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GRADUAL LOSS OF GENETIC DIVERSITY OF *Ambrosia artemisiifolia* L. POPULATIONS IN THE INVADED RANGE OF CENTRAL SERBIA

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As an invasive allergenic weed, *Ambrosia artemisiifolia* L. causes serious public health and economic problems in invaded ranges of Europe. Over the last two decades, while expanding toward southern parts of Serbia, this common ragweed has become a very troublesome plant species in the whole country. Considering the importance of genetic studies in understanding of invasive species, our main objectives in this study were to analyze the genetic diversity and genetic structure of *Ambrosia artemisiifolia* populations from Central Serbia, a relatively recently invaded region. Comparing values of genetic measures obtained by microsatellite analyses, a number of differences were detected in genetic diversity between sampled populations. Allelic richness-r (ranged from 5.42 to 7.80), the mean number of alleles per locus-N_A (5.8-8.4) and the mean number of rare alleles per locus-N_R (2.8-5.8) have quite similar ranges across populations. We observed greater genetic variability in populations from the northern part of investigated area than in southern populations. Based on pairwise Fst values, AMOVA results and PCo Analysis, moderate differentiation among population was detected, while the STRUCTURE analysis clearly separated SR-Kru and SR-Les. Data obtained for analyses of differentiation and gradual losses of genetic diversity of sampled populations provides useful information about invasion dynamics of common ragweed in recently invaded region.

Key words: *Ambrosia artemisiifolia*, Central Serbia, genetic diversity and structure, microsatellites

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INTRODUCTION

Common ragweed (*Ambrosia artemisiifolia* L.), an allergenic invasive weed introduced from North America, is spreading throughout all continents. The species produces highly allergenic pollen in vast amounts causing serious medical problems for humans (BOHREN, 2008), and is also to blame for damage to economy, particularly in agriculture. Unlike any other plant, *Ambrosia artemisiifolia* has raised the awareness of invasive plants in Europe (GERBER *et al*., 2011). First records of this species in Serbia date back to the 1950's in Northern Serbia. In this part of the country, *Ambrosia artemisiifolia* became abundant, reaching high population densities (BOŽA *et al*., 2006). Today however, as it spreads toward southern parts of Serbia, common ragweed represents an increasing problem in the whole country (VRBNIČANIN and JANJIĆ, 2011).

Analyses of the genetic variability of invaded populations provide insight into the history of introduction and establishment phases of invasion (CIOSI *et al*., 2008). Also, for invasive species, the factors which influence population genetic structure are often related to its history of invasion (WATTS *et al*., 2010). Over time, invaded populations can reach a genetic equilibrium where the loss of alleles as a result of drift is balanced by the introduction of new alleles through gene exchange (ELLSTRAND and SCHIERENBECK, 2000). In contrast, in recently established populations, genetic structure is often influenced by evolution events, such as bottleneck or founder effects, rather than gene flow (BOHONAK, 1999). Levels of genetic diversity and genetic structure for newer invasive populations are affected by the recent expansion of the species, the number of founders and by anthropogenic factors, i.e. passive dispersal (DLUGOSCH and PARKER, 2008; WATTS *et al.,* 2010).

During the course of human-mediated introductions, all non-indigenous invasive species have experienced population founding events. Presence of this evolution force predicts that invasive species undergo reduction in genetic variability (DLUGOSCH and PARKER, 2008). However, a common appearance in invasions seem to be multiple introductions (KOLBE *et al.,* 2004; BOSSDORF *et al*. 2005; LAVERGNE and MOLOFSKY, 2007; KANG *et al.,* 2007; HAUFBAUER and SFORZA, 2008; PRENTIS *et al*., 2009), which can rescue invaders from loss in diversity, bringing together unusually large amount of variability and novel genetic combinations (DLUGOSCH and PARKER, 2008). Analyzing genetic diversity and genetic structure of *Ambrosia artemisiifolia* populations, various groups of authors confirmed the existence of both of these patterns. GENTON *et al.* (2005a) revealed high population genetic diversity as a result of multiple sources that introduced common ragweed in France. They also observed reduced values of genetic diversity measures (e.g. allelic richness and variance of allele size) in populations distant from the putative original area of introduction. Results obtained by GLADIEUX *et al*. (2011), GAUDEUL *et al*. (2011) and LI *et al.* (2012) are consistent with a scenario of multiple introductions of *Ambrosia artemissifolia* in different parts of Europe, as well as in China. In the reported studies (GLADIEUX *et al.*, 2011; GAUDEUL *et al*., 2011) showing separate introduction events in East and West Europe, Serbia was represented with only two populations. The origin of common ragweed in our region remained unclear, since these populations were classified under two different groups (Eastern and Central European).

