

Health Condition of European Beech (*Fagus sylvatica* L.) According to Provenances in International Provenance Trial

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ABSTRACT

The performance of European beech (*Fagus sylvatica* L.) is severely threatened by abiotic and biotic stresses, but the resilience of its provenances from distinct geographic areas has not been sufficiently studied. Therefore, the performance of European beech was investigated in an international provenance trial consisting of twenty-one natural populations originating from Central Europe and Southeast Europe located on Medvednica Mountain (Croatia). The performance of European beech was investigated by characterizing I) damage types, II) crown damage intensity, III) damage frequency, and IV) clustering of provenances based on damage types. Anthracnose, galls, chewing damages, and aphids were recorded on leaves, and canker on the trunk and branches. The crown damage intensity was minute (less than 10%) for all types of disease. Anthracnose was the most common damage, followed in descending order by galls, canker, chewing damages and aphids. When the types of damage were analysed using descriptive statistics (frequency analysis and clustering), significant differences were recorded between provenances ($\chi^2=322.19$, $p<0.0001$). European beech provenances were classified into four clusters. Aphids and galls caused the least and the highest damage, respectively, in each cluster, except for Cluster 4, where anthracnose caused the highest damage. The results of this study showed generally good health condition of European beech provenances originating from Central and Southeast Europe in the period of the investigation.

Keywords: European beech; pests; diseases; international provenance trial

INTRODUCTION

European beech (*Fagus sylvatica* L.) is a deciduous, monoecious, anemophilous tree species in the *Fagaceae* family. As the dominant tree species in Europe, European beech has a large distribution range extending from Sicily in southern Italy (ca. 37.7N) to Bergen in southern Norway (ca. 60.7N) (Fang et al. 2006). It covers approximately 14 million hectares of forest land in Europe (Wühlisch 2010). In Croatia,

European beech is a native tree species, accounting for 47 percent of the forest area. Its natural structure has been preserved in pure or mixed stands (Vukelić and Rau 1998). Moreover, it has a wide ecological valence at altitudes in Croatia; ranging from the Mediterranean vegetation region at about 20 m above sea level to the sub-mountainous vegetation region at about 1570 m above sea level (Pilaš et al. 2016). The wide geographical distribution of European beech indicates high adaptability to climatic, local edaphic,

and soil hydrological parameters (Rennenberg et al. 2004, Bolte et al. 2007, Gessler et al. 2007, Kramer et al. 2010;). Studies on the genetic composition of European beech populations indicated that ecotypes are formed by both local and macroclimatic factors (Gömöry et al. 2007, Ivanković et al. 2008a, 2008b Mátyás et al. 2009). Considering the influence of climatic factors on the distribution range of European beech, climate change-based models predicted that European beech can expand its northern range while losing habitat at its southern distribution area (Bréda et al. 2006, Gessler et al. 2007, Kramer et al. 2010).

The adaptability and growth characteristics of European beech were investigated in a beech provenance trial series. The study was conducted as part of the European Union project "European Network for the Evaluation of the Genetic Resources of Beech for Appropriate Use in Sustainable Forestry Management" (AIR3-CT94-2091) of the Institute of Forest Genetics, Grosshansdorf, Germany (Muhs and Wühlisch 1993, Wühlisch 2008). Croatia contributed to the International Beech Provenance Trial project by supplying beechnuts and by establishing three international European beech provenance trials (Gračan 2001, Ivanković 2010).

European beech is thought to be threatened by changing climatic conditions (Gessler et al. 2007), although it is less affected by severe diseases than other forest tree species (FAO 2009). Still, the damages caused by biotic and abiotic stresses to European beech threaten its survival (Bahnweg et al. 2005, Tomiscek 2006, FAO 2009, Gossner et al. 2014, Unterseher et al. 2016). For example, defoliation of European beech was caused by a combination of climate change, edaphic factors, and various biotic factors (FAO 2009). In addition, sun exposure or logging accidents caused cracks in beech bark accompanied by secondary fungal and insect infestations (Tomiscek 2006). However, fungal colonization was reduced more during the hot and dry seasons than during ozone treatment or sun exposure (Bahnweg et al. 2005). Local stand conditions in interactions with fungi in the phyllosphere, as well as leaf physiology influenced the diversity and composition of the leaf mycobiome (Unterseher et al. 2016). Microclimatic conditions had an important influence on the variability of herbivory (Gossner et al. 2014).

