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RESEARCH ARTICLE

Clustering of Field Maple Populations from Different Ecological Conditions in Bosnia and Herzegovina Based on Discriminant Analysis of Morphological Traits

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ABSTRACT

Acer campestre L. is naturally distributed in most of Europe. The species can serve as a valuable model for researching the sensitivity of populations to habitat fragmentation. This research aims to determine whether there is a differentiation of field maple populations based on their division by ecological-vegetation regions, precipitation amounts, temperatures, and altitudes of the populations. The material was collected from 25 populations in Bosnia and Herzegovina, from different ecological-vegetation areas, with twelve trees per population and five fruits and ten leaves per tree. Then, 10 fruit properties and 19 leaf properties were analyzed. A discriminant analysis was performed for population groups based on the ecological-vegetation area, amount of precipitation, average annual air temperature and altitude. The discriminant analysis results showed the distinguishing of groups of populations in the sub-Mediterranean area, and groups of populations with an average annual temperature of 14.00 to 15.99 °C. There was no clear separation of the groups according to the average annual precipitation or altitude. The analysis of the connection established that the influence of environmental factors is more pronounced compared to geographical factors. The key ecological variable that determines morphological separation was temperature and to a lesser extent precipitation. The results of this research will be used in planning of afforestation of suitable, unvegetated land and extreme habitats in the southern and southwestern parts of Bosnia and Herzegovina with *Acer campestre*.

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1. Introduction

Field maple (*Acer campestre* L.) is naturally distributed in most of Europe, except its northern parts. This species in its natural distribution does not build pure stands, but is often a subdominant species in many forest stands in Europe (Zecchin et al., 2016). Considering its low commercial importance, field maple is not susceptible to different silvicultural treatments and often grows in spontaneously formed, semi-natural populations. For these reasons, the species can serve as a valuable model for the purpose of researching the sensitivity of populations to habitat fragmentation, given that it grows at different levels of population fragmentation (Chybicki et al., 2014).

According to the available literature, research on the morphological and population-genetic variability of *Acer campestre* is very scarce, and other aspects of the species have been investigated through numerous studies. Leinemann and Bendixen (1999) studied the heritability of isoenzyme variants in field maple in Bavaria, Germany. In Italy, Ferrini and Nicese

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(2003) investigated the influence of different substrates with compost on the growth and physiology of leaves of the species. Coudun et al. (2006) surveyed the influence of soil nutrients on the distribution of the species in France. In Romania, Drăghici and Abrudan (2011) studied the effects of different stratification methods on seed germination of the species. Šimala and Masten Milek (2013) investigated whitefly species from the genus *Aleurochiton* Tullgren on maples in Croatia, and identified *Aleurochiton acerinus* on *Acer campestre*. Milenković et al. (2014) studied the morphological and molecular identification of *Phytophthora* species on maple species in Serbia, including *Acer campestre*. Kabaš et al. (2014) investigated the bioclimatic niche for four species of maple (including field maple) in Serbia and Kosovo.

Genetic studies of this species are few. Ducci et al. (2010) researched the genetic structure of field maple populations in Italy using biochemical markers, and Chybicki et al. (2014) used microsatellite markers in Poland.

In Bosnia and Herzegovina, the most attention was paid to the taxonomic processing of field maple. Thus, Malý (1906), who studied maples in Bosnia and Herzegovina, devoted considerable attention to the species. Some attention to this issue was also given by: Pax (1885, 1886), Hayek (1927), Plavšić (1941), and Fukarek (1953). The most significant contribution to the taxonomic problem of this species was made by Drenkovski (1979) for the area of the former Yugoslavia, who investigated the *Acer campestre–Acer marsicum* complex in detail.

The morphological variability of *Acer campestre* populations according to fruit traits in Bosnia and Herzegovina was investigated by Kvesić et al. (2019), and leaf traits by Kvesić et al. (2020a, 2020b, 2021). The genetic variability of field maple populations in Bosnia and Herzegovina was investigated by Kvesić et al. (2020b).

The research of field maple as a subdominant tree species provides an excellent opportunity to determine the influence of ecological and geographical factors, climate changes, natural distribution, and habitat fragmentation on the morphological and genetic variability of forest populations. The morphological and genetic variability of field maple populations established so far can be used to preserve this species and its genetic diversity, then in its breeding and monitoring of its reproductive material. In addition, there is a significant area of unvegetated land and extreme habitats in the southern and southwestern parts of Bosnia and Herzegovina, which can be afforested with *Acer campestre* due to its great adaptability to different ecological conditions.

This research aimed to determine whether there is a differentiation of field maple populations based on their division by ecological-vegetation regions of Bosnia and Herzegovina, precipitation amounts, temperatures, and altitudes of the populations.

2. Materials and Methods

Material for analysis of the morphological variability of field maple populations was collected in 25 populations throughout Bosnia and Herzegovina, covering the various ecological and geographical aspects in which the species grows. The sampled populations belonged to different ecological-vegetation areas (Stefanović et al., 1983), indicated in Table 1 and Figure 1.

In terms of phytocenological affiliation, the populations inhabited different types of forest communities (Stefanović et al., 1983), but in most cases, they were habitats of different progradation-degradation stages. Most of the sampled populations were in fields, meadows, and pastures where field maple trees existed independently (Posušje, Rama, Livno, B. Petrovac, Ljubuški, Mostar, Gacko, Trebinje, Višegrad, Kakanj, Olovo, and Žepče). The open habitats of fields and meadows primarily represented the final stages of degradation of the various forest communities that existed in the mentioned areas. Certain populations were located within the forests of pedunculate oak and common hornbeam (Carpino betuli–Quercetum roboris) /Bosanska Dubica, Derventa, Banja Luka, Bijeljina, Tuzla and Bratunac/. Some of the populations were located within the forests of sessile oak and common hornbeam (Querco-Carpinetum) /Sarajevo, Kreševo, Jajce and Bihać/. The Rogatica population was located within common beech and common hornbeam (Ostryo-Fagetum) forests. The Ključ and B. Grahovo populations belonged to Italian maple and common beech (Aceri obtusatum-Fagetum) forests.