Considering the abundance and negative impacts of this allergenic-weed species in Serbia, a number of studies on distribution and density of ragweed populations and pollen in the country have been conducted (BOŽA *et al*., 2002; BOŽA *et al*., 2006; ŠIKOPARIJA *et al.,* 2006; ŠIKOPARIJA *et al*., 2009; KONSTANTINOVIĆ *et al.,* 2011; ANAČKOV *et al*., 2012). Considering the importance of genetic studies in improving our understanding of the processes underlying the

success of invasive species, preliminary research of genetic diversity of *Ambrosia artemisiifolia* populations from the territory of Serbia has been conducted (KOČIŠ TUBIĆ *et al.,* 2011, 2012). However, a more detailed genetic characterization of common ragweed across Serbia has not yet been conducted. For these reasons, our main objectives in the present study were to analyze genetic diversity and genetic structure of *Ambrosia artemisiifolia* populations from the relatively recently invaded region of Central Serbia. We used neutral nuclear microsatellite markers to establish relationships among common ragweed populations and to gain insights into the population dynamics of *Ambrosia artemisiifolia* in the considered area.

MATERIALS AND METHODS

Population sampling and DNA extraction

We collected a total of 134 samples of *Ambrosia atremisiifolia* from 6 populations from the territory of Central Serbia (Figure 1). Approximately 22 adult individuals of the species were randomly chosen (>1m apart) from each populations. The data of the populations are presented in Table 1.

