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Bud burst and flowering phenology in a mixed oak forest from Eastern Romania

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Abstract. Bud burst and flowering phenology have been observed in year 2008 in a natural white oak species complex situated in eastern Romania. A total of 300 mature individuals was mapped and identified based on leaf morphology. The community consists of four oak species: *Quercus pedunculiflora, Q. robur, Q. pubescens* and *Q. petraea.* A set of 28 individuals could not be unambiguously classified to one or another species. Data on bud burst showed a normal distribution and the differences among species were small. The "very late" flushing was recorded on 15th of April, three weeks later when compared to early flushing individuals. The time period between the bud burst and the complete development of leaves was nearly the same in all oak species, varying on average, between 18.4 and 20.6 days. The spatial distribution of phenological groups within the complex appears to be non-randomly, because in many parts of the study plot exist groups in which most of the trees belong to the same phenological category. Our results indicate an overlap in flowering time for all oak species which occur in the area. The data support the hypothesis that interspecific gene flow is possible between closely related oak species.
Keywords. *Quercus*, bud burst, flowering, oak

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Introduction

Bud burst and flowering are complex processes controlled by both genetic and environmental factors (climate, physiography etc.) (Danciu & Parascan 2002). Phenological observations on tree species are useful for forest management practices (Marcu 1971) and an important indicator of variability and climate change (e.g. Menzel 2000, Chmielewski & Rötzer 2001, Richardson et al. 2006). Especially the flowering phenology can be an effective reproductive barrier and influences the patterns of hybridization between closely related species (e.g. Bacilieri et al. 1996, Gerard et al. 2006). Data on phenology is also needed when searching for candidate genes in forest tree species (Vornam et al. 2009).

In Romania, in compared with other European countries (e.g. Germany, Great Britain, Czech Republic), phenological observations in forest tree populations were made discontinuously, systematic records being made in the last years in plots of the IPC Forests and

FENOFOR network (Teodosiu et al. 2005). Phenological data on oak species - pedunculate oak (*Quercus robur*) and sessile oak (*Q. petraea*) - were recorded for short time periods in several stands and arboreta across the country (Bălănică & Tomescu 1953, Tomescu et al. 1967, Teodosiu et al. 2005). However, no data about bud burst, flowering and leaf fall are reported for *Q. pedunculiflora* K. Koch., a lesser known oak taxon occurring in S-E Europe (Georgescu & Morariu 1948, Schwarz 1993).

A bimodal floral phenology distribution would be regarded as a complete prezygotic barrier between *Q. pedunculiflora* and *Q. robur* and it would support the hypothesis of the existence of two separate species. On the contrary, an unimodal distribution would rather suggest that the flower phenology does not constitute a reproductive barrier between them and the two taxa are part of the same species. The aim of this study was to examine the leaf unfolding and flowering phenology at a fine scale in a mixed forest with *Q. robur* and *Q. pedunculiflora*. The phenological observations, particularly on flowers, in these two oak taxa, are part of a large genetic and morphological investigation which aims to clarify the taxonomical status of *Q. pedunculiflora*. This species is considered either as separate species (Georgescu & Morariu 1948, Schwarz 1993) or as intraspecific unit of *Q. robur* (e.g. Petit et al. 2002, Broshtilov 2006).

Materials and methods

The studied tree species are monoecious, i.e. they produce on the same tree both male and female flowers. As most of the temperate tree species, they are wind-pollinated. *Q. robur* prefers nutrient-rich and wet soils whereas *Q. pedunculiflora* is more adapted to xeric conditions (Şofletea & Curtu 2007).

The study plot is located at Fundeanu forest (45°58'41"N, 27°41'26"E, altitude 230 m above see level), Grivița Forest District, Galați Forest Directorate, in eastern Romania. A total of 300 adult oak individuals were sampled, numbered and mapped by using a high precision Leica SR 20 GPS unit (Figure 1).