Each of the 25 populations was represented by 12 trees. For the purposes of morphological research, five healthy and undamaged fruits and ten healthy and undamaged leaves were collected from each individual. Fruits and leaves were collected from normally developed trees with a lighted crown (solitary trees or trees at the edge of the forest) so that their phenotype was fully expressed without modification changes conditioned by breeding measures, that is, to show the recent state of the species (Franjić, 1996; Kajba, 1996; Idžojtić et al., 2006; Mikić, 2007; Ballian et al., 2010; Zebec et al., 2010). Fully developed leaves were collected from the same place of the short shoot, the first pair of normally developed leaves looking from the tip of the short shoot. A total of 1,500 fruits and 3,000 field maple leaves from individuals of exclusively generative origin were collected and morphometrically processed. The trees were at least 50 meters apart to reduce the likelihood of potential relatedness.

Field maple fruits and leaves were collected in August and September 2014. After collection, the leaves were herbarized. Morphometric measurements of leaves and fruits were made with a digital electronic meter (caliper) with an accuracy of ±0.01 mm.

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Table 1. List of studied populations.

Region	Area	Area label	Population	Latitude	Longitude	Altitude m a.s.l.
Pripanonian	North Bosnian	1.1.	Derventa	45°01'05"	17°59'29"	115
		1.1.	Tuzla	44°29'39"	18°40'50"	245
	Northwest-Bosnian	1.2.	B. Luka	44°48'25"	17°13'36"	185
		1.2.	B. Dubica	45°04'44"	16°41'25"	170
Transitional Illyrian-Mesian	Lower Drina	2.1.	Bijeljina	44°49'01"	19°01'59"	95
		2.1.	Bratunac	44°14'48"	19°06'38"	220
	Upper Drina	2.2.	Višegrad	43°45'17"	19°23'17"	375
		2.2.	Rogatica	43°46'55"	19°00'28"	515
Inner Dinarides	Cazin's Krajina	3.1.	Bihać	44°51'29"	15°53'17"	315
	West Bosnian limestone-dolomite	3.2.	Ključ	44°29'08"	16°53'34"	685
		3.2.	B. Petrovac	44°37'37"	16°14'57"	620
	Central Bosnia	3.3.	Kreševo	43°53'01"	18°04'22"	570
		3.3.	Jajce	44°22'33"	17°17'36"	345
		3.3.	Kakanj	44°04'42"	18°06'06"	450
		3.3.	Sarajevo	43°55'13"	18°22'48"	690
	Zavidovići-Teslić	3.4.	Žepče	44°25'27"	18°03'01"	225
		3.4.	Olovo	44°08'18"	18°33'11"	510
Mediterranean-Dinaric	Sub-Mediterranean-high mountainous	4.1.	Rama	43°49'37"	17°30'28"	625
		4.1.	B. Grahovo	44°11'12"	16°22'16"	845
		4.1.	Gacko	43°13'28"	18°34'44"	1115
	Sub-Mediterranean-mountainous	4.2.	Posušje	43°27'10"	17°22'22"	710
		4.2.	Livno	43°51'19"	16°56'31"	750
	Sub-Mediterranean	4.3.	Ljubuški	43°10'11"	17°37'00"	50
		4.3.	Mostar	43°11'40"	17°49'00"	225
		4.3.	Trebinje	42°47'26"	18°09'25"	240

