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SPATIAL AND TEMPORAL VARIABILITY OF FLOWERING IN THE PEDUNCULATE OAK (*Quercus robur* L.)

PROSTORNA I VREMENSKA VARIJABILNOST CVJETANJA HRASTA LUŽNJAKA (*Quercus robur* L.)

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Summary

Climate change, as well as biotic and abiotic stress environmental factors and the exploitation of oak forests have the greatest impact in reducing the pedunculate oak areas. These factors on one side reduce the pedunculate oak living area, while on the other side they create unfavorable conditions for its renewal. In the last decades, there has been an attempt to change this situation, primarily by planting appropriate provenances and more resistant varieties. Knowledge of the phenological variability is one of the essential elements useful in separation of genotypes better adapted to changing environmental conditions.

One of the phenological studies of the pedunculate oak implied the analysis of the pollination time as one of the intermediate phase of the flowering phenophase. Observations were carried out in two populations at two locations in the area of Belgrade (Serbia), "Ada Ciganlija" and "Bojčinska šuma", a total of 58 trees (29 trees per location) in three consecutive years (2004, 2005, 2006). According to the ANOVA results differences between locations and years were statistically significantly (all $p < 0.0001$). Time of pollination in the location of "Bojčinska šuma" was earlier compared to location "Ada Ciganlija" in all three years of observation. The obtained time difference gives rise to the assumption that there are different varieties of the pedunculate oak: the "early" and the "late" one. According to the phenological pattern of population, the majority of trees maintain the same trend from year to year, i.e. remain in the same phenological group (labeled as "early", "average" and "late"), or change it for one phenological level, suggesting the genetic influence on the expression of this trait. Since those populations are located in similar environmental conditions, obtained differences between them can be regarded as a consequence of intraspecific variability of the pedunculate oak and of the genetic structure of population.

KEY WORDS: phenological variability of flowering, *Quercus robur*, pollination.

INTRODUCTION
Uvod

The causal relationship between climate and vegetation is only one link in the chain of ecosystem functioning. In recent decades the climate changes is evident, which is largely the consequence of anthropogenic activities. Global warming causes many changes in functioning of ecosystems (Visser and Holleman, 2001). Expected reactions are the moving of rank of geographic distribution, migration, adap-

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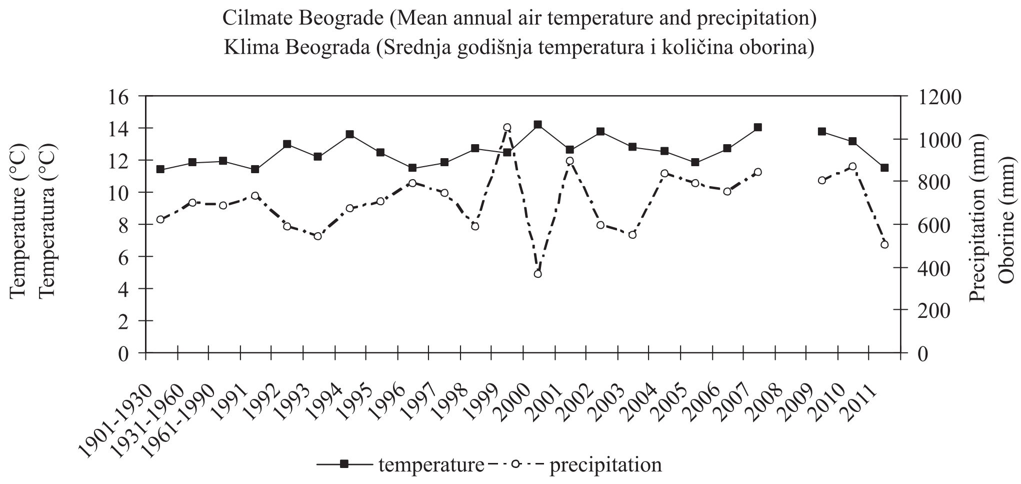


Figure 1. Data on mean annual temperature and precipitation in the area of Belgrade (Serbia) - data by Republic Hydro meteorological Service of Serbia.

