-BK-20007-21-3-L	401–550
7208	October 2009	NFR 38 White Carpathians and Vizovice hills Czech Republic	CZ-2-2A-BK-3349-38-4-Z-G-155	551–600
7209	October 2009	NFR 38 White Carpathians and Vizovice hills Czech Republic	CZ-2-2B-BK-3415-38-4-Z-G156	551–600
P1	October 2010	Lesko-Srednie Wielkie Poland	83a	510–540
P2	October 2010	Krasiczyn-Korytniky Poland	131a	320–65
P3	October 2010	Rymanow Poland	15a	360

NFR = Natural Forest Region.

January 2012 (crop 2011). As such we obtained data for 13 tests for each crop.

Germination tests (ČSN 48 1211 2006) were done using a peat-sand substrate (1:1 by volume). Four replicates of 100 seeds of each seed lot were mixed with a peat-sand substrate (one volume of seed to two volumes of substrate, 30 (±2)% mc) for germination in 17 × 12 cm boxes at 3±2°C. The boxes were fitted with translucent lids and were briefly opened weekly to check the germinants. Beechnuts with visible, protruding radicles were considered as germinated and discarded after counting. Germination counts were made weekly from the first week after sowing until no germination occurred for two consecutive weeks. Then, all the remaining seeds were cut and the dead (rotten), empty and fresh seeds were recorded.

Germination rate was determined by calculating mean germination time (MGT) according to the formula: MGT = $\sum (t_i.n_i)/(\sum n_i)$ where t_i is the number of weeks from the beginning of the germination test, and n_i is the percentage of germinating beechnuts on week t_i (Fernandéz et al. 1997). A tetrazolium test, (ČSN 48 1211 2006) was used to determine the viability of four replicates of 100 beechnuts each from each seed lot. The germination percentage (GERM) or viability (VIAB) was calculated on the basis of the total number of filled seeds.

Experimental design and statistical analyses

A completely random design was used for the experiments with three beechnut seed lots from the 2009 crop and three from the 2010 crop. The germination and viability tests started during the first week of each month. To check the reliability of a germination test result, the average germination percentage of the replicates was rounded to the nearest whole number and compared with the Table 5B Part 1 of the International Rules for Seed Testing (two-way test at the 2.5% significance level). The result was considered reliable, if the difference between the highest and the lowest replicate do not exceed the tolerance indicated (International Seed Testing Association 2012). The same procedure was applied for viability test results with the Table 6B (International Seed Testing Association 2012).

All three variables (GERM, MGT and VIAB) were analysed against time (T; real value, T = *year* + (*month*-1)/12, where *month* is order index of the month). Even if the variables GERM and VIAB had binomial distributions, these distributions would be nearly identical to normal distributions because the number of cases (seeds) in the analyses was large (n = 100). The general trend in a variable (var) was determined by linear regression analyse var'(T) = a + bT based on the least sum-of-square method. This took into account the aging of beechnuts during the testing period (12 months). All residuals R(T) = var(T) – var'(T) were calculated (variables var = GERM_RES, MGT_RES or VIAB_RES). Significance of differences among single analyse periods (months) was tested using one-way analyse of variance (ANOVA). The LSD test was used to determine possible differences between variable residuals calculated in two months. All analyses were done using Statistica version 8 software package (Hill and Lewicki 2007).

Because the data represents a time series, these sets were analysed as one-dimensional spatial data. Evaluation of semi-variograms is the basic method suitable for detecting autocorrelation in a random field (e.g. Schabenberger and Gotway 2005). Semi-variances were calculated according to the equation:

$$\hat{\gamma}(D) = \frac{1}{2N(D)} \sum_{N(D)} \left(v_{m+D,r1} - v_{m,r2} \right)^2$$

where $v_{m,r}$ is the observed value of the variable in r-th repetition in month m, D is time distance and $N(D)$ is number of respective data pairs with the time distance D. Because observed variables have the Gaussian distribution, the ratio $\hat{y}(D)/\hat{y}(0)$ is F-distributed with $N(D)$ and $N(0)$ degrees of freedom. The semi-variograms were evaluated using the ratio of semi-variance and nugget variance $\hat{y}(0)$. It is possible to detect a periodicity in the time series under such circumstance when $\hat{y}(D \leq D_0)/\hat{y}(0) > F(\alpha)$ and concurrently when $\hat{y}(D > D_0)/\hat{y}(0) < F(\alpha)$ for the certain time-distance D_0. Values $\hat{y}(D)/\hat{y}(0)$ were plotted against distance D together with respective critical values of the F test by error probability 5% and 95%.

Results

The mean GERM of the beechnuts harvested in 2010 (P1-P3) was higher (87.9%) and less variable (SD 4.1%) compared to the lots collected in 2009 (74.7% and 11.1%, respectively). The beechnuts of the 2010 crop were less dormant as they germinated faster (MGT 7.92 weeks) than seeds of lots 5102–7209 from the 2009 crop (MGT 11.17 weeks). The VIAB of all lots varied less than GERM or MGT (Table 2).

Germination (GERM)

A significant decline in beechnut GERM during the 12 months of storage was noted only in one seed lot collected in 2010 in Poland (P2, p = 0.0002) with the slowest GERM (Fig. 1). Two seed lots (P3, p = 0.19 and 7209, p = 0.13) showed an insignificant decrease, while the GERM of the other seed lots changed only slightly (Fig. 1). The highest GERM was observed in tests done in the spring (April and May in lots 5102–7209) or in the summer (July in the lots P1-P3). Other germination peaks were found in lots 5102–7209 and P1 in the autumn (September) (Fig. 1). The ANOVA test for the residuals of germination was significant only for seed lots 7209 and P3 (Table 3). These two seed lots exhibited different linear trends (less regression coefficient b) in germination compared to other seed lots.