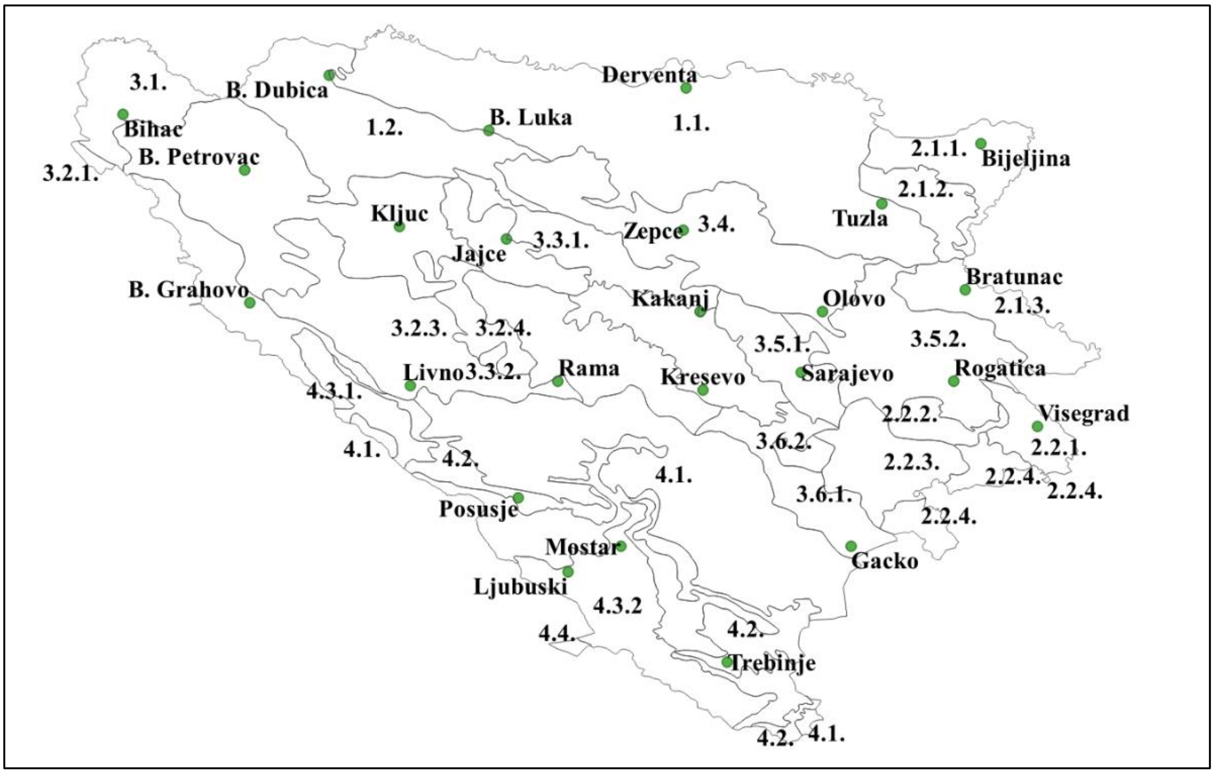

Figure 1. Geographical location of the investigated populations and belonging to ecological-vegetation areas.

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2.1. Investigated Morphological Traits

The morphometric analysis included 10 fruit traits (Figures 2 and 3) and 19 leaf traits (Figure 4). Out of a total of 10 fruit traits, eight traits were measured (Figures 3 and 4) and two were derived.

a) Measured fruit traits
1. Fruit petiole length (F_1),
2. Fruit wing with the nut length (F_2),
3. Fruit wing width (F_3),
4. Fruit wing without the nut length (F_4),
5. The length of the fruit wing from the widest part of the wing (F_5),
6. Nut length (F_6),
7. Nut width (F_7),
8. Fruit angle (α) (F_8).

b) Derived properties of the fruit
1. The ratio of fruit wing width and fruit wing length with the nut ($F_9=F_3/F_2$),
2. The ratio of nut width and nut length ($F_{10}=F_7/F_6$).

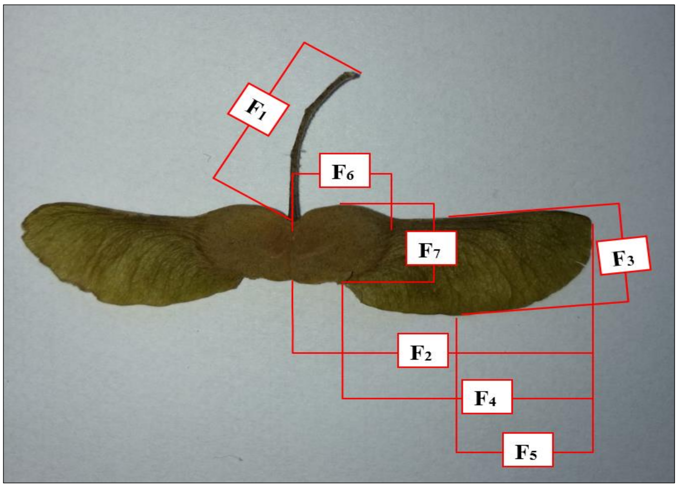

Figure 2. Analyzed morphological characteristics of the fruit (F_1-F_7).

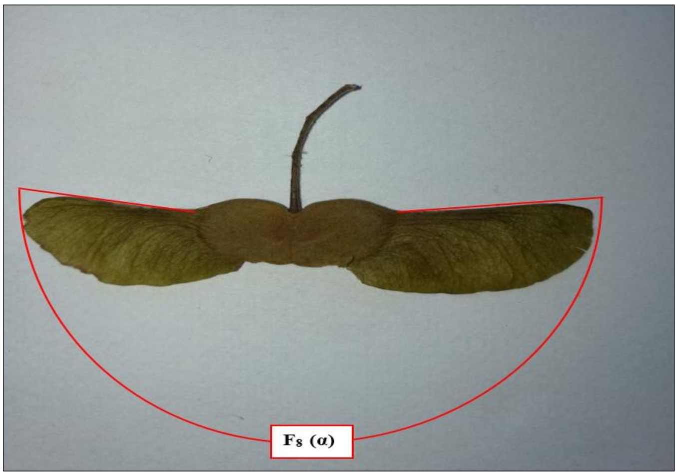

Figure 3. Analyzed morphological characteristics of the fruit (F_8).

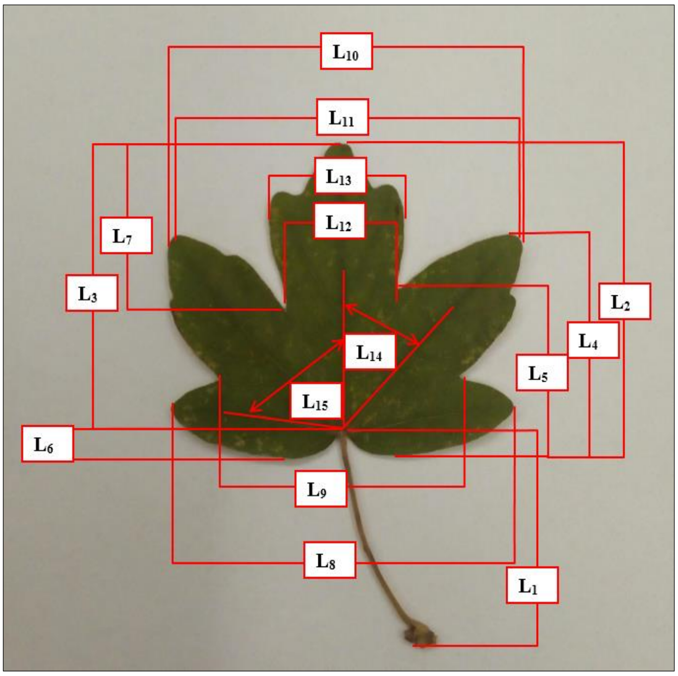

Figure 4. Analyzed morphological characteristics of leaf.