In Croatia, the health condition of European beech is influenced by biotic factors, insects, and fungi. The detected insects were bark beetles (*Typhorychus bicolor* and *Ernoporus fagi*), sucking insects (*Cryptococcus fagi*, *Phyllapsis fagi*), gall midge (*Mikiola fagi*), and pests of cambial tissue and wood-boring beetles (*Trypodendron domesticum*), long-horned beetle (*Hylecoetus dermestoides*), beech buprestid (*Agrilus viridis*), and small weevil (*Rynchaeus fagi*) (Hrašovec 2003). The beech fungal diseases included root Phythophthora infection, Nectria bark diseases, leaf diseases, red heart and decay fungi (Diminić 2003, Arač 2010, 2016, Pernek et al. 2011). The drought was identified as the most significant abiotic factor threatening European beech in Croatia (Harapin 2003). Particularly, the predominant climatic parameter affecting the crown condition of European beech trees on Medvednica Mountain were annual temperature and precipitation during the vegetation period (Potočić et al. 2008, Seletković et al. 2009).

The aim of the study was to provide insight into the differences in hypersensitivity to pests and diseases between provenances of international European beech provenance trial, considering local climatic conditions. It investigated: I) damage types, II) crown damage intensity, III) damage frequency, and IV) clustering of provenances based on damage types.

MATERIALS AND METHODS

Study Site

International European beech provenance trial is situated on Medvednica Mountain (45° 53'N, 15° 55'E, elevation 730 m, slope with west-northwest exposure). It consists of twenty-one natural populations (150 plants each) originating from Central Europe (DE, CH, AT, and HU) and Southeast Europe (HR, BA, SR, and RO) (Table 1). It was established by the Croatian Forest Research Institute in March 2007 (Ivanković et al. 2008a, 2008b, Bogunović et al. 2020).

Health Status Assessment

The damage types in the European beech provenance trial were recorded by an overall visual tree assessment in July 2015. The presence of surviving and visually healthy trees was documented. For symptomatic trees, the overall crown, trunk, and branches were assessed for damage types. The damage types were classified according to symptoms caused by insect pests and pathogenic fungi. The following labelling was used for the damage types: colour change and curling of leaves caused by aphids (A), chewing damages (CD), galls (G), anthracnose (A), canker on the branch and trunk (C). The intensity of crown damage types was expressed as a percentage of the affected crown and scaled with an ordinal scale: 1=slight crown damage (<10%), 2=moderate crown damage (10%-50%), 3=severe crown damage (>50%). Canker infestation on the tree trunk and branch bark was recorded as follows: 0 represented the absence of a canker and 1 represented the presence of a canker.

Data Analyses

Descriptive statistics were carried out for the variables analysed. The data were calculated as follows and expressed as percentages: (I) survival = surviving trees / total planted tree; (II) health = healthy trees / surviving trees; (III) intensity of damage per provenance = damaged trees / surviving trees, and (IV) average intensity of damage (slight, moderate and severe) of each provenance. The calculations and graphs were performed with Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA). For statistical analyses, the sum of damaged trees per type of damage was calculated regardless of damage intensity. Chi-square statistics was used to test the distribution of damage types between European beech provenances, without data for aphids (Sokal et al. 1995). The data for aphids were excluded from the calculation due to small numbers. The statistical analyses for each type of damage are shown in frequency distribution tables and the mosaic maps. The optimal

Table 1. Data of the international European beech provenance trial (Medvednica Mountain, Croatia).