Because the semi-variances were mainly insignificant, time variability was not expected. Some exceptions were observed in lots P2, P3 and 7209, where certain time fluctuations ("periodicities") were determined (Fig. 2, 3). Sometimes (e.g. P2, P3), the fluctuation period was less than a year (Fig. 2). The reliability of germination results was not accomplished in all the repeated tests. The highest variation in GERM among replicates was observed in lot 5102 for three tests that started in April, July and August, twice in the lot 7209 for test in April and September and once in the lot 7208 (January), P2 (December) and P3 (August), respectively (data not showed). In these cases, the observed differences among replicates were out of tolerance. All seed lots from the 2009 crop (5102, 7208 and 7209) showed higher variability compared to lots collected in 2010 (P1-P3).

Table 2. Basic statistics of the observed variables. Number of cases in each row is N = 52

	Seed lot	Mean	Minimum	Maximum	Std.Dev.
Germination (%)	5102	63.6	36	78	7.8
	7208	86.5	76	94	3.4
	7209	74.0	60	90	5.7
	P1	86.1	79	93	3.2
	P2	88.8	80	95	3.8
	P3	88.7	78	99	4.5
Viability (%)	5102	81.4	71	91	4.6
	7208	83.9	75	97	4.2
	7209	75.0	63	92	6.4
	P1	83.0	74	93	4.3
	P2	86.1	77	93	4.2
	P3	82.7	74	91	3.8
Mean germination time (weeks)	5102	14.05	12.72	15.42	0.59
	7208	6.34	5.54	7.28	0.41
	7209	13.13	12.30	14.31	0.54
	P1	7.07	4.93	9.06	0.77
	P2	10.11	8.62	10.94	0.58
	P3	6.60	4.66	9.18	0.75

Mean germination time (MGT)

Trends in MGT differed between data sets P1-P3 (increase; significant in P2, p = 0.0022) and 5202–7209 (decrease; p = 0.016, 0.0085 and 0.0089 in seed lots 5202, 7208 and 7209, respectively) (Fig. 4). Thus, lot P2 was characterised by a significant decline in germination (Fig. 1), attributable to a significant slowing in germination (Fig. 4) and also by significant variation (fluctuation). Compared to the initial values of MGT at the start of the experiments (Table 1) this lot was more dormant then the other lots from the same harvest. The fastest germination (minimum MGT) was observed in July or August for lots 5102–7209, and in January for the lots P1-P3. Highly significant differences among months were confirmed by the residuals of MGT for all seed lots (Table 3). Almost all semi-variances were highly sig-