Out of a total of 19 leaf traits investigated, 15 traits were measured (Figure 4), and four traits were derived.

a) Measured leaf traits
1. Leaf petiole length (L_1),
2. Maximum leaf blade length (L_2),
3. Central nerve length (L_3),
4. Leaf blade length to the top of the lateral lobes (L_4),
5. Leaf blade length to the incision of the lateral lobes (L_5),
6. Leaf blade base incision depth (L_6),
7. Central lobe length to the incision of lateral lobes (L_7),
8. Leaf blade width between the tips of the lower lobes (L_8),
9. Leaf blade width between the incision of the lower lobes (L_9),
10. Maximum leaf blade width (L_{10}),
11. Leaf blade width between the tips of the lateral lobes (L_{11}),
12. Leaf blade width between the incisions of the lateral lobes (L_{12}),
13. Central lobe width (L_{13}),
14. Angle (α) between the nerves of the central and lateral lobes (L_{14}),
15. Angle (β) between the nerves of the central and lower lobes (L_{15}).

b) Derived leaf traits
1. The ratio of the maximum leaf blade length and maximum leaf blade width ($L_{16}=L_2/L_{10}$),
2. The ratio of maximum leaf blade length and the length of the central lobe to the incisions of lateral lobes ($L_{17}=L_2/L_7$),

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3. The ratio of leaf blade width between the tips of the lower lobes and leaf blade width between the incisions of lower lobes ($L_{18}=L_8/L_9$),
4. The ratio of leaf blade width between the tips of lower lobes and leaf blade width between the tips of lateral lobes ($L_{19}=L_8/L_{11}$).

To verify the presence of specific morphological groups, we made groups of populations based on: belonging to the ecological-vegetation areas (according to Stefanović et al. 1983), amount of precipitation, average annual air temperature, and altitude. In this way, we wanted to identify the most important factors influencing the separation of the studied population groups. The multivariate analysis of variance was performed to examine if ecological-vegetation areas have a statistically significant effect on all investigated traits of fruits and leaves of *Acer campestre.* Discriminant analysis was performed to show grouping of groups of populations formed on basis of ecological-vegetation areas, amount of precipitation, average annual air temperature, and altitude. Discriminant analysis is one of the techniques of multivariate analysis by which populations are classified into subgroups with similar characteristics based on a large number of measured traits (Čabaravdić, 2012). Discriminant analysis was performed in the statistical program SPSS 20.0, and graphical representations in the program PAST 3.18.

Cluster analysis was done for additional representation of the grouping of ecological-vegetation areas. Cluster analysis based on hierarchical agglomerative clustering was applied. The method of complete linkage was used as the method of connection, that is, the method of the furthest neighbor (complete linkage-furthest neighbor). The standard Euclidean distance was used as a measure of distance. The analysis was performed in the PAST 3.18 program.

3. Results

3.1. Multivariate Analysis

Results of multivariate analysis for the effect of ecological-vegetation areas on all investigated traits are shown in Table 2. Wilks' Lambda showed Sig. value of 0.000, which indicates that values of examined traits are significantly dependent on Ecological-vegetation areas.

Table 2. Multivariate analysis of effects of ecological-vegetation areas on the investigated traits.

Effect		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	1.000	746141.727[b]	27.000	263.000	0.000
	Wilks' Lambda	0.000	746141.727[b]	27.000	263.000	0.000
	Hotelling's Trace	76600.101	746141.727[b]	27.000	263.000	0.000
	Roy's Largest Root	76600.101	746141.727[b]	27.000	263.000	0.000
Ecological-vegetation area	Pillai's Trace	2.724	3.772	270.000	2720.000	0.000
	Wilks' Lambda	0.020	4.745	270.000	2499.579	0.000
	Hotelling's Trace	6.577	6.363	270.000	2612.000	0.000
	Roy's Largest Root	3.644	36.709[c]	27.000	272.000	0.000

a. Design: Intercept Ecological-vegetation area
b. Exact statistic
c. The statistic is an upper bound on F that yields a lower bound on the significance level.

The mutual separation of groups of populations from different ecological-vegetation areas based on the first two discriminant axes is shown in Figure 5. From the graphic representation, it is evident that a group of the sub-Mediterranean ecological-vegetation area has been separated based on the first axis with which the measurement properties of the leaf are most significantly correlated. The group of populations of the sub-Mediterranean area is connected with the groups of other ecological-vegetation areas exclusively through the group of populations of the sub-Mediterranean-mountainous area.

Figure 6 shows separation of population groups in terms of average annual rainfall (mm). There is no clear separation of population groups, but mutual overlapping of groups 4 and 5 on the one side and groups 1, 2, and 3 on the other side is visible. The overlapping of groups 1, 2, and 3 with groups 4 and 5 is very little pronounced.

Figure 7 shows separation of groups of populations formed by average annual air temperature. There was a clear separation of group 4 (14.00-15.99 ºC) from the other groups, based on the first axis with which most of the measured properties of the leaf were most significantly correlated. Out of 36 individuals included in the group 4, only two individuals overlap with individuals of other groups, while 34 individuals were completely separated from the others. The mutual overlapping of the individuals of three remaining groups (1, 2 and 3) was quite pronounced, but least in the individuals of the third group (12.00-13.99 ºC).