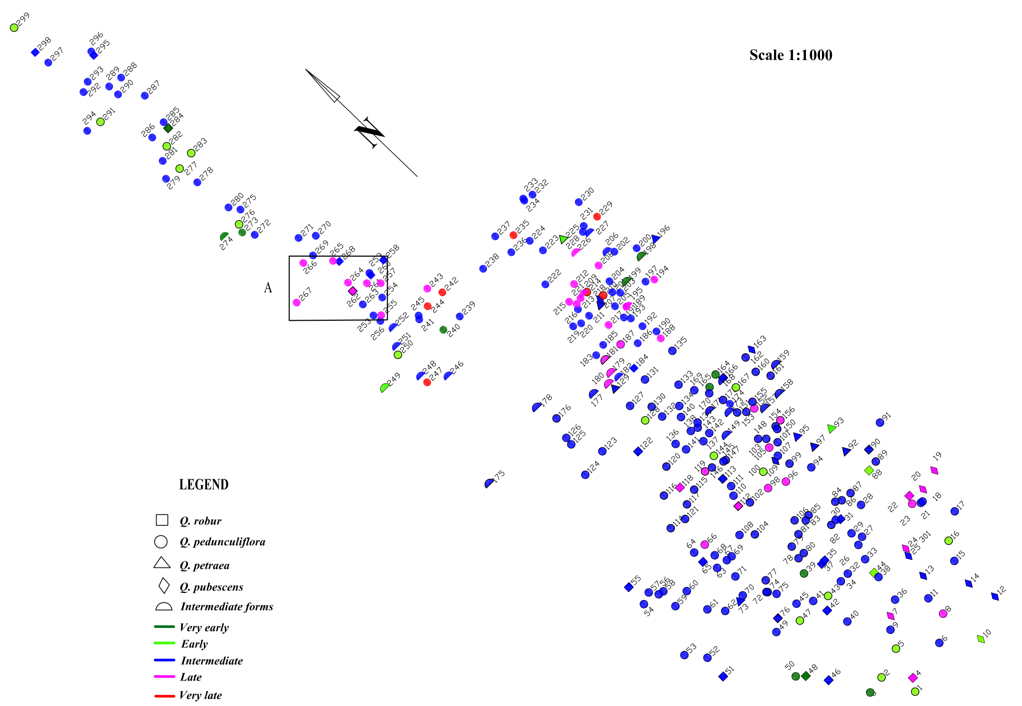

Figure 1 Map of the study site and distribution of oak tree on bud burst categories in year 2008

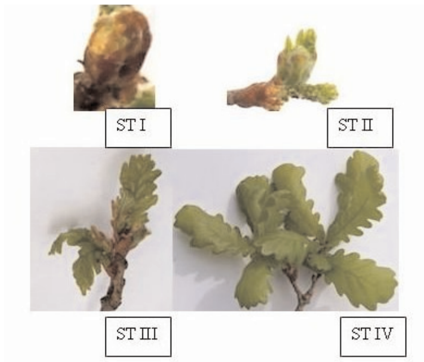

Figure 2 Different developmental stages for leaf unfolding

The raw data were processed with Leica Geo Office software, and the tree distribution map was drawn with AutoCAD software. All trees were classified as species or morphologically intermediate based on leaf morphological characters (Georgescu & Morariu 1948, Dumitru - Tătăranu et al. 1960, Tutin et al. 1993, Stănescu et al. 1997). Within the study plot we identified 222 *Q. pedunculiflora* individuals, 28 *Q. robur* individuals, and 28 individuals which could not be unambiguously classified as *Q. robur* and *Q. pedunculiflora*. We named these individuals as morphological intermediates. Additionally 10 *Q. petraea* individuals and 12 *Q. pubescens* were also found (Figure 1).

Phenological observations of bud burst and flowering were made every two-three days in spring of 2008. The observations were made in upper part of the crown, whenever possible. For some trees, they were done in the middle part of the crown. The observations were made from the same direction (Preuhsler 1999). Based on the field observations, for bud burst, the trees were grouped in five classes: very early flushing, early flushing, normal, late