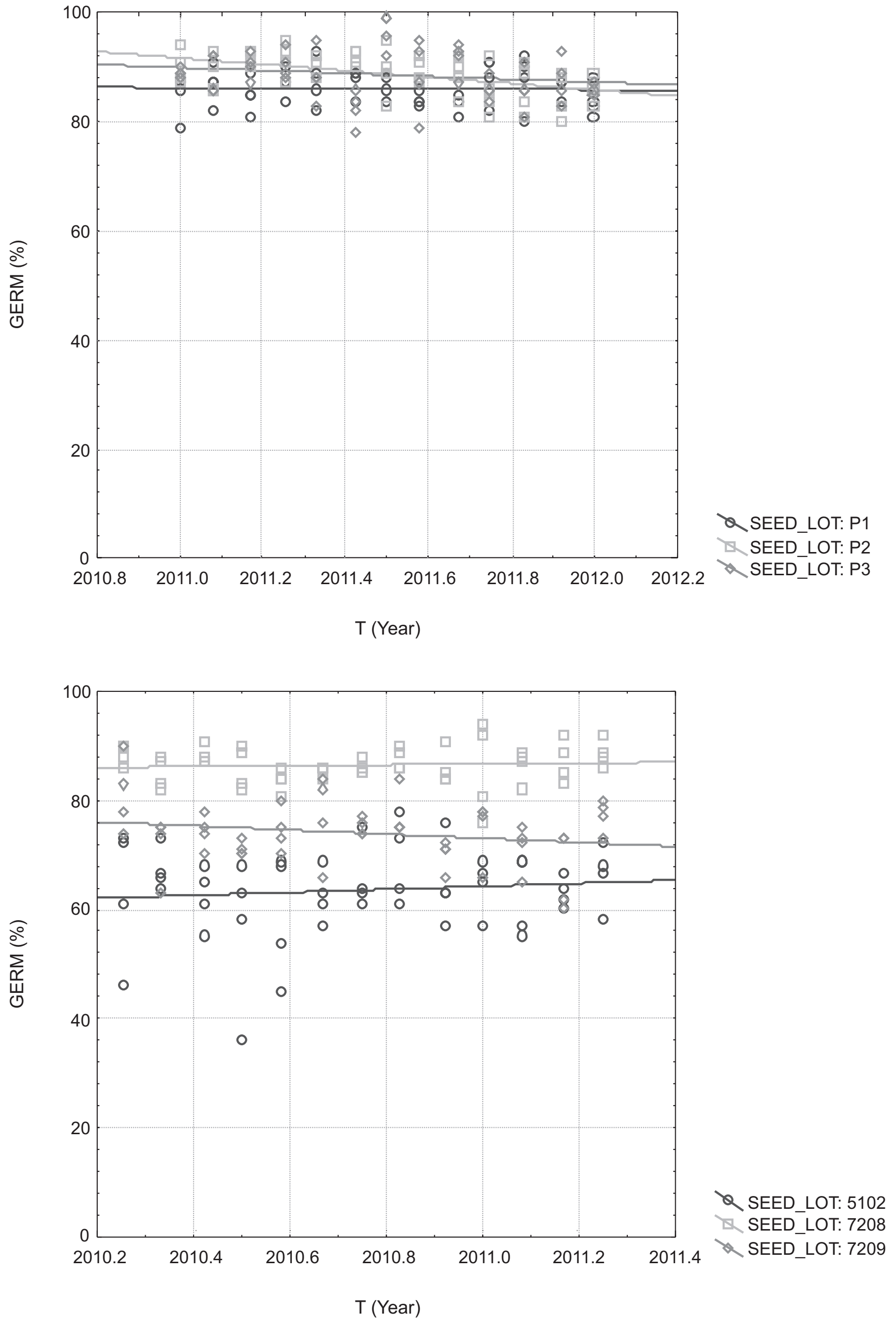


Fig. 1. Basic trends in European beech (*Fagus sylvatica)* seed germination (GERM; in per-cent) against time (T) using the least sum-of-square method: the seed lot P1: GERM = 832.7 –0.3712×T, r = –0.036, p = 0.80; P2: GERM = 11828.7 – 5.8364×T, r = –0.488, p = 0.0002; P3: GERM = 5450.2 – 2.6655×T, r = –0.185, p = 0.19; 5102: GERM = –5466.7 + 2.7504×T, r = 0.111, p = 0.43; 7208: GERM = –1489.6 + 0.7838×T, r = 0.072, p = 0.61; 7209: GERM = 7684.6 –3.7849×T, r = –0.210, p = 0.13

Table 3. Analyse of variance for differences among month viability and germination analyses of European beech (*Fagus sylvatica*) seed lots used in the experiments. Dependent variables are represented by residuals according to linear regression trend. Statistically significant values are marked by the sign *

Seed lot	Error probability (p)		
	Germination	Mean germination time	Viability
5102	0.79	<0.0001*	0.0002*
7208	0.76	<0.0001*	0.024*
7209	0.042*	<0.0001*	0.0019*
P1	0.59	<0.0001*	0.59
P2	0.70	<0.0001*	0.060
P3	0.0038*	<0.0001*	0.015*

nificant (Fig. 5); these results agree with those for the ANOVA tests (Table 3). Semi-variances increased to the maximum at time-distance D equal to 10–11 months (Fig. 5, 6).

Viability (VIAB)

A significant increase in VIAB (determined using the tetrazolium test) during the experiment period was observed only for seed lot P1 (p = 0.0089) while VIAB of the other seed lots decreased slightly (P3, 5102–7209) or increased slightly (P2) (Fig. 7). The highest VIAB was found in August for lots 5102–

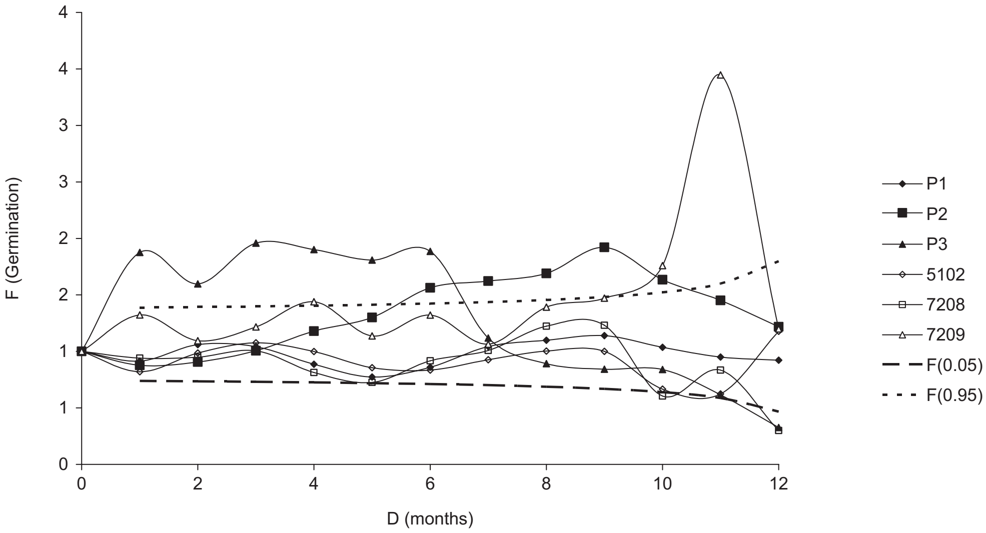


Fig. 2. Ratio of semi-variances to variance (F) plotted against time-distance (D) for germination of European beech (Fagus sylvatica) seeds by lots. The bold curve shows an example of a significant periodicity (lot P2)