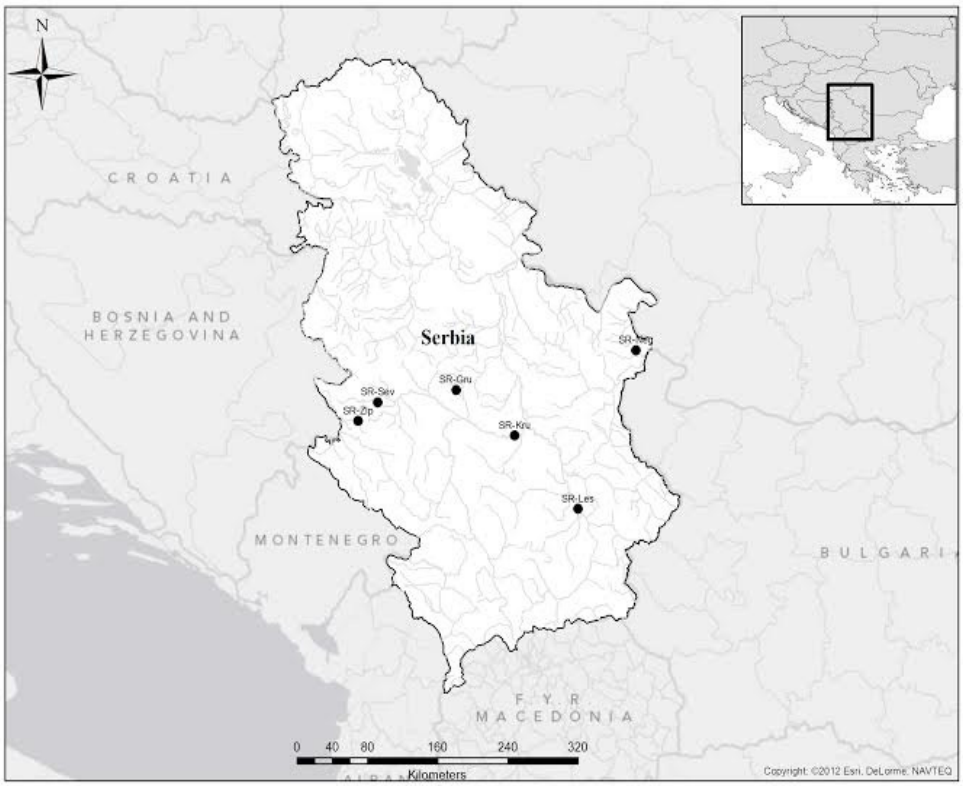

Figure 1. Map of sampled populations of *Ambrosia artemisiifolia*

Table 1. Sampled populations of Ambrosia artemisiifolia in present study

Location	Pop. code	Habitat	N	Latitude	Longitude	Date
Gruza	SR-Gru	roadside	22	43°55□31.35□□N	20°41□41.06□□E	June 2012
Sevojno	SR-Sev	Along the railway	22	43°50□24.88□□N	19°53□23.18□□E	August 2010
Negotin	SR-Neg	roadside	22	44°12□02.26□□N	22°32□00.34□□E	June 2012
Zlatiborski put	SR-Zlp	roadside	22	43°42□34.81□□N	19°41□17.49□□E	August 2010
Krusevac	SR-Kru	roadside	22	43°36□23.68□□N	21°17□33.32□□E	June 2010
Leskovac	SR-Les	roadside	24	43°05□32.54□□N	21°56□17.50□□E	June 2012

pop. code-population code, N-number of sampled individuals, latitudinal and longitudinal coordinates, date of collection

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Complete genomic DNA was extracted from fresh leaf material using a modified CTAB protocol (PADMALATHA and PRASAD, 2006) and DNA concentration was quantified applying a Bio-Spec spectrophotometer (Shimadzu) of each samples.

Microsatellite analysis

Five microsatellite loci: *Amb12*, *Amb16, Amb30, Amb82* (GENTON *et al.* 2005b) and *Ambart04* (GenBank accession no. FJ595149, Molecular Ecology Resources Primer Development Consortium 2009) were amplified. PCR was performed using Applied Biosystems Verity thermal cycler in a 20 µl final reaction volume containing 0.2 µl 5U Dream *Taq* DNA polymerase (Fermentas) and 2 µl 10x*Taq* buffer (including 20mM $MgCl_2$), 2 µl 2mM dNTPs, 1 µl 0.1µM each primer, 11.8 µl H_2O and 2 µl (caa. 50 ng) genomic DNA. Amplification cycles for *Amb12*, *Amb16, Amb30, Amb82* microsatellites included an initial denaturing of 94˚C for 4 min, 35 cycles of 94˚C for 30 s, 30 s at the primer-specific annealing temperature, 72˚C for 45 s and the final extension step of 72˚C for 5 min. The PCR thermal profile for *Ambart04* was: 95˚C for 15 min, followed by 30 cycles of 95˚C for 30 s, 54˚C for 45 s, 72˚C for 45 s, with a final extension at 72˚C for 5 min. Amplified products were genotyped using 6% denaturing PAA electrophoresis with modified silver staining (SAMBROOK and RUSSEL, 2001). Individuals were declared null (nonamplifying at a locus) and treated as missing data after two amplification failures.

Data analysis

We tested Hardy-Weinberg (HW) equilibrium and linkage disequilibrium to meet the assumption of further analysis of genetic diversity and structure using GENEPOP version 4.0.1 (ROUSSET, 2008). P values were corrected for significance levels using sequential Bonferroni method. GenAlEx version 6.5 (PEAKALL and SMOUSE, 2012) was used to estimate the mean number of alleles per locus (N_A), number of private alleles (N_P), observed heterozygosity (Ho) and unbiased expected heterozygosity (He). Allelic richness (r), mean number of rare alleles per locus (N_R, frequency < 0.1) and inbreeding coefficient (Fis) were calculated in FSTAT version 2.9.3 (GOUDET, 2001).

