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Maturation of Oriental beechnuts (*Fagus orientalis*)

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Abstract: In this study, both the morphological maturation and germination ability of the Oriental beechnut were investigated two months prior to seed dispersal to find out the appropriate period of ripened beechnut collection. Beechnuts were collected in the seed stand, Dokurcun-Adapazari, on August 21, September 9, and September 25 on trees, and on October 16, 2003, from the ground after the major seed fall. Germination percentages were 18.0%, 80.5%, 92.0%, and 94.7% on August 21, September 9, September 25, and October 16, respectively. Similarly, both beechnut weight and the embryo:beechnut weight ratio significantly increased with time and reached an approximate maximum level at September 25, 2–3 weeks prior to seed dispersal. This outcome indicates that ripened beechnuts can be collected from the trees, 15–20 days prior to major seed dispersal. The study also indicates that ripened Oriental beechnuts have physiological dormancy and need about 8–10 weeks chilling at 3°C for germination.

Additional key words: seed, beech, trees

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Introduction

Large amounts of beechnuts have been collected for the regeneration of the Oriental beech (*Fagus orientalis* (Lipsky) in Turkey. The collection time is particularly crucial for beechnuts to be stored over long-term periods. Therefore, both the maturation and development of beechnuts should be well understood to determine this optimal collection time. The best time for seed collection is when nutrient transfer from plants to seeds ceases and seeds reach their maximum dry weight and physiological maturity (Kozlowski 2002).

Three stages are encountered in seed development: rapid gain of fresh weight, rapid gain of dry weight, and loss of fresh weight because of maturation drying (Bewley and Black 1994). The duration of these periods depends on both the tree species and the prevailing environment. Environmental stresses, such as water deficits, low or high temperatures, nutritional deprivation, and shade, affect seed development in terms of amount, quality, weight, chemical composition, viability, and vigor.

During seed development, seeds obtain their germination capacity in different stages depending on their species; germination capacity also increases with an increase in dry weight. Undeveloped seeds take longer to germinate due to insufficient nutritional reserves (Bewley and Black, 1994).

Both morphological and physiological development have been studied in certain forest trees, such as *Pseudotsuga menziesii* (Ching and Ching 1962), *Picea orientalis* (Ürgenç 1965), *Pinus sylvestris* and *P. nigra* (Boydak 1984), *Cedrus libani* (Özdemir et. al. 1986), *Acer platanoides* (Hong and Ellis 1990), and *Ulmus wallichiana* (Phartyal et al. 2002). Cones of *P. menziesii* reach their maximum size, maximum fresh weight, and maximum dry weight at the beginning of June, July, and August, respectively (Ching and Ching 1962). During the seed development of *U. wallichiana*, germination percentages and dry weight increase at maximum levels coinciding with maximum dry weight 56 days after flowering (Phartyal et al. 2002).

Oriental beech is one of the major forest trees in Turkey. Beech forests generally originate from sprouts, which make the beech forests more vulnerable to environmental changes. Therefore, regeneration of the beech forests from seeds of selected stands is currently a high priority for foresters.

In this study, both the morphological maturation and germination ability of beechnuts were investigated two months prior to major seed dispersal from August 21 to mid-October. During beechnut development, changes in germination capacity were tested and the weights of beechnuts, embryos, and testae as well as embryo: beechnut weight ratios were measured.

Materials and Methods

Beechnuts were collected in the seed stand, Dokurcun (40°30’E, 30°44’N), on August 21, September 9, and September 25 from at least 10 randomly selected trees prior to seed dispersal, and on October 16, 2003, from the ground after the major seed fall.

Beechnuts in cupules: The cupules collected on the trees were left to dry at 20°C. When the cupules began to open (1–10 mm) after 2–3 days, the fresh beechnuts were extracted from the unopened cupules and the moisture content (MC) of the beechnuts, two replicates of 5 gr, was measured at 104 ± 1°C for 17 h. Four replicates of 25 randomly selected cupules were opened completely and the beechnuts were removed and cut longitudinally; embryo development was simply recorded. Beechnuts were classified into four groups: sound, insect infected, empty, and undeveloped. The ratios of sound, insect infected, empty, and undeveloped embryos were calculated as the mean of four replicates.

Morphological maturation: Beechnuts were air-dried to aproximately 10% moisture content at 20°C and 100 replicates of randomly selected beechnuts were weighed individually. The testae were then removed and the embryos of each beechnut also weighed individually. The weight of the testae for each beechnut was calculated by substracting the weight of the embryo from the weight of the beechnut. For each weighed beechnut, the embryo:beechnut weight ratio (EBR) was calculated by dividing the embryo weight by the beechnut weight.