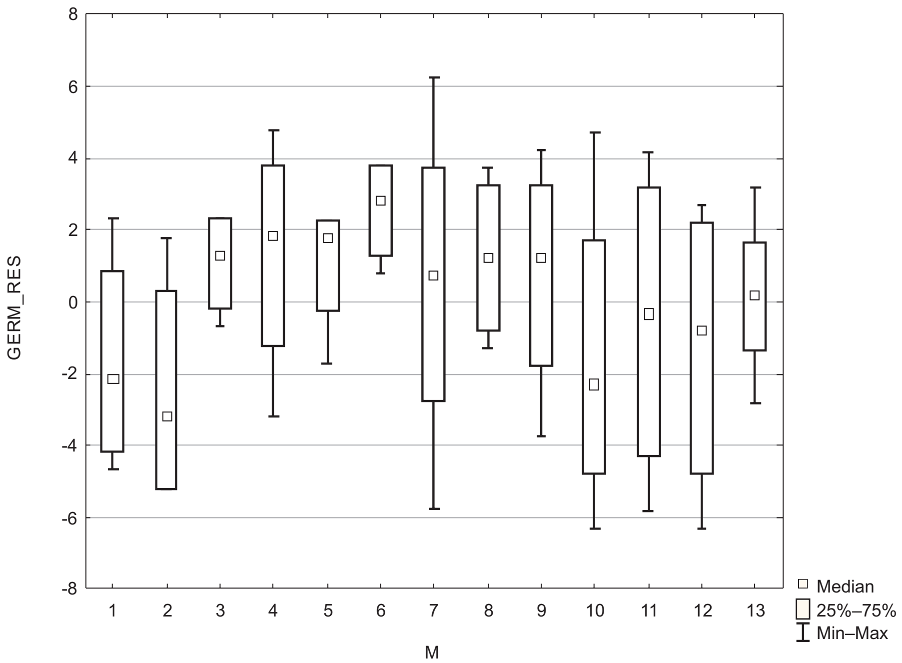
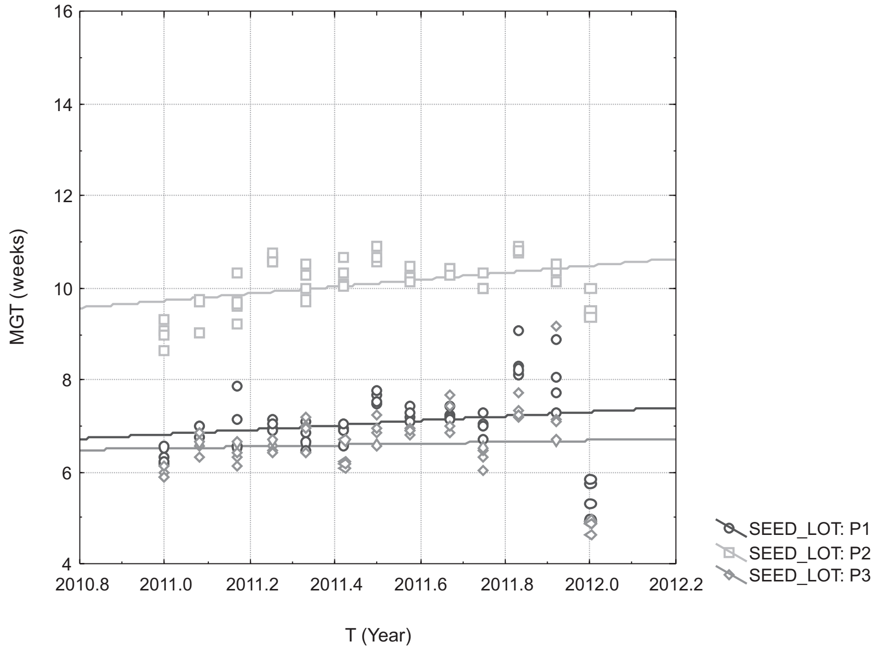


Fig. 3. Residuals of germination (GERM_RES; in per-cent) to linear regression of the European beech (Fagus sylvatica) seed lot P2 which shows a significant periodicity (compare with Fig. 2). M – number of months of the analyses (1= January 2011; 13 = January 2012)