Using ARLEQUIN version 3.0 (EXOFFIER *et al.,* 2005) we obtained Fst indices with their significance along 10,000 permutations. The significances of fixation index were corrected for multiple population comparison by Bonferroni adjustments. Genetic differentiation was

defined as low for Fst<0.05, moderate for 0.05<Fst<0.15, high for 0.15<Fst<0.25, and very high for Fst>0.25. FSTAT version 2.9.3 (GOUDET, 2001) was applied to calculate global estimator of genetic differentiation Fst.

To assess the distribution of genetic variation between and within populations, we performed analysis of molecular variance AMOVA implemented in ARLEQUIN version 3.0 (EXOFFIER *et al.,* 2005). Genetic distances among populations were measured with GENETIX version 4.05 (BELKHIR *et al*., 1996 - 2004) using the chord distance of CAVALLI-SFORZA and EDVARDS (1967) and the resulting distance matrix was subjected to a Principal Coordinate Analysis -PCoA in GenAlEx version 6.5 (PEAKALL and SMOUSE, 2012).

The Bayesian clustering method was used to elucidate the genetic structure among populations using STRUCTURE version 2.3.3 (PRITCHARD *et al*., 2000). The model applied in the analysis assumes the existence of K clusters. We took advantage of an admixture ancestry model under the correlated allele frequency model. The Markov chain Monte Carlo simulation was run 5 times for each value of K (1-10) for one million iterations after a burn-in period of 100,000. All other parameters were set at their default values. ΔK methods (EVANO *et al*., 2005) were used to choose the most likely number of genetic clusters (K). The graphical display of genetic structure was produce by the software DISTRUCT (ROSENBERG, 2004).

RESULTS

Tests for linkage disequilibrium between all pairs of loci across populations using GENEPOP found no significant genotypic disequilibrium in the pooled data ($P > 0.05$ for all after Bonferroni corrections). Genotyping a total of 134 individuals of common ragweed for five microsatellite loci, we found that all loci were polymorphic with the number of alleles per locus from 4 for the *Amb12* locus to 24 for the *Amb82* locus. Number of alleles at loci *Amb30*, *Ambart04* and *Amb16* was 11, 13 and 10, respectively. All considered loci were used for further analysis. The proportion of missing values (alleles) in the dataset was only 1.64%.

Comparing values of genetic measures, differences in genetic diversity between analysed populations were detected (Table 2). Allelic richness (based on minimal sample size of 16 diploid individuals), mean number of alleles per locus and mean number of rare alleles per locus have quite similar pattern of values distribution per populations, with the greatest values in SR-Gru population and the lowest values in population of Leskovac. All analysed populations possess private alleles (allele unique to one population). We detected 6 private alleles in SR-Zlp population, 3 in SR-Kru population, followed by populations SR-Gru and SR-Neg with 2 alleles, and 1 unique allele was found in SR-Sev and SR-Les populations.

In sampled populations heterozygote deficiencies were founded, with mean values of observed heterozigosity (Ho) 0.476 ± 0.055, unbiased expected heterozigosity (He) 0.702 ± 0.026 and with all positive values of inbreeding coefficient ranging from 0.198 to 0.463.

The overall Fst value (Fst = 0.055, test in FSTAT) indicated a moderate level of population differentiation. Approximately half (46.7%) estimates of Fst calculated between pairs of populations showed moderate genetic differentiation (pairwise Fst ranged from 0.051 to 0.144). The residue (eight comparisons out of fifteen) of the pairwise populations displayed low genetic differentiation (pairwise Fst<0.05). The greatest genetic differentiation, regarding to the Fst value 0.144, was found between SR-Kru and SR-Les populations. P values after Bonferroni correction have confirmed significant genetic differentiation between SR-Kru population and all the other populations (Table 3).