Slika 1. Podaci o srednjoj dnevnoj temperaturi i količini oborina za područje Beograda (Srbija) - podaci su dobiveni iz Republičkog Hidrometerološkog Zavoda Srbije.

tation or extinction of certain species (Alberto et al., 2011). Therefore, maintaining high genetic variability is a valuable resource for the adaptation of species that takes place through the process of conservation of genes and the process of mating (Baliuckas and Pliura, 2003; Aitken et al., 2008).

Global warming and climate change was recorded in the area of Serbia as well (Figure 1). In the period 1971 - 2000. there has been registered an upward trend in the mean annual temperatures that caused an average shift of the beginning of the spring and summer by 2.5 days per decade (Popović et al., 2009). Changing the time of the beginning of seasons directly affects the phenophase of flowering, and therefore it is important to study this phenophase as a part of the reproductive biology of species (Gomez-Casero et al., 2007). The change of environmental factors also influence the variability of phenophase occurrence (start, end and duration), at a population level and an individual level as well. Individual variability in the beginning of the occurrence of certain phenophases, or their duration, is useful for the selection of plants for resistance to temperature extremes (Gološin et al., 2005), and to pathogen impact (Klaper et al., 2001), and it makes species more potential to adapt to new climate change and thus contribute to its survival. Uniformity of flowering time between the selected individuals is one of the conditions that guarantee the production of seeds of genetically better quality which should be taken into account in the establishment of seed plantations (Franjić et al., 2011). Beside selection, practical importance of the phenological research is to make prediction models of expected changes for the needs of urban ecology, allergology and agrometeorology (Richardson et al., 2006; Šestan, 2012).

The beginning and duration of phenophases largely depend on the temperature, and then on the photoperiod, precipitation, radiations, CO_2 increase, etc. (Kremer, 2002; Hájková et al., 2010; Hanousková, 2010). According to the model predictions of climate change if at the end of the 21st century there was a duplication of CO_2 in the air, the oak pollen season would begin a month earlier (Garcia-Mozo et al., 2006). Phenological rhythm also depends on the local changes in the weeks right before the activation of a phenophase (Badeck et al., 2004; Bednorz and Urbaniak, 2004).

The aim of this research was to define the pattern of all three flowering phenophases among and within analyzed locations i.e. populations, as well as the monitoring of changes during three successive years. In this context, there has been searched for the answers to the following questions: Is there a difference in pollination as a part of flowering phenophase among locations for each of the years analyzed; Is there a difference among years for each of the locations analyzed; What is the phenological pattern of the analyzed populations compared to the percentage presence of trees with different flowering time; And whether the trees remain in the same group ("early", "average", "late") each year or they change it?

MATERIAL AND METHODS
Materijal i metode

Species – Vrsta

Pedunculate oak (*Quercus robur* L.) belongs to a very numerous and important genus *Quercus*, family *Fagaceae* (Nixon, 1993). It is one of the most respected forest tree species, whose areas have been significantly reduced in the last century (Thomas et al., 2003; Balboa-Murias et al., 2006; Broshtilov, 2006; Helama et al., 2009; Tikvić et al., 2011). It occurs in the temperate zone of the northern hemisphere, in almost all of Europe. In Serbia, the largest areas under pedunculate oak forests are in the valleys of major rivers: Sava, Danube and Morava. It is characterized with very pronounced morphoanatomical, physiological and phenological variability (Batos, 2010). It has great economic importance in forestry, ecology, medicine, pharmacy and so on (Barbehenn et al., 2006; Rakić et al., 2007).

Pedunculate oak is an anemophilous and monoecius species. The flowers on the same tree are spatially separated. Male flowers (catkins) appear at the beginning of leafing in April or May, and are located in the grounds of this year's or at the top of last year's shoot. Female flowers occur somewhat later than male ones (7.5 days, according to Bacilieri et al., 1994), and are grouped at the top of this year's shoot. In pedunculate oak the protandry is usual (Gomez-Casero et al., 2007), but there also may be found the protogyny (Franjić et al., 2011). Elongation phase of tassels occurs simultaneously with the differentiation of female flowers, while the receptivity of female flowers coincides with maturation time and dispersion of pollen. In the annual cycle of the pedunculate oak the flowering is usually in the spring, but there has been also registered a rare occurrence of summer flowering (Bobinac et al., 2000). On summer shoots pollen is very rarely formed, and it is significantly smaller in size than pollen from regular vernal flowering (Batos et al., 2012; Bobinac et al., 2012). The assumption is that the summer flowering can not be explained only by climate change, but as a primitive ancestral characteristic of *Fagaceae* family, where the oaks belong (Borzan, 2000).