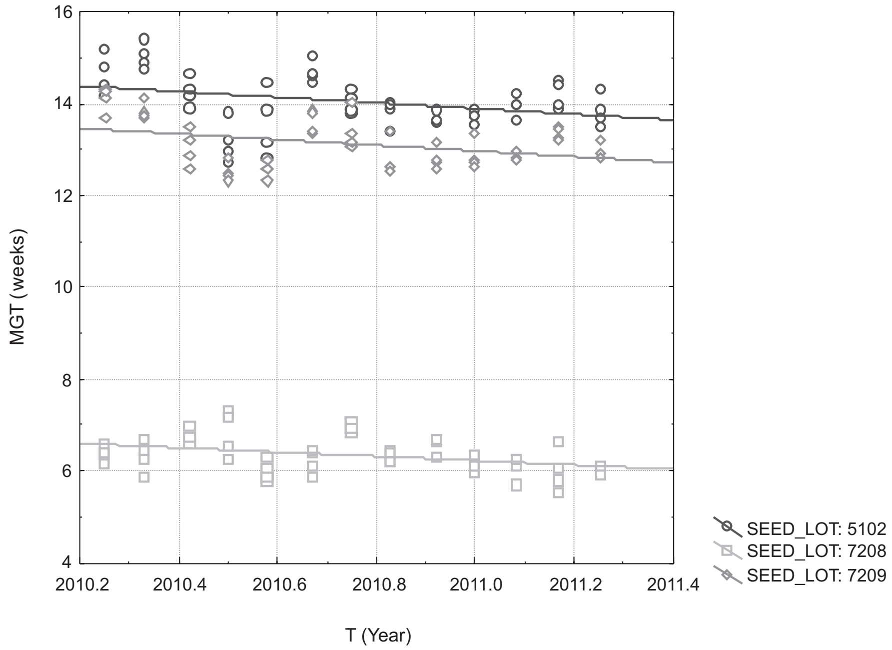


Fig. 4. Basic trends in European beech (Fagus sylvatica) seed mean germination time (MGT; in weeks) against time (T) using the least sum-of-square method: the seed lot P1: MGT = –976.88 + 0.4892×T, r = 0.200, p = 0.15; P2: MGT = –1521.67 + 0.7615×T, r = 0.415, p = 0.0022; P3: MGT = –333.40 + 0.1690×T, r = 0.071, p = 0.62; 5102: MGT = 1250.72 –0.6150×T, r = –0.331, p = 0.016; 7208: MGT = 943.83 –0.4662×T, r = –0.361, p = 0.0085; 7209: MGT = 1256.86 –0.6185×T, r = –0.359, p = 0.0089

7209 while for lots P1 and P2 maximum VIAB occurred both in the winter and in May or June (Fig. 7).

Significant differences in VIAB among months were determined in all three lots from 2009 harvest (5102–7209) and in the lot P3 from the 2010 crop (Table 3). Nevertheless the highly variable data on VIAB showed some periodicity in lots 5102 and 7209 (Fig. 8, 9), where values $\hat{y}(D)/\hat{y}(0)$ were higher than the test criterion for D less than 11 and 9, respectively, and lesser then the criterion for D above or equal these limits.

Discussion

Germination is completed at different times for each seed within a population leading to a distribution in germination times and hence a characteristic

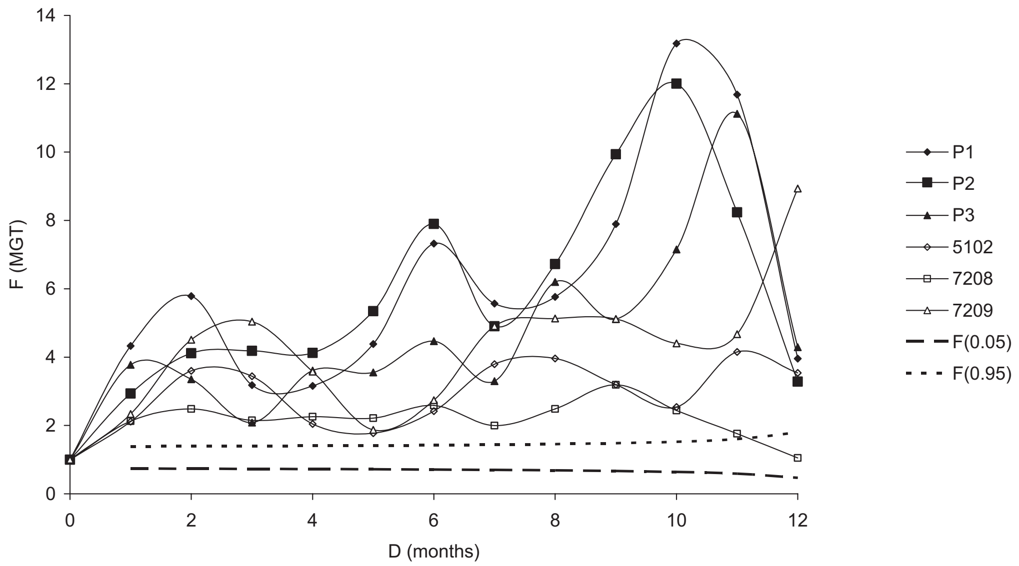


Fig. 5. Ratio of semi-variances to variance (F) plotted against time-distance (D) for mean germination time (MGT) of European beech (Fagus sylvatica) seed lots. The bold curve shows an example of a significant periodicity (P2)

cumulative germination curve for any specific seed lot. Factors affecting germination of stored seeds are water availability, temperature, dormancy (ABA hormone level) and ageing of individual seeds. A population of seeds is always more or less heterogeneous. This heterogeneity within a single population has many origins (Black et al. 2006). The germinative properties of seeds depends up several factors such as their maturity stage at harvest or shedding, their size, their position on the mother plant, their degree of dormancy, the quality of pollination, the treatments applied to the seeds after harvest, and the conditions and the duration of storage. As a consequence, all the seeds from a population do not complete germination at the same time. The rate of germination is also markedly dependent on dormancy (Black et al. 2006).

In most studies, greater fluctuation was seen in germination rate mainly expressed by germination energy values (e.g. Řeháčková 1954; Procházková 2002; Debnárová and Šmelková 2008, 2008a). This