Table 2. Population statistics for Ambrosia artemisiifolia using five microsatellite loci

Pop. code	r	N_A	N_R	N_P	Ho	He	Fis	H
SR-Gru	7.80	8.4	5.8	2	0.500	0.707	0.314	**
SR-Sev	7.49	8.0	5.0	1	0.439	0.743	0.428	**
SR-Neg	7.10	7.4	4.0	2	0.410	0.737	0.463	**
SR-Kru	6.41	6.8	4.4	3	0.502	0.667	0.270	**
SR-Zlp	6.36	6.8	4.2	6	0.568	0.689	0.198	*
SR-Les	5.42	5.8	2.8	1	0.434	0.668	0.368	**
Mean±SD	6.76±0.87	7.2±0.94	4.37±1.01	2.5±1.87	0.476±0.059	0.702±0.033	0.340±0.099	**

r-allelic richness based on minimal sample size of 16 diploid individuals, N_A-mean number of alleles per locus, N_R -mean number of rare alleles per locus (frequency < 0.1), N_P -number of private alleles, Ho-observed heterozigosity, He-unbiased expected heterozigosity, Fis-WEIR and COCKERHAMS (1984) estimators of inbreeding coefficient, HW test-Hardy-Weinberg test (*P<0.05,**P<0.01), SD-standard deviation

Table 3. Pairwise Fst values between all analysed populations in this study (lower-left matrix) and their significance (upper-right matrix)

	SR-Neg	SR-Les	SR-Gru	SR-Zlp	SR-Sev	SR-Kru
SR-Neg		**	*	NS	*	**
SR-Les	0.064		*	NS	NS	**
SR-Gru	0.048	0.048		NS	NS	**
SR-Zlp	0.032	0.028	0.033		NS	**
SR-Sev	0.051	0.046	0.032	0.026		**
SR-Kru	0.103	0.144	0.073	0.101	0.066	

* P < 0.05,**P<0.01 after Bonferroni correction; NS-nonsignificant population differentiation

Analysis of molecular variance AMOVA indicates that 6.01% of genetic variation resided between common ragweed populations from Central Serbia, while the bulk of the genetic variation was within the populations with 93.99 percentages (Table 4).

Table 4. Analysis of molecular variance of common ragweed populations from Central Serbia

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	P-value
Among populations	5	33.534	0.11122	6.01	
Within populations	262	455.847	1.73987	93.99	
Total	267	489.381	1.85110		
Fixation index Fst 0.06009					$<10^{-6}$

The Principal Coordinate Analysis (PCoA) showed a strong the first two axes which together explained 57.8% of the genetic variance among populations and clearly separated analysed samples, except SR-Sev and SR-Gru populations which displayed reduced genetic distance to each other and positioned in the center of the principal coordinate plot. The PC3 (explaining 16.2% of total variation) brought closer population of SR-Neg to previously mentioned two populations. (Figure 2)

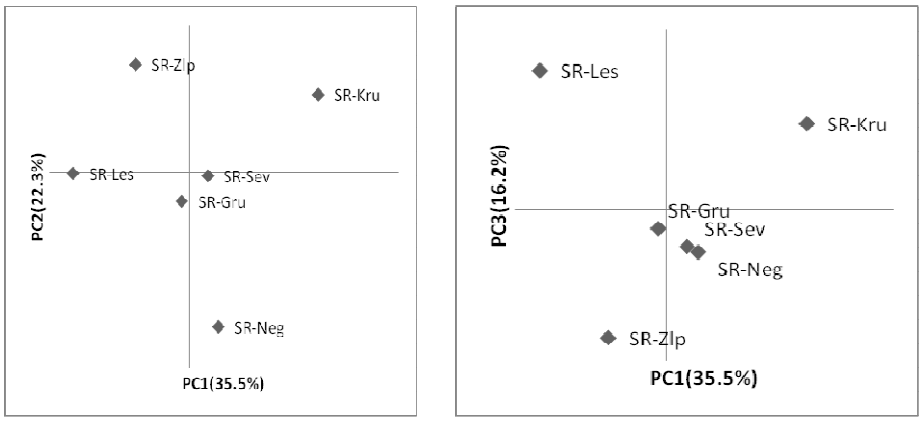

Figure 2. Principal Coordinate Analysis (PCoA) of chord distance among populations. The first, second and third principal coordinates account for 35.5, 22.3 and 16.2% of the variation, respectively