Locations – Lokaliteti

The study was conducted in two populations of the pedunculate oak in two locations in the area of Belgrade (Serbia): "Ada Ciganlija" (AC) and "Bojčinska šuma" (BS). Populations are found in habitats that are suitable for the pedunculate oak, are of mixed structures, uneven-aged, coppice and seed origin, aged 60–120 years. Belgrade area is characterized with moderate-continental climate. The annual courses of temperature and precipitation are harmonized so that the months with the highest temperatures get the highest amount of rainfall which corresponds to the vegetation period.

Location "Ada Ciganlija" belongs to the city forest and is situated on the peninsula of the same name at the Sava riverside, in the habitat of ass. *Fraxino angustifoliae – Quercetum roboris* Jov. and Tom. 1979, on the soil type *fluvisol calcaric*. The construction of dams and other melioration works changed the habitat conditions (groundwater level), which caused a negative impact on the vegetation of the peninsula, especially on pedunculate oak. According to Lang's climate classification features of the area (Unkašević et al., 2002), calculated value of the rain factor for this area for the current period (the last decade) is L = 55, which defines this area as an area of steppes and savannas, and as a humid climate.

Location "Bojčinska šuma" is a suburban forest park which is a relic of the old swamp-lowland pedunculate oak forests that have been very widespread in this part of the river Sava coastal basin. It is the habitat of ass. *Carpino – Quercetum roboris* Rausch 1969. with soil type *planosol dystric*. The value of the rain factor in this area is L = 59, which defines it as a boundary between the steppe and savanna areas and low forests, and as a humid climate.

Climate data were obtained from the yearbook Report of the Republic Hydrometeorological Service of Serbia for the area of Belgrade.

Phenophase – Fenofaza

Monitoring of the flowering phenophase was performed on a total of 58 (29 per site) trees of good growth and health, nearly of the same age. Observations were conducted once a week during spring (April, May) in three consecutive years (2004, 2005, 2006.). The first week of observation has been counted from the week in which has been noticed the earliest beginning of the flowering in the analyzed locations. Compared to the beginning of the phenophase there has been made a conditional grouping of trees on three phenological groups (Bacilieri *et a*l. 1994): "early" trees that had a beginning of the phenophase in the first week of observation (the first week of April), "average" trees that had a beginning of the phenophase in the second week of observation (the second week of April), and "late" trees that had a beginning of the phenophase in the third week of observation or later (the third week of April or later).

Statistical analyses – Statistička obrada

For statistical analysis we used the number of days that represented the sum of days from the beginning of a calendar year until the date of registration of the observed process in the year of observation (Hemery et al., 2005). There have been used adequate procedures of the software package SAS 9.3 (SAS Institute, 2002) and the software package STATISTICA (Version 10). Beside the basic parameters of descriptive statistics to define the difference between the mean values of the flowering phenophase of populations within each of the observed years, we applied the Scheffe's test. An estimation of the statistical significance of different levels of sources of phenotypic variation has been conducted using the analysis of variance (ANOVA). The locations have been treated as fixed factors (for clearly defined), while the tree has been treated as a random factor (because the election of trees within the location has been random).

RESULTS
Rezultati

Pedunculate oak begins to flower earliest on 7th April and no later than 2nd May in the analyzed locations, taking into acco-

Table 1. Basic statistical parameters of time of flowering of the analyzed sites
Tablica 1. Osnovni statistički parametri vremena cvatnje na analiziranim nalazištima