Table 1 Oak tree distribution on phenological classes for bud burst at Fundeanu

Morphological group	Number of individuals	Phenological class									
		Very early flushing		Early flushing		Intermediate		Late flushing		Very late flushing	
		No.	%	No.	%	No.	%	No.	%	No.	%
Q. pedunculiflora	222	7	3,2	17	7,6	165	74,3	26	11,7	7	3,2
Q. robur	28	2	7,1	2	7,1	19	67,9	5	17,9	-	-
Intermediates between *Q. robur* and *Q. pedunculiflora*	28	4	14,3	-	-	17	60,7	7	25	-	-
Q. petraea	10	-	-	2	20	8	80	-	-	-	-
Q. pubescens	12	-	-	1	8,3	6	50	4	33,4	1	8,3
TOTAL	300	13	4,3	22	7,3	215	71,7	42	14	8	2,7

Table 2 Mean length of the interval between bud burst and complete leaf development on phenological classes at Fundeanu

Cr. No.	Morphological group	Phenological class					General mean (days)
		Very early flushing	Early flushing	Intermediate	Late flushing	Very late flushing	
		Mean period (days)					
1	*Q. pedunculiflora*	29,4	25,3	18,4	16,7	14,9	19
2	*Quercus robur*	28,5	26	19,8	17,2	-	20,4
3	Intermediates between *Q. robur* and *Q. pedunculiflora*	28	-	18,1	16,8	-	19,2
4	*Q. petraea*	-	26,5	16,4	-	-	18,4
5	*Q. pubescens*	-	32	21	18,2	16	20,6

flushing and very late flushing, respectively. Leaf development was observed over four stages - Figure 2. For the flowering phase, only the maturation of the male flowers was recorded. Male flowers were considered mature when the catkin begins to release the pollen. A binocular was used for the observations of the flowers.

Results

Bud burst

The histograms of phenological categories for flushing indicate a unimodal distribution for *Q. pedunculiflora, Q. robur* and morphologically intermediate trees, respectively (Table 1). The distribution seems to show the same form even for *Q. pubescens* and *Q. petraea*, which are represented in the study plot by a small number of individuals. The large fraction of trees having intermediate values is typical for polygenic traits such as bud burst (White et al. 2007). Moreover, the phase of bud burst occurred simultaneously for *Q. robur* and *Q. pedunculiflora* as well as for the intermediate forms. *Q. petraea* and *Q. pubescens* are missing from the very early group, and *Q. petraea* is also not present among the late and very late flushing individuals, probably due to the small sample size.

The data presented in Table 2 indicate that the period between the bud burst and the complete development of leaves was very similar among different oak morphological groups. On average, the length of this phase was about 20 days (on average, between 18.4 days for sessile oak and 20.6 days for downy oak). Irrespective of taxonomic group, the period becomes shorter from the early flushing group towards the late flushing group. The dynamic of leaf unfolding is higher in late and very late flushing individuals as compared to early flushing individuals. This situation could be explained by temperature conditions during spring 2008. Based on our field observations it was a considerable temperature increase which coincided with the date of flushing for specimens from the late and very late group. Unfortunately we did not have access to the meteorological data for confirming this hypothesis. The flushing period consisted of more than 25 days for all individuals belonging to very early group. The longest period for an individual from the late or very late group was 19 days.