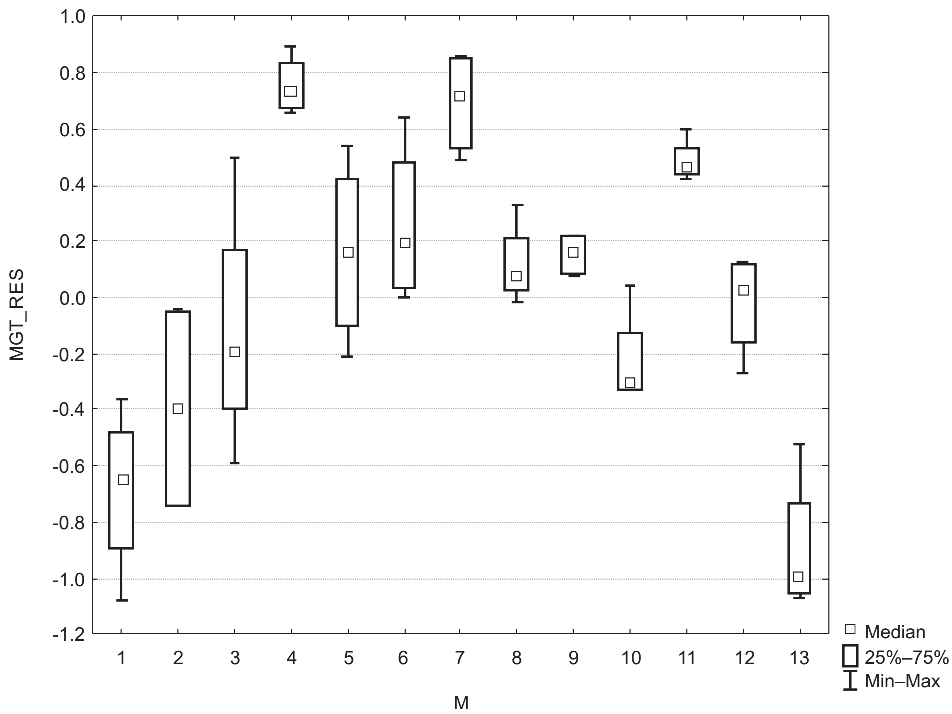


Fig. 6. Residuals of mean germination time (MGT_RES; in weeks) to linear regression of the European beech (Fagus sylvatica) seed lot P2 which shows a significant periodicity (compare with Fig. 5). M – number of months of the analyses (1= January 2011; 13= January 2012)

corresponds to the results of this study (Table 3, Fig. 5, 6) as principally the MGT that reflects germination rate and dormancy release significantly differed within some lots in time (month) of the analysis during seed storage. In partial agreement with earlier results (Schmidt 1930; Baldwin 1935; Řeháčková 1954; Barnett and Mamonov 1989; Procházková 2002; Wang 2003; Debnárová and Šmelková 2008, 2008a) we observed maximal GERM during in spring tests (April or May), but also for some seed lots in summer (July) or early autumn tests (September) (Fig. 1). However, the results of monthly germination tests over 12 months under standard conditions showed no consistent trend of endogenous control of germination periodicity in beech seeds and the observed fluctuation was more likely caused by other (external) factors which is in agreement with findings and conclusions of others such