Locality Lokalitet	Time of flowering Vrijeme cvatnje					
	Ada Ciganlija			Bojčinska šuma		
Year Godina	2004	2005	2006	2004	2005	2006
min-max minimum-maximum	98–123	101–118	101–111	98–105	98–111	105–111
var. rank varijacijski rang	25	17	10	7	13	6
x±Sx Srednja vrijednost i greška	113.3±1.21	112.6±1.10	107.4±0.62	99.0±0.45	104.4±0.68	108.1±0.54
S±Ss Standardna devijacija i greška	6.54±0.86	5.95±0.78	3.34±0.43	2.45±0.32	3.67±0.48	2.94±0.38
V±Sv Koeficijent varijacije i greška	5.77±0.75	5.29±0.69	3.11±0.40	2.48±0.32	3.52±0.46	2.72±0.35

unt all trees and years of observation. The average value of three years of monitoring the difference between the date of the latest and the earliest flowering in the location of "Ada Ciganlija" was 17.3 days, and in the location of "Bojčinska šuma" 8.7 days. This means that the flowering time lasted averagely longer in the location of "Ada Ciganlija" for about a week than in the location of "Bojčinska šuma", which makes the population in the location of "Ada Ciganlija" less homogeneous (Table 1). Observing each year particularly, the difference between the dates of the earliest and latest flowering in the location of "Ada Ciganlija" is 25 (2004), 17 (2005) and 11 days (2006) and in the location of "Bojčinska šuma" 7 (2004), 14 (2005) and 6 days (2006) (Figures 2, 3).

Among the locations there has been confirmed a significant difference in flowering time (mean value of number of days until pollination start for AC is 111.12[a] compared to 103.83[b] for BS). On average for three years of observation, this difference counted 7.3 days.

According to the Scheffe's test results differences between years of observation (2004, 2005 and 2006) have been confirmed for both locations AC and BS (Table 2, small caps a,

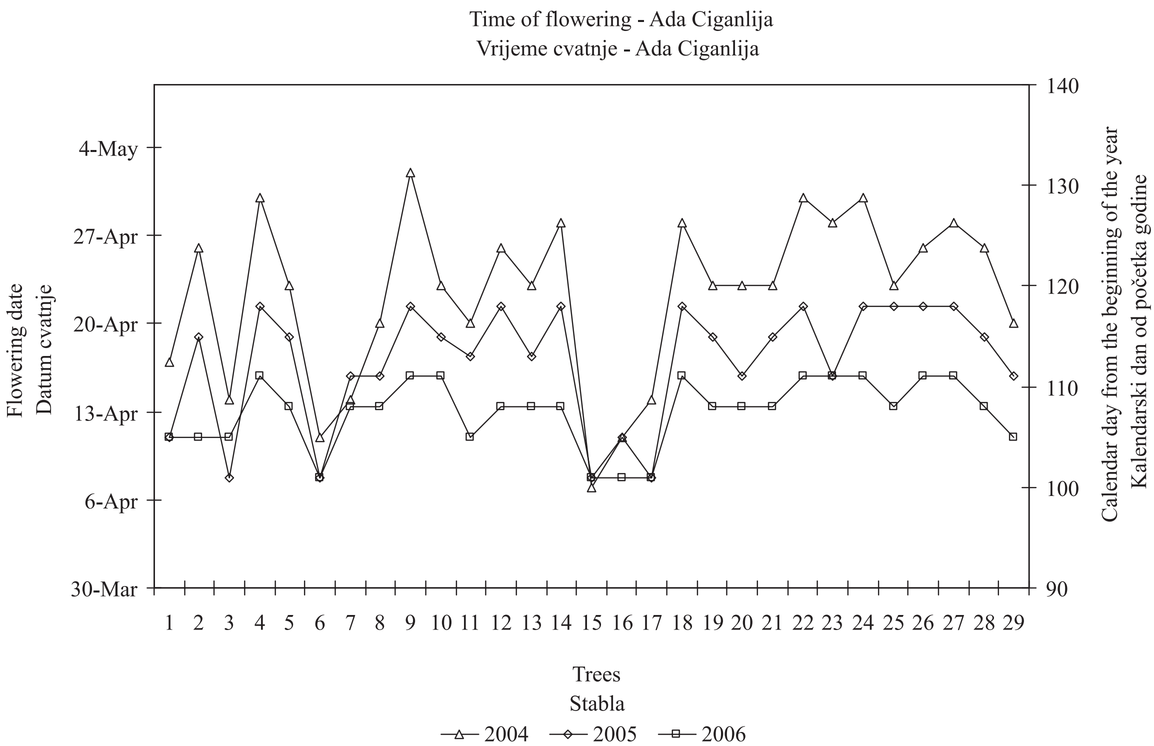


Figure 2. Flowering time of analyzed oak trees over the years of observation on the "Ada Ciganlija."
Slika 2. Vrijeme cvatnje analiziranih stabala hrasta lužnjaka tijekom godina promatranja na "Adi Ciganliji".