Figure 8 shows the mutual separation of population groups concerning their altitude position (m). It can be seen that there is no clear separation of population groups and that none of the groups stood out independently from the others. The overlapping of individuals is very pronounced in all the formed groups, and the groups mostly occupy the central part of the coordinate system. It can be concluded that the individuals in group 1 and group 2 are the most scattered.

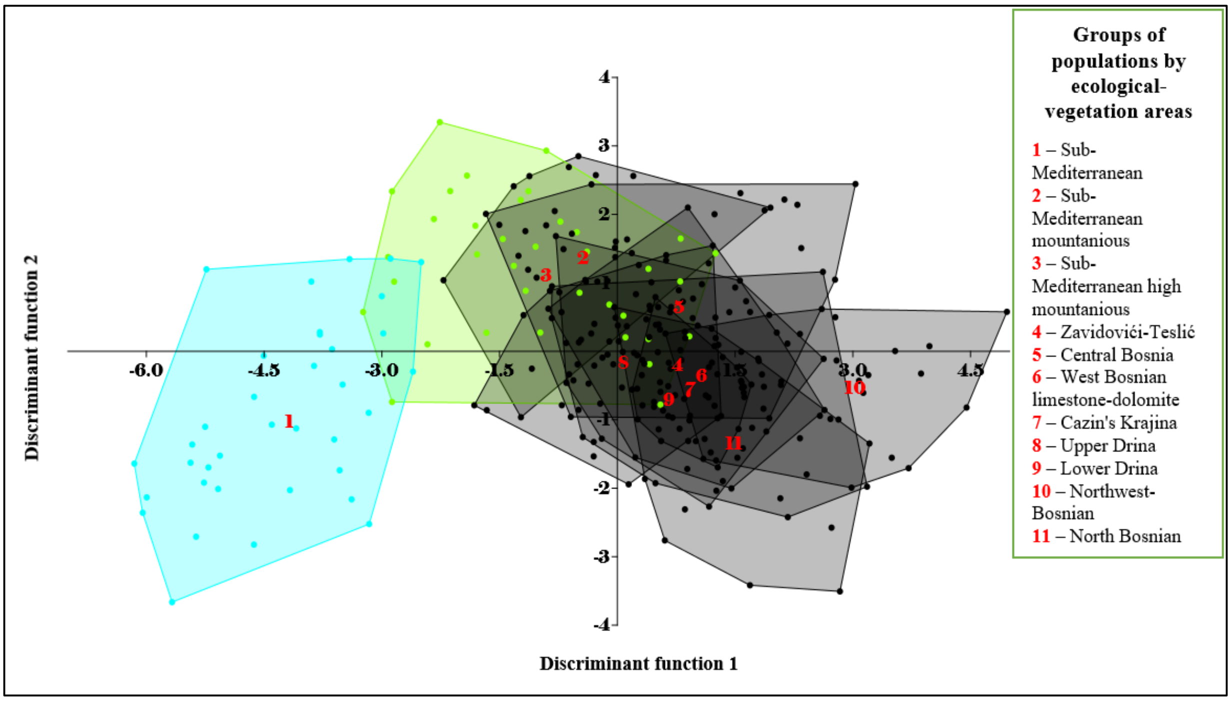

Figure 5. Scatterplot of separation of groups of ecological-vegetation areas based on the first and second discriminant functions.

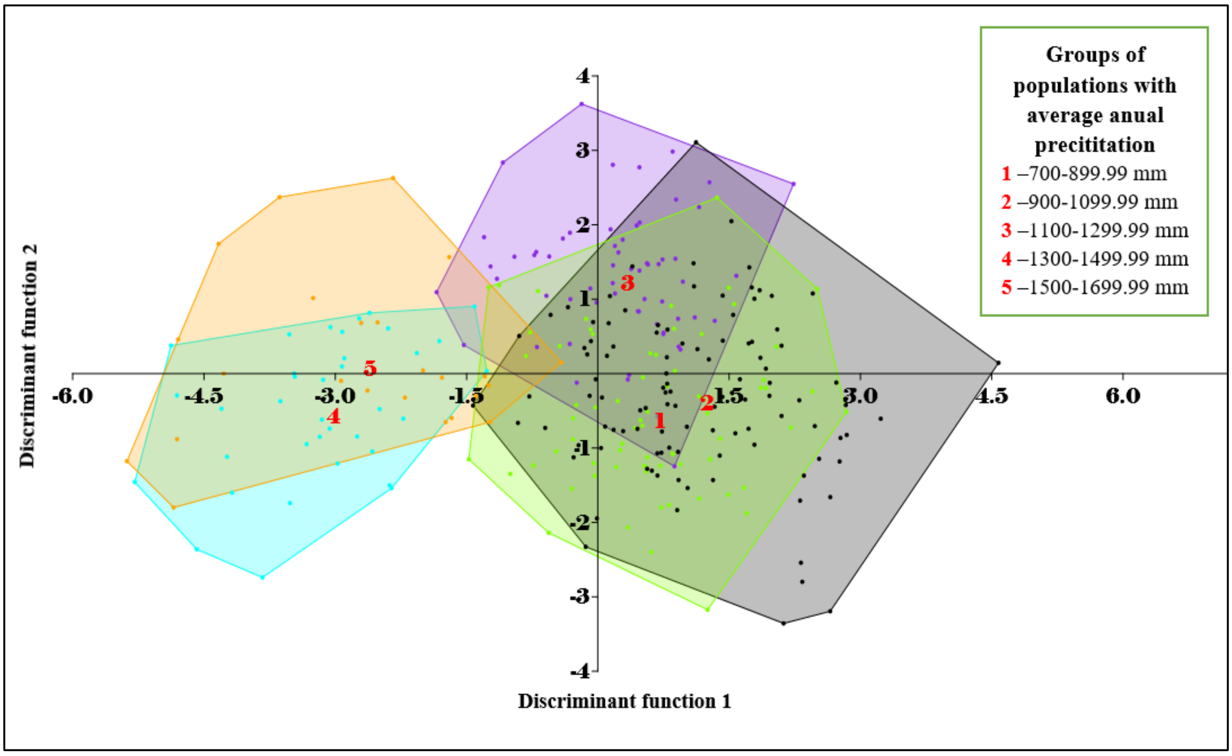

Figure 6. Scatterplot of separation of population groups according to the average annual amount of precipitation (mm) based on the first and second discriminant functions.