Germination ability during maturation: The beechnuts collected on August 21, September 9, September 25, and October 16 were germinated at 3°C (ISTA 1996). Two hundred (4*50) fresh beechnuts with high MC were used for the germination tests. The beechnuts weren’t dried before the germination tests. Germination tests were carried out in 15-cm diameter petri dishes on two filter papers. The beechnuts were considered germinated when the radicles protruded at least 3 mm. Germination was recorded once per week. The tests were terminated when no germination was observed for at least two weeks. Samples from all collection dates (August 21, September 9, September 25, and October 16) were also taken for germination tests at 15°C without prechilling to check for dormancy existence during development.

Mean germination time (MGT) was measured as the number of weeks for germination at 3°C. Germination percentage (GP) and MGT were calculated according to the following formulas:

$$GP(\%) = \frac{\sum n_1}{N} \times 100$$

Where GP (%) is the germination percentage, n_i is the number of germinating beechnuts on day of inspection, i, and N is the total number of incubated beechnuts for the test.

$$MGT = \frac{\sum (t_i \cdot n_i)}{\sum n_i}$$ (Bewley and Black 1994)

Where MGT is the mean germination time, t_i is the number of weeks from the beginning of the test, and n_i is the number of germinating beechnuts recorded on week $t_{(i)}$.

Statistical Analyses: The differences between germination percentage, MGT, and percentage sound, insect infected, empty, and undeveloped seeds on different collection dates were analyzed by one-way ANOVA. Both GP and percentage sound, insect infected, empty, and undeveloped seeds were transformed by arcsine square root prior to analyses to normalize error distribution. When a significant effect was detected, the groups were identified using Duncan’s New Multiple Range test (GP and MGT) and Tukey’s test (other parameters).

Results

Beechnuts in cupules

The percentages of sound beechnuts on trees were 75.5%, 68.0%, and 73.0% on August 21, September 9, and September 25, respectively (Table 1). There were no statistical differences between the collection dates for sound, empty, and undeveloped beechnuts. However, there were the significant (p = 0.01) differences for insect-infected beechnuts. Infection increased from August 21 to September 9 but decreased

Table 1. The characteristics of beechnuts extracted from cupules (Tukey's test)

Collection time	Sound ± s.d. (%)	Insect-infected ± s.d. (%)	Empty ± s.d. (%)	Undeveloped-deformed ± s.d. (%)	MC of fresh beechnuts (%)
August 21	75.5 ± 5.0 a[1]	7.0 ± 2.6 a	11.0 ± 5.3 a	6.5 ± 1.9 a	40.5
September 9	68.0 ± 5.2 a	13.0 ± 3.5 b	14.5 ± 3.8 a	4.5 ± 2.5 a	38.9
September 25	73.0 ± 6.8 a	5.0 ± 2.6 a	14.0 ± 2.8 a	8.0 ± 2.8 a	38.3
Average	72.2 ± 6.1	8.3 ± 4.4	13.2 ± 4.0	6.3 ± 2.7	39.2

[1]no statistical differences between the same letter in the same column ($p < 0.01$)

Table 2. The weights of beechnuts, embryos, and testae and the embryo:beechnut weight ratio according to collection date (Tukey's test)

Collection date	N	Beechnut weight ± s.d. (g)	Embryo weight ± s.d. (g)	Testa weight ± s.d. (g)	Embryo:beechnut weight ratio ± s.d. (%)
August 21	100	0.182 ± 0.025 a[1]	0.088 ± 0.017 a	0.094 ± 0.010 a	47.92 ± 3.60 a
September 9	100	0.226 ± 0.034 b	0.128 ± 0.026 b	0.097 ± 0.014 a	56.48 ± 4.95 b
September 25	100	0.286 ± 0.069 c	0.189 ± 0.053 c	0.097 ± 0.020 a	65.60 ± 4.30 c
October 16	100	0.301 ± 0.063 c	0.205 ± 0.051 c	0.097 ± 0.019 a	67.38 ± 5.40 c

[1]no statistical differences between the same letter in the same column ($p < 0.01$)

from September 9 to September 25 due to early dispersal of insect-infected beechnuts.

There were generally two beechnuts per cupule (~93%). However, certain cupules had one, three, four, five, or six beechnuts. No significant differences were found in the MC of fresh beechnuts during the development period (Table 1).

Morphological maturation

The weights of beechnuts and embryos increased during development. There were no significant increases in weight between September 25 and October 16. There were also no noteworthy increases in the weights of testae from August 21 to the time of seed fall (mid-October).

The embryo:beechnut weight ratio (EBR) increased significantly from August 21 to September 25, but there were no significant increases from September 25 to major seed fall in mid-October (Table 2).

Germination ability during maturation

Non-chilled beechnuts collected on August 21, September 9, September 25, and October 16 did not germinate at 15°C.