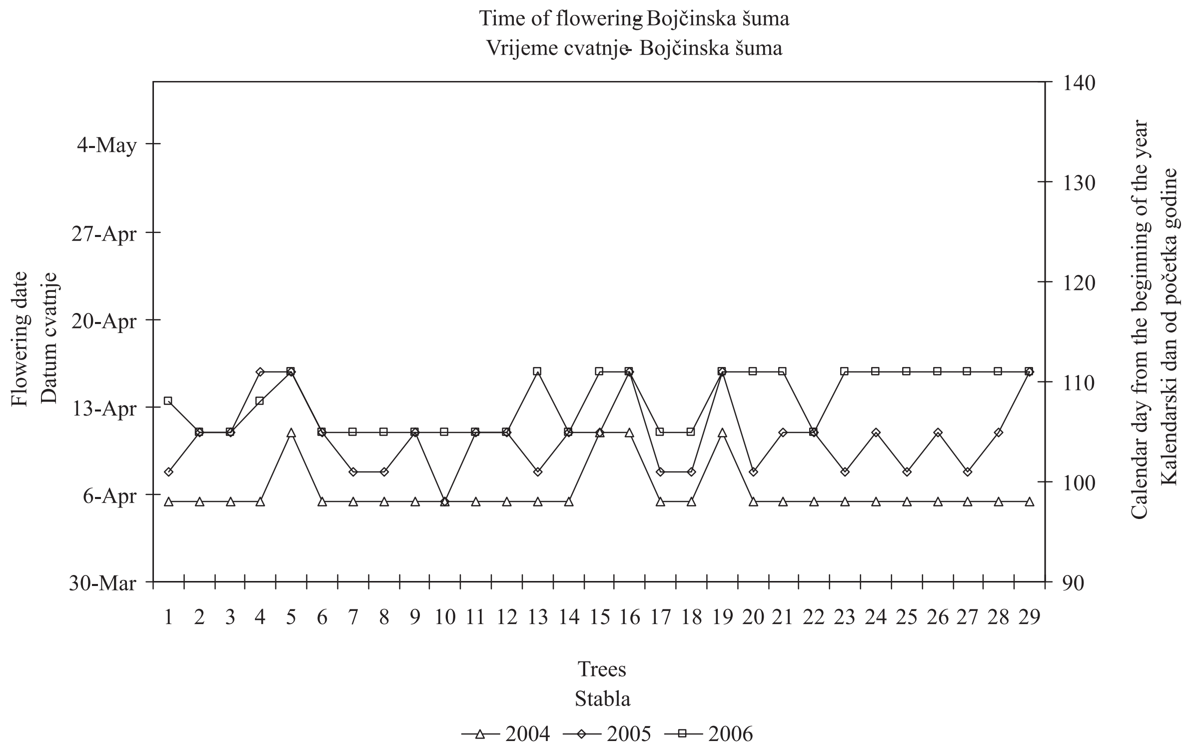


Figure 3. Flowering time of analyzed oak trees over the years of observation on the "Bojčinska šuma."
Slika 3. Vrijeme cvatnje analiziranih stabala hrasta lužnjaka tijekom godina promatranja u "Bojčinskoj šumi"

b, c in first and second rows). The flowering has begun significantly earlier in the 2006 in order to 2005 and 2004 for AC locations (107.4 *vs.* 112.6 and 113.3; respectively), while for location BS the flowering has begun significantly earlier in the 2004 in compared with 2005 and 2006 (99.0 *vs.* 104.4 and 108.1; respectively). The average values for all three years were statistically significant different between localities (Table 2, the small caps a, b in last column), the flowering has begun significantly earlier in the BS *vs.* AC (103.8 *vs.* 111.1; respectively). According to the ANOVA results differences between locations, years, trees were statistically significantly (all p <0.0001) (Table 3).

According to applied phenological differentiation in the location of "Ada Ciganlija", the largest number of trees has been in the group of "late" trees that have been flowering in the third week of April and later (69.0 % in 2004; 79.3 % in 2005; 31.0 % in 2006). In the location of "Bojčinska šuma" in the first year of observation (2004) the maximum number of trees belonged to the group of "early" trees (86.2%), while in the second and the third year there have been mostly "average" trees (44.8 % in 2005 and 51.7 % in 2006) (Figure 4a).