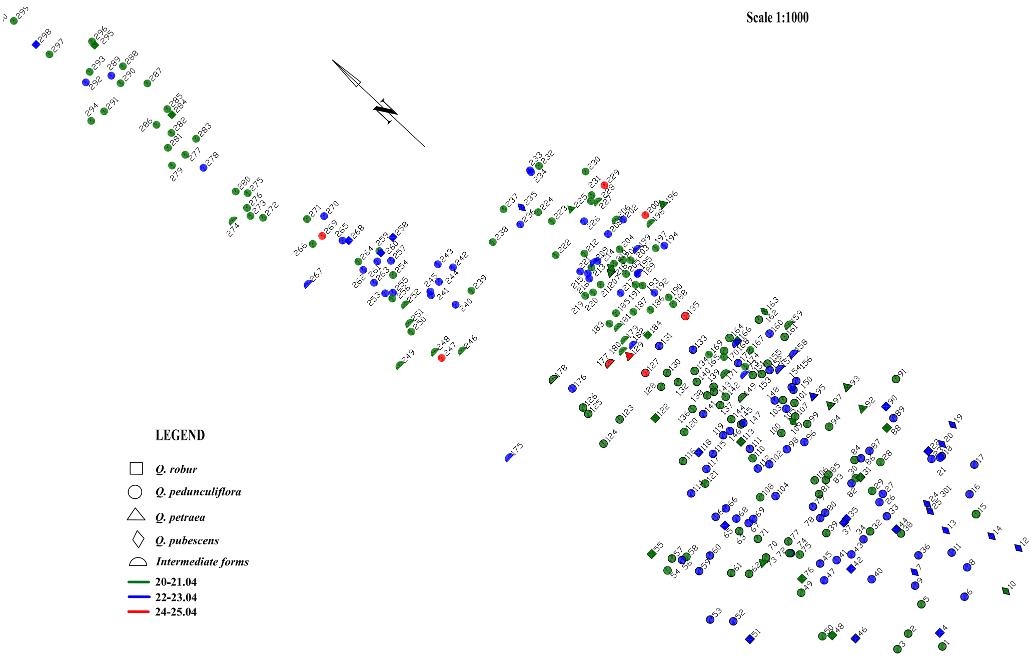

Figure 3 Maturation of male flowers within the study plot

Table 3 Maturation of male flowers at Fundeanu in year 2008

Cr. no.	Morphological group	Number of trees	Maturation of male flowers					
			20-21 April		22-23 April		24-25 April	
			No.	%	No.	%	No.	%
1	*Q. pedunculiflora*	222	123	55,4	92	41,4	7	3,2
2	*Q. robur*	28	12	42,9	16	57,1	-	-
3	Intermediates between *Q. robur* and *Q. pedunculiflora*	28	14	50	13	46,4	1	3,6
4	*Q. petraea*	10	8	80	1	10	1	10
5	*Q. pubescens*	12	4	33,3	8	66,7	-	-

Table 4 Distribution of individual trees on bud burst and flowering phenological classes

Cr. no.	Morphological group	Flowering period / No. of trees	Bud burst phenological classes		
			very early and early	intermediate	late and very late
1	*Q. pedunculiflora*	20-21 April / 123	21	98	4
		22-23 April / 92	3	63	26
		24-25 April / 7	-	4	3
2	*Q. robur*	20-21 April / 12	3	9	-
		22-23 April / 16	1	10	5
		24-25 April / -	-	-	-
3	Intermediates between *Q. robur* and *Q. pedunculiflora*	20-21 April / 14	3	10	1
		22-23 April / 13	1	6	6
		24-25 April / 1	-	1	-
4	*Q. petraea*	20-21 April / 8	2	6	-
		22-23 April / 1	-	1	-
		24-25 April / 1	-	1	-
5	*Q. pubescens*	20-21 April / 4	1	3	-
		22-23 April / 8	-	3	5
		24-25 April / -	-	-	-

The earliest flushing date recorded in 2008 at Fundeanu was 24 March while the latest date was 15 April. In both instances there were *Q. pedunculiflora* individuals. It results more than three weeks differences among oak trees growing under similar conditions.

Flowering phenology

The observations concerning flowering were confined to the maturation of male flowers. The point of pollen shed is easily recorded (Preuhsler 1999) which it is not the case of the receptiveness of female oak flowers. As in the case of leaf unfolding, for most of the oak trees, the flowering takes place simultaneously in all morphological groups present at Fundeanu (Table 3 and Figure 3). In 2008, the first date for the flowering was recorded on 20 April. The length of the male flowering period was 6 days in *Q. pedunculiflora* and intermediate forms, and only 4 days in *Q. robur* and the two other species: *Q. petraea* and *Q. pubescens*.

When analysing the phenological group for flushing and flowering in the same tree a strong correspondence was found (Table 4). Thus, in the group of individuals which flowered in the first two days there were the majority of very early and early flushing trees (85, 7%) and only 10% from the late or very late flushing group. Moreover, only a small portion of early flushing individuals (14,3%) but a high fraction of late flushing individuals are among those that flowered in the next two days (22-23 April). During the last days (24 and 25 April) only intermediate and late flushing individuals flowered.