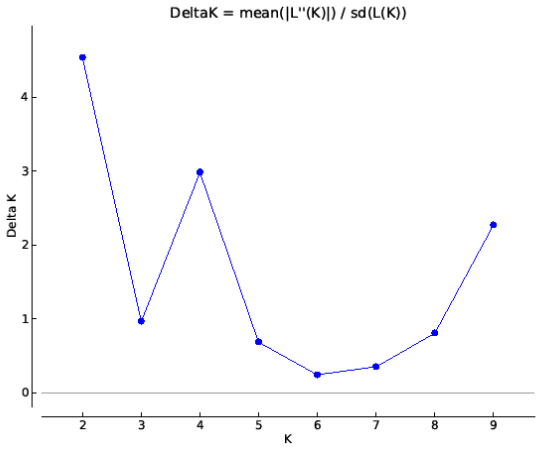

Figure 3. ΔK, as a function of K, the number of clusters

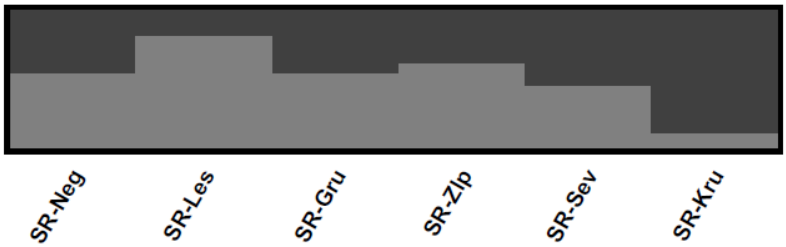

Figure 4. Proportion of membership coefficient with K=2 for 6 populations of common ragweed obtained using STRUCTURE software

In the Bayesian clustering analysis, ΔK indicated that two clusters best explained the genetic structuring of examined populations (Figure 3). The populations were roughly assigned to two clusters which were displayed by different colors (Figure 4). Individuals from SR-Les population had high membership in gray cluster and individuals from SR-Kru population strongly assigned to dark-gray cluster, indicating a presence of genetic differentiation in the dataset. The SR-Neg, SR-Gru and SR-Sev populations contributed almost equally to two clusters, while SR-Zlp population had slightly higher membership coefficient in the gray cluster. Levels above K=2 (secondary peak K=4, third peak K=9) (Figure 3) produced no new clusters among populations, but instead introduced some heterogeneity in membership coefficients (not shown).

DISCUSSION

In this study, we analyzed genetic diversity and genetic structure of *Ambrosia artemisiifolia* populations in the invaded range of Central Serbia. Within-population diversity indices obtained by microsatellites analyses revealed that genetic diversity in the common ragweed population from Gruza (SR-Gru) was the highest among the populations in the investigated area. Similarlly to SR-Neg, the SR-sev population also had a relatively high genetic variability. The southern populations from Krusevac and Zlatiborski put showed relatively lower genetic diversity than the above three populations. They also displayed quite similar genetic variability when compared to each other. The SR-Les population, located at the southern edge of the analysed invaded area (Figure 1), exhibited the lowest genetic variability compared to the other examined populations. Based on the data, we observed that common ragweed populations experienced a gradual loss of genetic diversity during their expansion (in the North-South direction) through the recently invaded region. A decline in genetic variability was also observed by GENTON et al. (2005a) who investigated range expansion of common ragweed in France, as well as in other species (CIOSI *et al*., 2008; WATTS *et al*., 2010; MEN *et al.,* 2013). This pattern of differences in allelic diversity among populations is expected with range expansion and colonization of new area via founder or bottleneck effects (DLUGOSCH and PARKER, 2008).