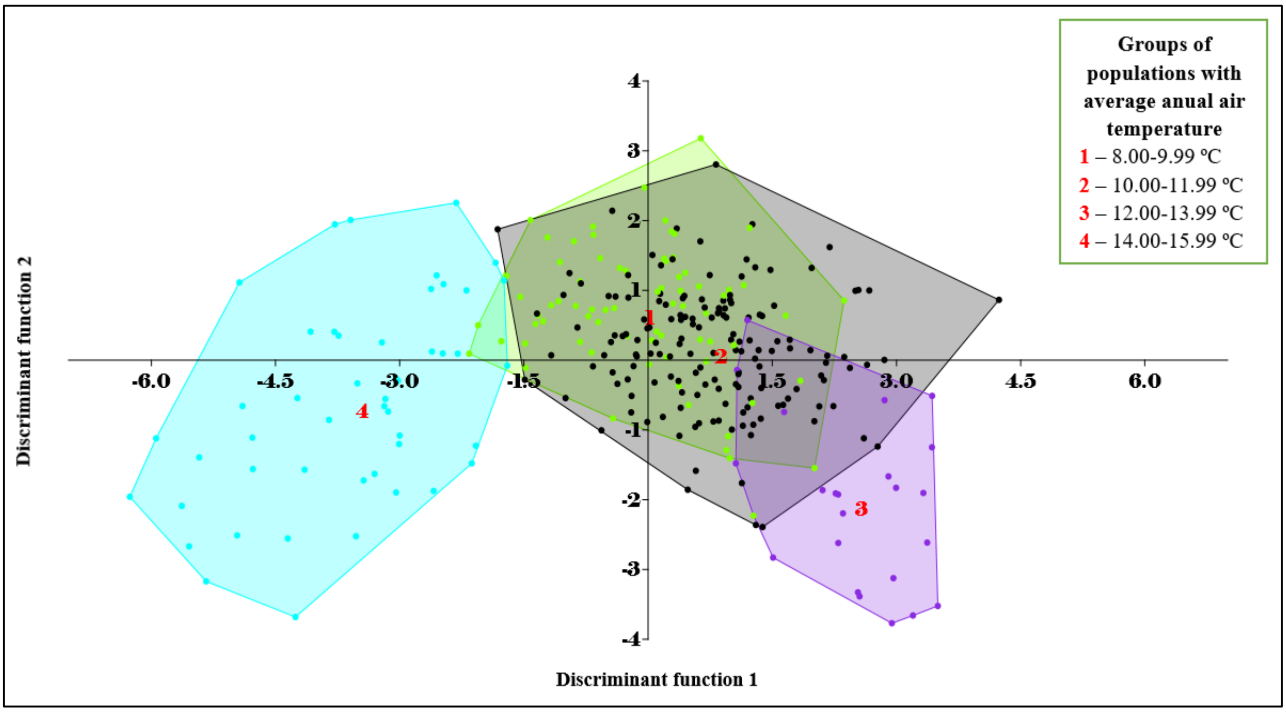

Figure 7. Scatterplot of separation of population groups according to the average annual air temperature (ºC) based on the first and second discriminant functions.