Discussion and conclusions

The earliest date of bud burst for *Q. robur* reported in Romania (Tomescu et al. 1967) was one week later than in our study. This date was recorded in southern Romania at Ciolpani-Ilfov forest. Early flushing individuals, however, two-three weeks later relative to our data, were also mentioned in other two pedunculate oak populations (Noroieni - Satu Mare - located in north-west Romania, and Rădeşti - Argeş in south Romania). Phenological observations carried out in Bucharest (Rădulescu 1938), in 1938, indicated the date of 31 March for the first flushing *Q. robur* individual, which it is one week later as compared with our results. At the same location late-flushing oaks were recorded two weeks later (30 April). The observations made in the same region - Moldova - in 1949 (Bălănică & Tomescu 1953) showed that the earliest *Q. robur* individual was flushing nearly five weeks later (3 May) than at Fundeanu in 2008. However, no other observations were made exactly at the same location and, therefore, these comparisons should be treated with caution.

Concerning the beginning of the flowering phase, other phenological studies performed in Romania (Rădulescu 1938, Tomescu et al. 1967) indicated the same date (Bucharest, 1938) or a two weeks delay in the majority of the cases for the time period: 1956-1965. In 2008 at Fundeanu the flowering process was explosive, because the majority of trees flowered in a four-day interval (53, 7% in the first two days and 43, 3% in the next two days, respectively). This situation happened probably due to a rapid and considerable increase of temperature.

Our results showed a very good correspondence between the phenological groups for bud burst and flowering. An early-flushing oak tree is flowering earlier, in the majority of cases, or normal, but never at the end of the flowering period. The same aspect based on observations made between 1956 and 1965 was highlighted (Tomescu et al. 1967). This fact is very useful in the field because the data about the flushing class is giving us roughly information about the time of the flowering for the same individual. However, the observations of floral development remain the only way for getting very accurate data. At Fundeanu, the length of flushing (approximately 20 days) and flowering (6 days on average) differed considerably because of their specific dynamics and weather conditions. The longer period of leaf unfolding conducted to a better stratification of trees into phenological groups. The longer periods of male flowering, from 10.1 to 12.0, were observed in 1989, in a mixed stand of *Q. robur* and *Q. petraea* in north-western France (Bacilieri et al. 1994).

The overlap found between the flowering period for *Q. robur* and *Q. pedunculiflora* suggests the lack of a strong prezygotic reproductive barrier between the two taxa. The presence of morphologically intermediate forms could be considered as a result of past introgressive hybridisation events between pure *Q. robur* and pure *Q. pedunculiflora*. Moreover, *Q. petraea* and *Q. pubescens* shed their pollen in the same time with *Q. robur* and *Q. pedunculiflora*, respectively, which points towards the possibility of interspecific gene flow within the whole white oak complex at Fundeanu. No basic differences in floral phenology between the most important European oak species, *Q. robur* and *Q. petraea*, were found in other parts of their natural distribution (Tomescu et al. 1967, Bacilieri et al. 1994, Bacilieri et al. 1995, Kleinschmit & Kleinschmit 2000).

The spatial distribution of oak trees on phenological groups seems to be non-random, as it results from Figure 1. In many parts of the study plot, groups of early or late flushing trees can be recognized. These bio-groups are dominated by trees which belong to one of the phenological categories (for example A in Figure 1) and they might originate from seeds produced by the same mother tree. In this context, these clumps might represent family structures (e.g. Dounavi 2000). The flowering times are often strongly heritable. Moreover, the stand originates from natural regeneration and the seed dispersal in oaks is confined mostly to the surroundings of the maternal tree (Petit et al. 2004).

To the best of our knowledge this is the first phenological study on oaks at a fine scale in Romania. Our findings, from the first year of observation, showed overlapping flowering dates which follow a unimodal distribution in all pure species and in the group of morphologically intermediates identified within Fundeanu white oak complex. At least at this particular site, we conclude that floral phenology does not represent a reproductive barrier between closely related oak taxa. Further work is needed to examine the intervals and dynamics of flowering by recording data over at least one more year. Moreover, genotyping the trees with the help of highly variable molecular markers and performing a paternity analysis will help us to estimate the degree of interspecific gene flow between oaks at Fundeanu.

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