In germination tests at 3°C, GP was low on August 21 as only 18% of beechnuts germinated. A relatively high percentage of beechnuts (80.5%) germinated by September 9. GP reached 92.0% and 94.7% on September 25 and October 16, respectively. There were no significant differences between September 25 and October 16 in terms of GP (Table 3).

MGT varied greatly according to collection date and maturation stage. There were significant differences between all collection dates. The fully mature beechnuts collected from the ground on October 16 germinated faster than all others (August 21, September 9, and September 25) (Fig. 1). Although GP on both September 25 and October 16 were at approximately the same level, there were significant differences (about 4.5 week) between September 25 and October 16 in terms of MGT.

Table 3. Effect of collection time on germination percentage and mean germination time at 3°C (Duncan's test)

Collection dates	Germination (%)	Mean germination time (weeks)
August 21	18.0 a[1]	21.2 a
September 9	80.5 b	19.4 b
September 25	92.0 c	15.2 c
October 16	94.7 c	10.5 d

[1]no statistical differences between the same letter in the same column ($p < 0.01$)

Discussion

Morphological maturation

The ratio of sound beechnuts in the cupules collected on August 21, September 9, and September 25 from the trees was relatively high (72.2%) since the cupules fallen on the ground weren't taken into account. Beechnut production studies for *F. orientalis* (Tosun 1992) and *F. crenata* (Yasaka et al. 2003) over 11 years showed that the proportion of sound beechnuts was generally low, averaging 35.6% (*F. orientalis*) and 24.5% (*F. crenata*). In both studies, the proportion of sound beechnuts was calculated over the total beechnut production time, including early shedded beechnuts, using seed traps.

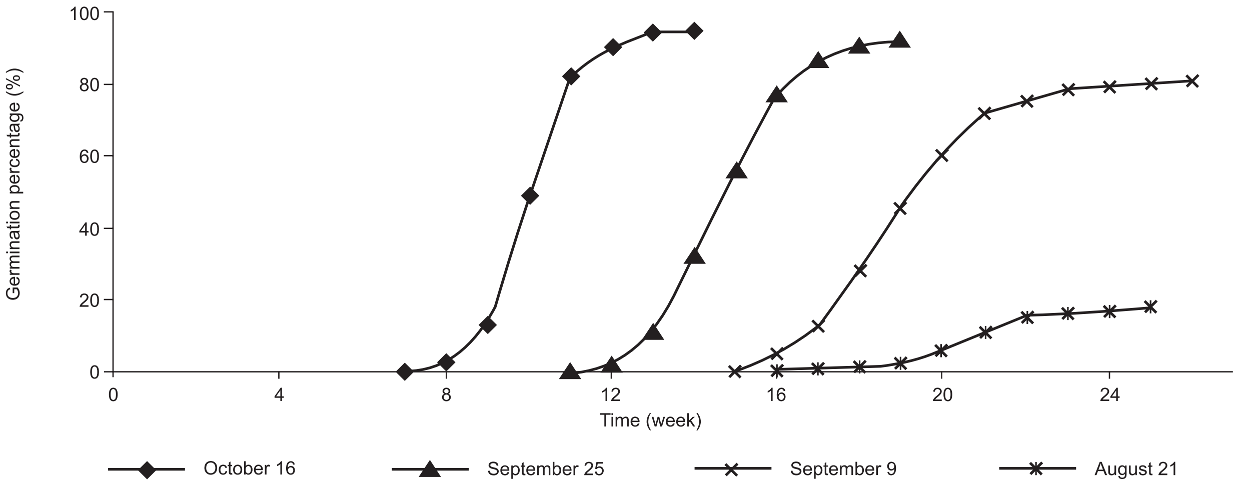

Fig. 1. Germination percentages in different collection dates

The proportion of insect-infected beechnuts significantly increased on September 9 and decreased by September 25 due to earlier dispersal of infected beechnuts prior to major seed dispersal. Similar observations for oriental beehnuts were also recorded by Tosun (1992). Insect damage is generally a widespread problem for beechnuts. For instance, the major cause of the low proportion of sound beechnut in *F. crenata* has been attributed to insect damage and, in some years, all beechnuts have been consumed by insects (Yasaka et al. 2003; Igarashi and Kamata 1997).

The proportion of empty beechnuts on the trees did not vary significantly during the observation period. Some empty beechnuts fell prior to August 21 primarily in cupules in which both beechnuts were empty and there were no cupules on the trees carrying two empty beechnuts on August 21, September 9, and September 25. The proportion of empty beechnuts varies between trees. Wang (2003) studied beechnut production in 91 trees and found significant differences between the trees and the ratios of empty beechnuts ranging from 4.8–40.9% (average 21.4%) likely due to self-fertilization.