A comparison of genetic variations between populations can provide useful clues for deduction of the putative pathways of spreading, as well as of origins of the introduced populations (DLUGOSCH and PARKER, 2008). Therefore it should not be unexpected that high

levels of genetic diversity combined with the strategic location of a population (region) indicate that the considered population or region is the most likely source of introduction of common ragweed into the target area (MEN *et al*., 2013). In the present study the SR-Gru and SR-Sev populations, situated in the north of the examined area, showed that region with the greatest genetic variability can be considered as sources of expansion of *Ambrosia artemisiifolia* to the south of the country. This interpretation is supported by analyses of population structuring and differentiation among Central Serbian populations of common ragweed.

Although we analyzed a relatively small geographic region in Serbia, we obtained a certain level of population structuring. In the introduced range, founder effects tend to increase inter-population differentiation. Separate introductions may establish differentiated gene pools in different sites through founder effects, subsequent drift and/or responses to selection (DLUGOSCH and PARKER, 2008; ROSENTHAL et al., 2008). Based on pairwise Fst values, AMOVA results and PCo Analysis, moderate differentiation among population was detected. Two populations with the greatest level of genetic variability (SR-Gru and SR-Sev) displayed the lowest genetic distance in the principal coordinate plot, as well as non-significant population differentiation regarding Fst value. This observation is not surprising due to good connectivity by roads between named populations. By PC3 genetic distance between the SR-Neg population, and the SR-Gru and SR-Sev populations was reduced, presenting region with relatively high genetic diversity. These three populations, which are placed in the north of the analysed area, displayed a similar share in the clusters obtained by Bayesing clustering approach. All the other populations showed greater genetic differentiation. The STRUCTURE analysis clearly separated the SR-Kru and SR-Les populations, which was in agreement with the relatively high value of Fst and their positions in the coordinate plot. The differentiation of the two populations can be explained by populations expressing a fraction of the genetic variation occurring in the source population(s) (MEN *et al.,* 2013). Indeed, in accordance with the membership coefficient of individuals, the SR-Les population may be one fraction (gray cluster in Figure 4) and the SR-Kru population the other (dark-gray cluster) fraction of SR-Gru and SR-Sev populations. These results, along with the relatively low population genetic diversity of the SR-Les population, indicated the population located on the edge of the analyzed area was the latest one established, relative to other samples.

In all of the analyzed populations we detected heterozygotes deficiency. Deviation from Hardy-Weinberg equilibrium could be a result of mixing of populations with different allele frequencies (Wahlund effect), or it could be caused by natural selection, inbreeding and/or null alleles (KARLSSON and MORK, 2005). In several previous genetic studies of common ragweed (GENTON *et al*, 2005a; GLADIEUX *et al.,* 2011; CHUN *et al*., 2010), null alleles were proposed as the most likely explanation for excess of homozygotes. We do not favour this possibility, since in our data they occur in a very low frequency and have limited contribution to heterozygotes deficiency. This is reasonable according to the relatively short history of evolution of common ragweed in the analyzed area: null alleles are caused by mutations in the flanking regions of the microsatellites that are expected to evolve more slowly than repeat number, implying a longer period of evolution. Inbreeding could be the cause of heterozygotes deficiency in *Ambrosia artemisiifolia* populations, but the most recent studies suggest the prevalent mode of reproduction is outcrossing (FRIEDMAN and BARRETT, 2008; LI *et al*., 2012). We may consider the detected heterozygotes deficiency as a consequence of mixing of genetically different populations. It may be concluded that our newly established populations have been established

through subsequent invasion events, from different source populations. Moreover, we have to consider founder effect as one of the potential factors causing heterozygotes deficiency. Gradual reduction of genetic measures values, especially allelic richness, points to founder effects. Allelic richness reflects the loss of rare alleles and therefore is more sensitive to founder effects than heterozygosity (DLUGOSCH and PARKER, 2008). However, in order to support conclusions about the exact mechanism underlying the gradual loss of genetic diversity from our study, more populations from the analyzed area and putative source populations from northern parts of Serbia should be genetically characterized.