Analyzing phenological pattern of the population it can be said that in the location of "Ada Ciganlija" for three years of observation the largest number of trees either changed the group for one level (58.6 %), or kept the same level (41.4%), whereas there were no trees changing the group

Table 2. The differences between the analyzed sites and years of observation during the flowering. Means followed by the same letters within a variable are not significantly different ($p \leq 0.05$) according the results of Scheffe's test.
Tablica 2. Razlike između analiziranih nalazišta i godina promatranja tijekom cvatnje. Srednje vrijednosti praćene istim slovom ne razlikuju se značajno za razinu značajnosti od $p \leq 0.05$, prema rezultatima Scheff-ovog testa.

Locality Lokalitet	Years observation Godina promatranja			
	2004	2005	2006	average
Ada Ciganlija	113.3[a]	112.6[a]	107.4[b]	111.1[b]
Bojčinska šuma	99.0[a]	104.4[b]	108.1[c]	103.8[a]

a b c – homogeneous groups (homogene grupe)

Table 3. The results of three way analysis of variance (ANOVA) for flowering time of oak with locality, years, trees, and their interactions.
Tablica 3. Rezultati trofaktorijalne analize varijance (ANOVA) vremena cvatnje hrasta lužnjaka za lokalitete, godine, stabla i njihovih interakcija.

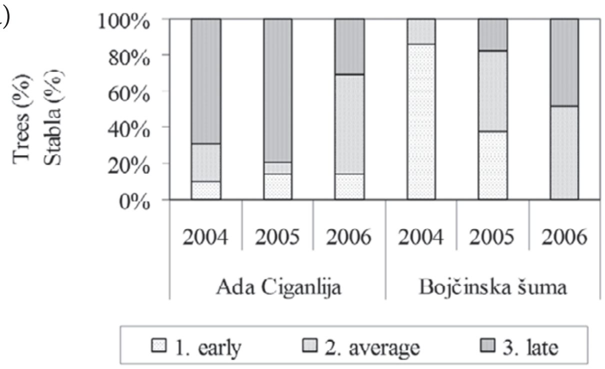
Source Izvor	DF	MS	F - Value	Pr > F
Locality Lokalitet	1	2310.09	153.93	<.0001
Year (locality) Godina	4	454.54	30.29	<.0001
Trees Stabla	28	42.72	2.85	<.0001
Error Greška	140	15.01		

for two levels (0.0 %). In the location of "Bojčinska šuma" also most of the trees changed the group for one level (62.1 %), whereas significantly fewer trees changed the group for two levels (37.9%), and there were no trees without the group change (0.0 %). According to total results, in the location of "Ada Ciganlija" all trees (100.0 %) kept the same trend from year to year, or they changed in only for one level. In the location of "Bojčinska šuma" all of trees (100.0 %) changed its group for one or two levels (Figure 4b).

Among those trees that, over the years of observation, have not changed phenological group in the "Ada Ciganlija" location (41.4 %), there have been mostly late flowering trees (70.0 %), early flowering have been 20.0 %, and 10.0 % of the intermediate ones. In the "Bojčinska šuma" location there have been no trees that, over the years of observation, showed stability and remained in the same phenological group.

According to the Annual Report of Republic Hydrometeorological Service of Serbia there has evidently been a slight rise in temperature in Belgrade area during the last 110 years. Also, the abrupt and sudden changes typical for the amount of precipitation have been noticeable (Table 1). Analyzed period of phenological observation in this paper (2004, 2005, and 2006) fits the average of it (110 years), and there have not been any sudden changes to reflect on the phenological cycle.

a)



b)

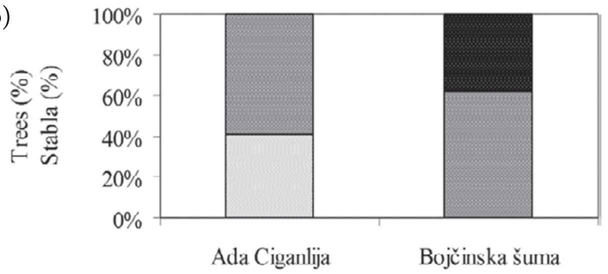


Figure 4a,b. Phenological pattern of oak populations analyzed in relation to the flowering (a: early, average, late; b: same level, changing groupe for 1 level, changing group for 2 level)