The level of embryo development was much lower than the average in some beechnuts extracted from the cupules. The beechnuts with these undeveloped embryos were heavier than the empty beechnuts but lighter than the normal sound beechnuts and, thereby, the EBRs of beechnuts with undeveloped embryos was clearly lower than the those of sound beechnuts. The presence of undeveloped beechnuts lowers the quality of the seed lot (Bewley and Black 1994). Soltani (2003) found that the heavy beechnuts had a high GP. Yilmaz (2005), on the other hand, found that there were big variation both between populations and within populations in terms of beechnut (*F. orientalis*) size. Fully filled, small beechnuts germinated at a higher rate than the larger and heavier beechnuts with lower EBRs even the smaller sound beechnuts that were lighter than the bigger beechnuts with undeveloped embryos. Removing the sound small beechnuts eliminate certain families with small beechnuts from the population (Yilmaz 2006), narrowing genetic variations (Hellum 1976). Air ventilation is a very effective way to separate sound beechnuts according to the stage of embryonic development.

MC of the beechnuts collected from the trees were 40.5% (August 21) and 38.3% (September 25), which are similar to the maximum MC of the mature sound beechnuts of approximately 40±1% MC (Yilmaz 2005).

The dry weights of beechnut clearly increased during the development until the end of September (Table 2). There were no significant increases from the end of September to the time of major seed fall in mid-October. Barthe et al. (2000) found that beechnuts primarily completed embryonic development and nutrient accumulation by the beginning of September at a 1200-m elevation in France. The current study showed that the maturation of oriental beechnuts is completed by the end of September. The development process and maturation time varies according to elevation, origin, and weather. On October 15, 2003, when all beechnuts and leaves fell in Dokurcun (40°30'E, 30°44'N) at a 1200-m elevation, the majority of beechnuts and leaves was on the trees at the same elevation in Yenice, Karabük (41°11'E, 32°23'N). Suner (1978) observed that the major seed fall was in the second half of October for three years in Düzce, Cide, and Akkus in Northern Turkey.

EBR remained fairly constant at the end of September through mid-October, suggesting morphological maturation 2–3 weeks prior to major beechnut shedding in a stand during a 1-year ripening period.

The earliest date (August 21) on which beechnuts were collected provides clear estimate about the sound beechnut ratio for the year. Similarly, Tosun (1986) stated that beechnuts become heavy from mid-August and the branches of the trees bend down.

Germination ability during maturation

Beechnuts collected during development or after dispersal did not germinate at 15°C without chilling, suggesting that dormancy occurs in beechnuts both during development and after dispersal. Whereas dormancy on August 21 and September 9 is both morphological (undeveloped embryo) and physiological, dormancy on September 25 and October 16 is mainly physiological because the beechnut had completed its morphological development by September 25.

Germination capacity reached high percentages on September 9 and achieved its highest level beginning at the end of September. Similar trends are also seen for morphological development and EBR (Table 2), which show close relationships between morphological and physiological development. Similarly, in the Taurus Cedar (*Cedrus libani*), seeds collected from May to October germinated at an increased percentage with collection time (Özdemir et al. 1986).

During beechnut development, the MGT (chilling duration + germination duration) was shortened due to dormancy loss duration (chilling requirement). Whereas there was a 16-day difference between September 9 and September 25, the difference in MGT was longer than four weeks as the chilling duration was shorter on September 25 and germination was earlier. Although morphological parameters and GP were similar on September 25 and October 16, there were clear differences in MGT on the same dates as the chilling plus germination durations were shortened significantly.

Air-dried beechnuts collected on September 25, for one week of drying to 10% MC at 20°C also germinated 12 days faster than fresh non-dried beechnuts collected on the same date (data not shown). Similarly, shortening of MGT by 2 weeks (Muller et. al. 1999) and 4 weeks (Thomsen 1997) were also seen in dried European beechnuts (*F. sylvatica*). These shortening effects of drying on the chilling requirement may be due to either after-ripening (Probert 2000; Murdoch and Ellis 2000) or an increase in seed cover porosity due to drying and hydration and, therefore, more efficient respiration. Therefore, beechnuts should be air-dried (~10% MC) prior to rehydration before either the prechilling treatment or sowing to shorten the chilling duration.

Desiccation tolerance of seeds varies during development. Drying sensitivity decreases as the seeds mature (Bewley and Black 1994; Kermode and Finch-Savage 2002). Mature dispersed beechnuts can be safely dried to 8–9% (Suszka et al. 1996; Koyama et al. 2002; Leon-Lobos and Ellis 2002) or 6% (Poulsen 1993; Yilmaz, 2008) and stored for several years. Further research is needed on desiccation tolerance and storage of oriental beechnuts during maturation.

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