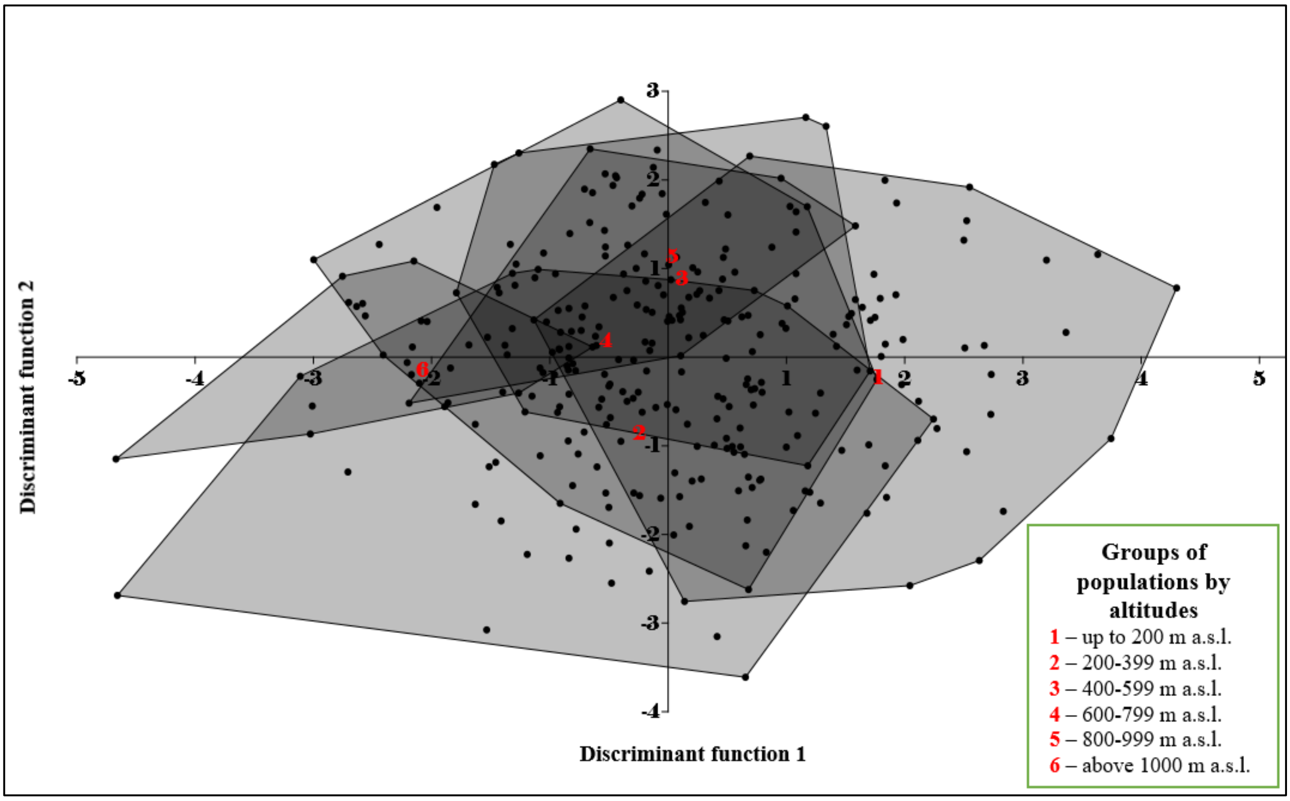

Figure 8. Scatterplot of separation of population groups according to altitude (m a.s.l.) based on the first and second discriminant functions.

3.2. Cluster Analysis

The results of cluster analysis regarding the belonging of populations to ecological-vegetation areas are shown in Figure 9. The complete connection method and Euclidean distance measure were used.

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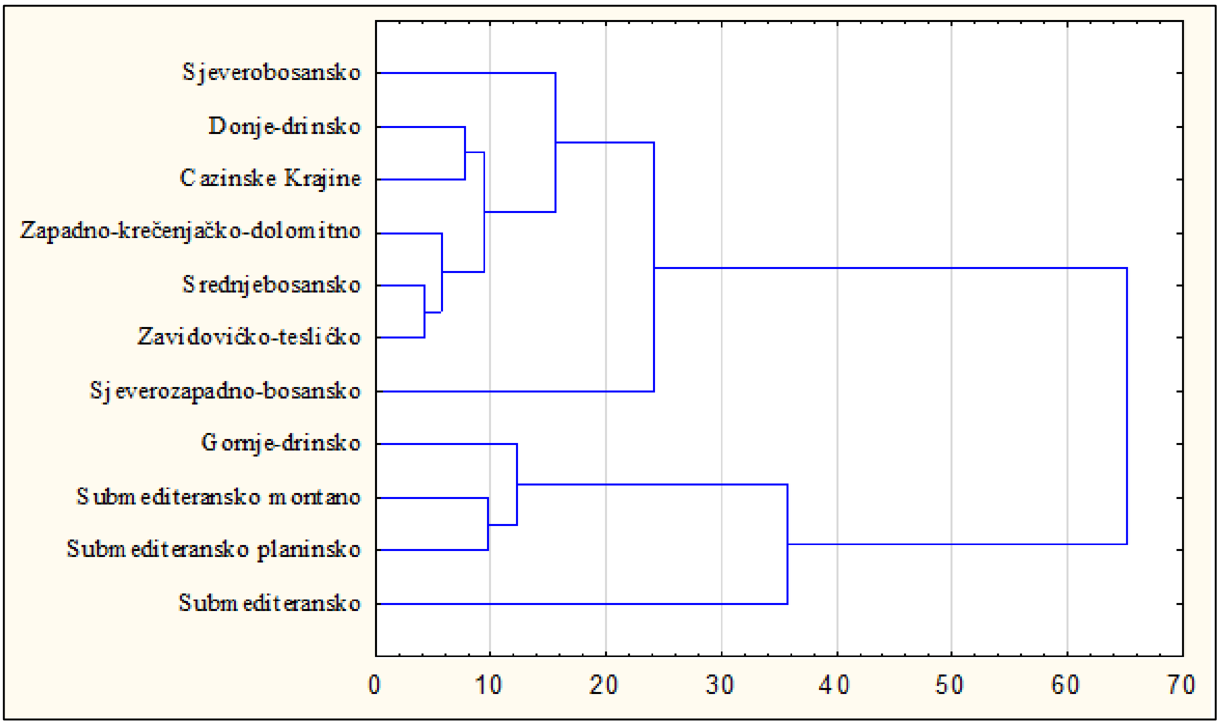

Figure 9. Dendrogram of connecting populations from different ecological-vegetation areas.

From the dendrogram of the connection of populations from different ecological-vegetation areas, it is evident that all investigated populations are grouped in 10 steps, i.e. on the basis of 10 clusters. The populations most similar to each other, which joined already at the Euclidean distance of 4.17, are the populations of Central Bosnia and the Zavidovići-Teslić ecological-vegetation area Populations combined from the two mentioned areas make the first cluster. The second cluster is formed when populations of western limestone-dolomite area connected with the populations of the first cluster, at the Euclidean distance of 5.67. The third cluster is formed when populations of Lower Drina area and the populations from the Cazin Krajina area merged, at the Euclidean distance of 7.79. The fourth cluster is formed by merging the populations of the previous two clusters at the Euclidean distance of 9.47. The fifth cluster consists of the populations of the sub-Mediterranean-montain area combined with the populations of the sub-Mediterranean-mountain area, at a Euclidean distance of 9.71. The populations from the fifth cluster are connected with populations of the upper Drina area at the Euclidean distance of 12.29, and the sixth cluster was formed. The seventh cluster is created by joining the populations of the fourth cluster and the populations of the North Bosnian ecological-vegetation area at the Euclidean distance of 15.58. The eighth cluster is formed by joining the populations of the seventh cluster with the populations of the northwestern Bosnian area, at the Euclidean distance of 24.21. The ninth cluster is formed by joining populations of the fifth cluster and populations of the sub-Mediterranean ecological-vegetation area, at the Euclidean distance of 35.68.