Slika 4a,b. Fenološki obrazac cvatnje hrasta lužnjaka u analiziranim populacijama (a: rana, prosjek, kasna; b: ista razina, promjena grupe za 1 razinu, promjena grupe za 2 razine)

DISCUSSION
Rasprava

Flowering time mostly depends on the species and areas in which it develops, and also on the climate, mostly temperature and precipitation (Bednorz and Urbaniak, 2004; Figueiredo Goulart et al., 2005; Gomez-Casero et al., 2007; Franjić et al., 2011). The impact of latitude on an oak flowering is best illustrated by the following examples. Flowering of several oak species in the area of Spain (37° 26' N) takes place from 26th February till 17th April (*Quercus sp*., Garcia-Mozo et al., 2000), or in the second half of March (42° 15' N), and in the area of Italy (43° 06' N) during the last two weeks of April (*Quercus robur, Q. suber, Q. ilex, Q. pyrenaica, Q. pubescens*, Rodríguez-Rajo et al., 2003). In northern areas, in Slovakia (48° 38' N) oak flowering occurs about 10th May (*Quercus robur*, Škvareninová et al., 2008), and even more in the north, in the area of Germany (53° 39' N) it happens even later, from 8th till 20th May (*Quercus robur*, Schueler et al., 2005). According to results presented here, oak flowering (*Quercus robur*) in Serbia (44° 49' N) occurs between 7th April and 2nd May, which compared to the literature data corresponds to the coordinates of the study area.

There is not much data about oak flowering in natural populations in the area of Serbia. According to Erdeši (1971), in south-western Srem oak flowering occurs between 8th April and 23rd May, with a difference among individual trees of 2–6 weeks on which basis has been allocated 4 varieties: var. *robustissima*: 8.04.–30.04.; var. *typica*: 11.04.–5.05.; var. *tardiflora*: 25.04.–9.05. and var. *tardissima* 10.05.–23.05. Compared to the above mentioned literature data and results presented here, analyzed pedunculate oak in the Belgrade area would belong to the earliest flowering forms of var. *robustissima*. However, it should be also taken into account that recorded slight rise in temperature since the beginning of the last century, certainly had an impact on a certain shift and earlier beginning of the pedunculate oak vegetation. Climate data (Popovic et al., 2009) and the value of the rain factor for the analyzed area in the last decade compared to data for the period of half a century ago (Radulović, 1982) point to climate change in the direction of increased aridity. Bearing in mind above mentioned constatation about the trend of moving vegetation forward, comparing with early and late phenological forms should be taken with some caution. From this point of view it would have been very useful and interesting to make a review of the pedunculate oak phenology in the same populations in which Erdeši (1971) conducted observations 40 years ago.

Analyzed pedunculate oak expressed an individual variability of flowering. A significant difference between trees with the earliest and the latest flowering in the location of "Ada Ciganlija" confirms this statement, as well as that it was more pronounced in this location. In the location "Ada Ciganlija" climate conditions are more favorable (it is warmer and with more precipitation), which could initiate earlier flowering in this locality, although it started earlier in the location of "Bojčinska šuma." This provides a basis for the assumtion that the pedunculate oak in the location of "Bojčinska šuma" belongs to a "early" oak form. Based on the analysis of the phenological pattern of the analyzed populations, according to which during the years of observation most trees did not change the group or changed only for one level, it could be assumed that the genetic influence on the expression of this trait was very evident, which would represent objective of future research. Among the trees that, over the years of observation, have not changed its phenological group in the "Ada Ciganlija" location, have mostly been late flowering, while in the "Bojčinska šuma" location have not been any trees that did not change its group.