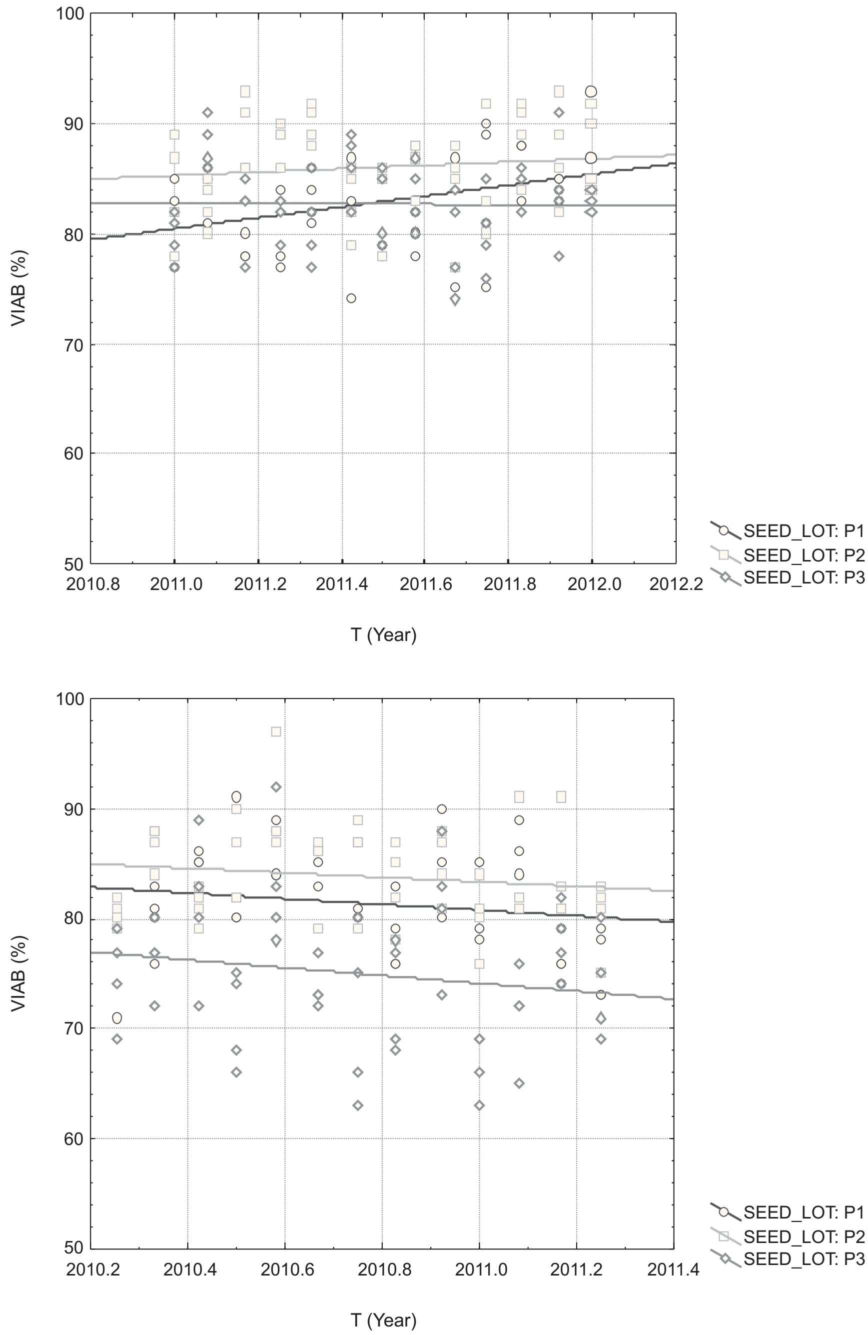


Fig. 7. Basic trends in European beech (Fagus sylvatica) seed viability (VIAB; in per-cent) against time (T) using the least sum-of-square method: the seed lot P1: VIAB = –9861.7 + 4.9439*T, r = 0.360, p = 0.0089; P2: VIAB = –2999.8 + 1.5341*T, r = 0.116, p = 0.41; P3: VIAB = 543.4 –0.2290*T, r = –0.019, p = 0.89; 5102: VIAB = 5310.0 –2.6003*T, r = –0.178, p = 0.21; 7208: VIAB = 4200.8 –2.0475*T, r = –0.153, p = 0.28; 7209: VIAB = 7201.2 –3.5441*T, r = –0.173, p = 0.22

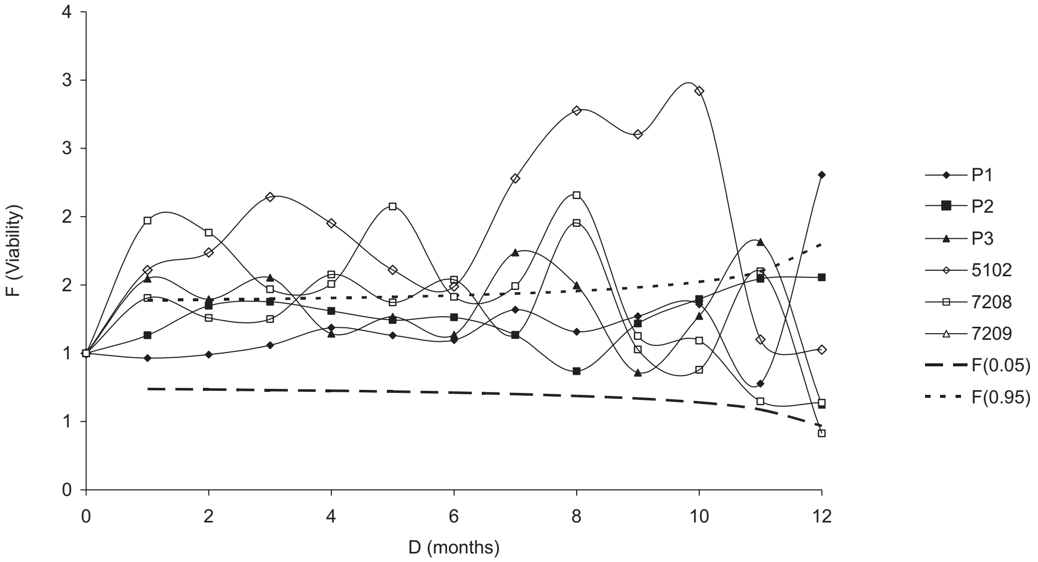


Fig. 8. Ratio of semi-variances to variance (F) plotted against time-distance (D) for viability of European beech (Fagus sylvatica) seed lots. The bold curve shows an example of a significant periodicity (lot 5102)

as Baldwin (1935), Řehácková (1954), Rostovcev et al. (1975) and Wang (2003).

Compared to germination tests, tetrazolium tests often overestimate viability. Furthermore, germination tests of beechnuts can also last up to five months (Gosling 1991; Procházková and Bezděčková 1999; Procházková et al. 2000, 2002) while viability results are available in a week. Viability tests give valuable information regarding whether or not beechnuts can be stored or sow as soon as possible. Viable seeds should show by their biochemical activity the potential to produce normal seedlings (International Seed Testing Association 2012), but not all viable seeds can and will germinate. Tetrazolium viability results are greatly affected by good training and especially by staff experience as the viability assessment is more subjective than the germination tests. As such we expected viability results to be higher than those for germination but our results of this study varied among the test seed lots even when the differences between average viability and germination were low (Table 2). As told previously, the viability of most