It is characteristic that the populations of the sub-Mediterranean ecological-vegetation area joined the mentioned populations for the first time only at the Euclidean distance of 35.68. It is evident from the diagram that after the formation of the eighth and ninth clusters, there follows a relatively large Euclidean distance that precedes the merging of these two clusters. Their joining occurs only at the Euclidean distance of 65.20. This clearly indicates the existence of significant morphological differences between the two last clusters.

4. Discussion

Morphological and genetic studies of field maple populations in Bosnia and Herzegovina have shown intra-population and inter-population variability (Kvesić et al., 2019, 2020a, 2020b, 2021).

The results of the research on the morphological variability of the fruit of 25 field maple populations in the territory of Bosnia and Herzegovina (Kvesić et al., 2019) showed that the most variable characteristic of the fruit was fruit petiole length and the most divergent population were Trebinje and Rogatica. Analysis of variance revealed statistically significant differences among field maple populations in Bosnia and Herzegovina for all analyzed fruit properties, and intra-population variability was higher than inter-population variability for most of the investigated properties (Kvesić et al., 2019).

Variance analysis for leaf traits of field maple populations from Bosnia and Herzegovina (Kvesić et al., 2020a) showed statistically significant differences amongg populations for all analyzed properties, and multiple testing showed greater inter-population than intra-population variability for most of the traits. In the research by Kvesić et al. (2021), leaf parameters were confirmed as the dominant carriers of morphological differentiation between field maple populations in Bosnia and Herzegovina. The sub-Mediterranean populations Trebinje, Ljubuški, and Mostar had smaller leaf areas than other populations.

Kvesić et al. (2020b) investigated the genetic variability of field maple in Bosnia and Herzegovina. The results indicated that the group of sub-Mediterranean populations differs from internal populations: they showed higher genetic diversity, form a separate cluster based on genetic distances, and revealed different shares of the gene pool identified by Bayesian population structure analysis.

The results of this research indicate the specific morphological structure of Bosnian-Herzegovinian field maple populations, and influence of ecological conditions, *i.e.*, belonging to different ecological-vegetation regions and altitudes.

From the graphic presentation of the mutual separation of population groups from different ecological-vegetation areas based on the first two discriminant axes, it is evident that there was a clear separation of the sub-Mediterranean ecological-vegetation area group. The group of populations of the sub-Mediterranean area was connected with the groups of other ecological-vegetation areas exclusively through the group of populations of the sub-Mediterranean-mountainous area. It means that group of populations from sub-Mediterranean-high-mountain area represents an intermediate group, and that the sub-Mediterranean group of populations would have been completely separated from the other groups if the research had not included the group of the sub-Mediterranean-high-mountain area. This is an interesting phenomenon considering that between the sub-Mediterranean and sub-Mediterranean-high-mountain areas, in the physical sense, there is a sub-Mediterranean-montane area. If we exclude the group of the sub-Mediterranean area, which stood out quite clearly, and the group of the sub-Mediterranean-high-mountain area, which is intermediate, it is evident that the other nine groups of ecological-vegetation areas, in terms of morphology, form a fairly homogeneous whole with a strong mutual overlap.

Discriminant analysis regarding the average annual rainfall showed an overlap of population groups with an average annual rainfall of 1300-1499.99 mm and 1500-1699.99 mm on the one side, and population groups with an average annual rainfall of 700-899.99 mm, 900-1099.99 mm and 1100-1299.99 mm.

Regarding the average annual air temperature, the discriminant analysis indicated the separation of the population group with the highest average annual temperatures (14.00-15.99 ºC).

Discriminant analysis regarding groups of populations from different altitudes did not show a clear separation of groups.

The cluster analysis confirmed the clear separation of populations from the sub-Mediterranean ecological-vegetation area in relation to other groups of populations.

The general morphological structuring of field maple populations in Bosnia and Herzegovina is conditioned by geographical and ecological factors. The analysis of the connection established that the influence of environmental factors is more pronounced compared to geographical factors. The key ecological variable that determines morphological separation is temperature and, to a lesser extent, precipitation. It is generally considered that the above-mentioned factors are determinants of the natural distribution of different species of maple, including field maple (Kabaš et al., 2014).

5. Conclusion

The multivariate analysis of variance showed statistically significant differences among groups of populations from different ecological-vegetation areas.

The results of the discriminant analysis revealed that the group of populations of the sub-Mediterranean area is connected with the groups of other ecological-vegetation areas exclusively through the group of populations of the sub-Mediterranean-high-mountainous area.

Discriminant analysis of population groups in terms of average annual rainfall showed no clear separation of population groups and that none of the groups stood out independently in relation to the others.

In addition, discriminant analysis of population groups based on average annual temperature indicated a clear separation of population groups with an average annual temperature of 14.00 to 15.99 °C, in relation to population groups from areas with lower average annual temperatures, which overlap each other.

According to the altitude position of the population groups, the discriminant analysis did not show a clear separation of any population groups.

The cluster analysis confirmed the clear separation of populations from the sub-Mediterranean ecological-vegetation area from other groups of populations.

The analysis of connection showed that the influence of environmental factors is more pronounced compared to geographical factors. The key ecological variable that determines morphological separation is temperature and to a lesser extent precipitation.

Conflict of Interest

There is no conflict of interest to declare.

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