Phenological asynchronization among individuals affects the flexibility and throuh adaptability helps its survival (Figueiredo Goulart et al., 2005). Bacilieri et al. (1994) emphasize that there is great individual variability in the initiation and duration of the pedunculate oak flowering. In this study they report that 49% of the trees remain in the same phenological group as the last year, 45% shift into the next group and only 6% of the trees change the group for two levels. Among those that did not change the group 26% belonged to the early flowering group, 26% to the late flowering, and the rest (48%) to an intermediate one. Since about 50% of the trees shift from one phenological group to another, quoted authors conclude that the ecological impact is also very strong, especially on the size and stability of populations, and that genetic differentiation among phenological groups is not possible. According to Matziris (1994), the beginning of flowering is under high genetic control, while the duration of flowering is more influenced by environmental factors. As mentioned author further states, it justifies an individual selection of the phenological characteristics, since they are synchronized with the seed production. The stronger effect of the flowering duration by enivornmetal factors is pointed out by Gomez-Casero et al. (2007) in a phenological analysis of three oak species (*Quercus ilex* L. ssp. *ballota* (Desf.) Samp., *Q. coccifera* L., *Q. suber* L.). The analysing the *Q. suber* L. in the region of Spain Elena-Rossello et al. (1993) concluded that the intrapopulation variability was the consequence of different ratio of the presence of male and female flowers, on which basis can be distinguished: –trees mostly with male flowers, –trees mostly with female flowers, –trees with both male and female flowers, and –trees without flowers but with a abundant biomass. In the study results of the pedunculate oak flowering in the area of Belgrade, a wealth of flowering was one of the criteria for the selection of trees for the observation of flowering, so that those results can be considered reliable for the assessment of genetic and environmental impact and analyzed variability.

CONCLUSIONS
Zaključci

During three years of observation, flowering phenopase of the pedunculate oak in the analyzed locations in the area of Belgrade takes place from 7th April to 2nd May, indicating very pronounced individual variability of the species. Analyzed locations have similar ecological conditions, so that the differences between them are the result of the population phenological pattern, which gives rise to the assumption of the presence of "early" and "late" varieties of the pedunculate oak. Population in the location of "Bojčinska šuma" develops in somewhat more humid conditions as a result of more humid climate and more hydromorphic soil type, although they have no significant effect on the analyzed traits. The results indicate the existence of individuals who maintain the same level in the beginning of flowering phenophase each year, which means its stability regardless the changes in climatic factors between years. As such, those results could be applied in modeling the response of the plant organisms to the micro and macro changes in the ecosystem, as well as in an individual selection for the establishment of plantations for various purposes (Valladares et al., 2006 and references therein).

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Zahvala

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Sažetak:

U radu je analizirana individualna i populacijska varijabilnost polinacije kao međufaze fenofaze cvjetanja lužnjaka. Istraživanja su obavljena na 58 stabala u dvije populacije lužnjaka na dva lokaliteta na području Beograda (Srbija) tijekom tri uzastopne godine (2004, 2005, 2006). Populacije se nalaze u oblasti umjereno-kontinentalne klime, na staništima koja odgovaraju lužnjaku, mješovite su strukture, raznodobne, vegetativnog i sjemenog porjekla, starosti 60–120 g. Promatranje je vršeno jedanput tjedno u travnju/svibnju. Lužnjak na analiziranim lokalitetima, uzimajući u obzir sva stabla i godine promatranja, počinje cvjetati najranije 7. travnja a najkasnije 2. svibnja. Cvjetanje na lokalitetu "Bojčinska šuma" događa se oko tjedan dana ranije u odnosu na lokalitet "Ada Ciganlija". Statističkom obradom potvrđen je značajan učinak lokaliteta, godine i individue. Prema fenološkom obrascu populacija i podeli u odnosu na vrijeme cvjetanja, većina stabala na lokalitetu "Bojčinska šuma" pripada fenološkoj grupi "rani" lužnjak, a na lokalitetu "Ada Ciganlija" grupi "kasni" lužnjak. Tijekom godina promatranja, najveći broj stabala ostajao je u istoj fenološkoj grupi ili se mijenjao samo za jednu razinu. S obzirom da se populacije nalaze u sličnim sredinama i stanišnim uvjetima, dobivene razlike mogu se smatrati posljedicom unutarvrsne varijabilnosti lužnjaka i genetičke strukture populacije. Genetička stabilnost fenofaze cvjetanja korisna je u odabiru "ranih" i "kasnih" formi lužnjaka u procesu individualne selekcije pod utjecajem promjene klimatskih čimbenika.