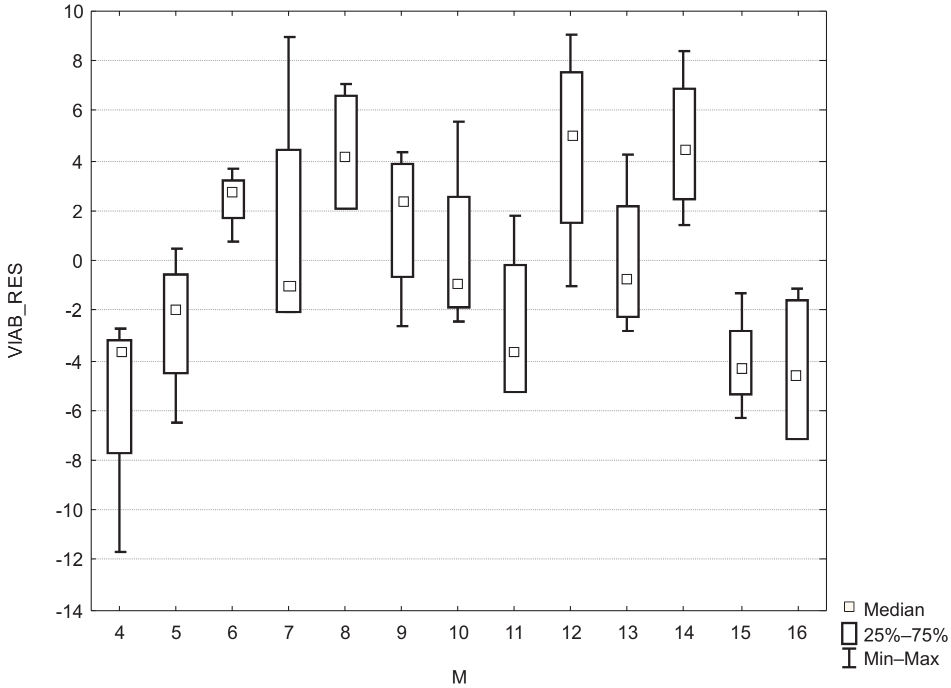


Fig. 9. Residuals of viability (VIAB_RES; in percent) to linear regression of the European beech (Fagus sylvatica) seed lot 5102 which shows a significant periodicity (compare with Fig. 8). M –number of months of the analyses (4= April 2010, 16= April 2011)

seed lots after 12 months of storage decreased and only in one lot (P1) the increase was significant. The reason for this is unknown.

Beechnuts are seeds with intermediate, physiological dormancy and are more sensitive to handling during harvesting, processing and testing. In the Czech Republic, the harvest can occur from stands with areas >10 ha but also >100 ha (Musil and Procházková 2011). Beechnuts are collected from the forest floor or nets suspended above the floor for several days or even weeks; so ripening and subsequent dormancy level within the lot can vary dramatically. Compared to agricultural seeds, seed lots of forest trees that originate from forest stands represent genetically and, to a certain extent, also physiologically heterogeneous populations. Also, obtaining representative samples from seed lots sometimes weighing more than 4.000 kg should be considered. During processing, beechnut lots are not always thoroughly mixed; which could result in differences among containers with stored seeds. However, the weight of seed lots used in this study was not more than 10 kg and working samples for germination and viability tests were carefully prepared according the procedure specified in the rules (International Seed Testing Association 2012).

Another source of fluctuating GERM or MGT might be even a minor failure during a germination test resulting in uneven moisture of the substrate since water availability is critically important (Black et al. 2006). In the present work the moisture content of the germination substrate varied from 28.0 to 32.0% (determined at the start of the test) and did not show any relation to variation in germination, e.g. lot 5102.

The differences in GERM could be attributed to beechnut quality as the highest fluctuation in GERM (with differences among replicates out of tolerance) was observed in lots 5102 and 7209 which had the lowest GERM. Also, dormancy might be regarded as another factor influencing the results of repeated germination tests as beechnuts of the same lots (5102 and 7209) were more dormant and germinated slower (Table 2). Thus, low GERM and deeper dormancy could be considered as important source of differences among germination tests.

The reliability of germination and viability tests are verified by comparing the differences between the highest and lowest replicates. When these differences are out of tolerance, then the test has to be repeated (International Seed Testing Association 2012). As the germination test for beechnuts can take up to 24 weeks it is not practical to repeat the test. The length of the germination test is the reason why the tetrazolium test is used to determine beechnut quality. However, a germination test is the best method for determining the stratification requirements of beechnut seed lots and as such it must be reliable.

After ripening is the progressive loss of dormancy in mature dry seed. Dry seeds (typically 5–15% moisture content) often may experience gradual dormancy loss as the result of dry after ripening, which commences on the mother plants and continues following seed shedding onto the soil. Chilling (optimum about 4°C), generally for several days or weeks (cold stratification) is effective in breaking dormancy in seeds of species adapted to germinate in the higher temperatures of late spring and summer. It is commonly effective in seeds of temperate woody species (Black et al. 2006). In beechnuts after ripening has been observed in air-dried seeds (*Fagus orientalis*, *Fagus sylvatica*) to 10% mc at 20°C that germinated a few weeks (2–4 wks) faster than fresh non-dried beechnuts (Muller et al. 1989; Suszka 1994; Thomsen 1997; Yilmaz and Dirik 2008). However, once beechnuts are dried to ca 10% mc and stored, then the after ripening has been completed and no significant changes in seed dormancy during the first year of storage have been observed (Procházková et al. 2012 unpublished annual research report). In this study were used seeds stored for up to 14 months, so after ripening should not impact dormancy release and germination speed.

Conclusions

There were determined differences in germination, germination rate and viability for the same seed lot of European beech seeds in monthly tests performed during one year of their storage under controlled laboratory conditions. Among important factors that might influence the reliability of the repeated test seem to be initial seed quality (namely germination and dormancy) and very careful adherence to test procedures. However, no consistent seasonal fluctuation in GERM, MGT or VIAB of the beechnuts was observed in the tests. A more or less significant increase in germination capacity and germination speed (dormancy release) was observed mostly in tests that started in the spring or early autumn while other lots germinated better in summer tests. The 12-months experimental period was limited by the availability of beechnuts and this prevented from obtaining additional data to see if any seasonal fluctuations exist. Additional studies (e.g. of ABA content) might help determine the cause of fluctuation in germination of stored beechnuts.

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