Our analyses of the data set presented in this study showed that populations in Central Serbia could have had successive introductions through sequential bottlenecks and founder events from the putative original populations. However, we cannot exclude the possibility of independent, separate introductions of examined populations from not hitherto sampled source populations. Presence of private alleles, which can be powerful in identifying source populations of invasions (GENTON *et al.*, 2005a), and which was detected in all examined populations in Central Serbia (e.g. SR-Zlp population had even 6 unique alleles), supports this conjecture. Invasions in newly invaded ranges, as shown in this study, may be unpredictable (the location and the time of introduction are generally unknown), making them difficult to study directly (GREVSTAD, 1999). There are therefore few detailed descriptions of population dynamics during early phases of invasions and founder events remain largely unstudied. Analysis of the genetic variation of recently introduced populations and putative source populations can be used to provide information about the invasion process (CIOSI *et al*., 2008). Hence, to determine the possible geographic origins of common ragweed in Central Serbia and its spread through country, additional research is required.

Did the expansion of common ragweed in Serbia proceed in the stepwise manner, where each population was colonized by a neighboring one, or did new populations appear as a result of independent colonization events? In order to answer this question we have to extend our research with samples of common ragweed from the initial area of introduction, namely Northern Serbia. Our preliminary results revealed relatively high genetic diversity of populations sampled from the territory of Banat area (KOČIŠ TUBIĆ *et al*., 2012), Vojvodina (KOČIŠ TUBIĆ *et al.,* 2009) and the neighboring regions (KOČIŠ TUBIĆ *et al*., 2011). Extending the sampling area should provide additional information of evolutionary processes and putative pathways of expansion of *Ambrosia artemisiifolia* in this part of Europe.

The present study represents the first more detailed genetic analysis of *Ambrosia artemisiifolia* populations of Central Serbia. Our results showing gradual losses of genetic diversity and moderate genetic structuring among populations contribute to better understanding of the biological invasion processes in recently invaded regions. In addition to knowledge already available in other areas of studying this aggressive weed, we hope our research provides useful information for better management of further expansion of *Ambrosia artemisiifolia* in Serbia, and for reduction of its negative impact.

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SUKCESIVNO SMANJENJE GENETIČKOG DIVERZITETA POPULACIJA *Ambrosia artemisiifolia* L. U CENTRALNOJ SRBIJI

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Izvod

Kao invazivni alergeni korov, *Ambrosia artemisiifolia* L. uzrokuje ozbiljne zdravstene i ekonomske probleme u Evropi. U poslednje dve decenije, šireći se prema južnim delovima Srbije, ambrozija je postala veliki problem u celoj zemlji. Obzirom na važnost genetičkih studija u razumevanju invazivnih vrsta, cilj ovog rada je bio analiza genetičkog diverziteta i strukture populacija *Ambrosia artemisiifolia* iz Centralne Srbije, regiona relativno skoro nastanjenog ovom biljnom vrstom. Poredeći vrednosti genetičkih parametara dobijenih analizom mikrosatelitskih markera, uočena je veća genetička varijabilnost u populacijama iz severnog dela ispitivanog regiona u odnosu na južne populacije. Na osnovu Fst vrednosti, AMOVA rezultata i PCo analize, detektovana je umerena diferencijacija među populacijama, dok je STRUCTURE analiza jasno izdvojila dve populacije (SR-Kru i SR-Les). Dobijeni podaci ukazuju na sukcesivan gubitak genetičkog diverziteta populacija ambrozije u regionu Centralne Srbije. Da bi potvrdili mehanizme širenja ambrozije u ovom regionu i otkrili moguće izvorne populacije, neophodno je proširiti ispitivani uzorak sa populacijama iz severnih delova Srbije, regiona inicijalne introdukcije. Ovo istraživanje predstavlja prvu detaljniju genetičku analizu ambrozije u Centralnoj Srbiji i dobijeni rezulati mogu doprineti razumevanju dinamike invazija u skoro nastanjenim regionima

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