No.	Provenance label	Provenance name	Country	Latitude (N)	Longitude (E)	Elevation (m)
1.	HR24	Dilj Čaglinski	Croatia	45° 17'	18° 01'	350
2.	HR25	Vrani kamen	Croatia	45° 37'	17° 19'	600
3.	HR26	Lovrin kal	Croatia	45° 19'	14° 23'	900
4.	HR27	Miletka	Croatia	45° 24'	15° 01'	800
5.	BA30	Tajan, Zavidovići	Bosnia and Herzegovina	44° 23'	18° 03'	700
6.	HU42	Valkonya	Hungary	46° 30'	16° 45'	300
7.	DE46	Pfalzgrafenweiler	Germany	48° 46'	8° 35'	700
8.	DE47	Schelklingen	Germany	47° 59'	9° 59'	650
9.	DE48	Hoellerbach	Germany	49° 01'	13° 14'	755
10.	DE49	Hasbruch	Germany	53° 08'	8° 26'	35
11.	AT56	Scharnstein	Austria	47° 54'	13° 96'	480
12.	BA59	Bugojno Vranica, Bistrica	Bosnia and Herzegovina	43° 33'	17° 49'	750
13.	BA60	Tešanj, Crni Vrh	Bosnia and Herzegovina	44° 33'	17° 59'	500
14.	BA61	Bosanska Krupa, Bastra Čorkvaća	Bosnia and Herzegovina	44° 45'	16° 14'	720
15.	BA62	Dinara Devrek Tefen	Bosnia and Herzegovina	44° 06'	16° 30'	950
16.	RO63	Alesd	Romania	47° 11'	22° 15'	490
17.	RO64	Alba-Iulia	Romania	46° 10'	23° 05'	860
18.	CH65	Sihlwald	Switzerland	47° 12'	7° 21'	1050
19.	RS66	Avala	Serbia	44° 23'	20° 45'	475
20.	RS68	Fruška gora	Serbia	45° 10'	19° 55'	370
21.	RS69	Cer	Serbia	44° 12'	19° 50'	745

number of clusters was identified by hierarchical cluster analysis using complete linkage and Euclidean distance. K-means non-hierarchical cluster analysis was used to determine subsets of European beech provenances based on damage types. All statistical analyses were performed using the STATISTICA 13.5 statistical software (TIBCO Software, Palo Alto, CA, USA). Map generation was done with the QGIS software, version 2.16 (<http://qgis.osgeo.org>).

Local Climate Condition on Medvednica Mountain

The Croatian Meteorological and Hydrological Service provided meteorological data (maximum, average and minimum monthly temperatures, monthly precipitation, days with snow and frost) from August 2014 to July 2015, based on records from the state hydro-meteorological station Puntijarka (15°58'E, 45°55'N, 988 m), the closest weather station to the study site. A measure of site aridity proposed by de Martonne (1926) was given by the following relationship: $I = 12P / (T + 10)$, where I was aridity index, and input parameters were local monthly precipitation (P) in millimetres and average temperature (T) in degrees Celsius.

The values of the aridity index (I) were estimated according to the scale given by de Martonne (36): Dry ($I < 10$); Semi-dry ($10 \leq I \leq 20$); Mediterranean ($20 \leq I \leq 24$); Semi-humid ($24 \leq I \leq 28$); Humid ($28 \leq I \leq 35$); Very Humid ($35 \leq I \leq 55$); Extremely humid ($55 < I$). Tables (S4 a, b, c) showed the meteorological data. These data confirm that the local climate could be described as Cfb according to the Köppen-Geiger climate classification, i.e. as a moderately warm, humid climate with warm summers and differences between the seasons (Zaninović et al. 2008). This climate has been termed „the climate of beech“ (Šegota and Filipčić 1996).

RESULTS

Types of Damage

The survey of the provenance trial started with determination of the number of surviving and healthy trees to provide data for descriptive statistics. The surviving ranged from 60% (RO6) to 94.67% (HU42) and healthy trees ranged from 4.67% (RO63) to 68.00% (DE47) (S1). The health

condition survey of the provenances was performed on the damaged trees. Four types of leaf damage were recorded: colour change and curling caused by aphids, chewing damages, galls, and anthracnose. Bark and wood damages recorded canker on the trunk and on branches. *Phyllaphis fagi*, a woolly beech aphid, was found on the underside of young beech leaves, causing yellowing and reddening as well as buckling of the leaf margins towards the underside (S2a). The winged and wingless viviparous beech leaf aphids were oval, pale yellow, and covered with dense waxy wool. Leaves chewing damages were present as bitten leaf sections (S2b) and leaf-mining (S2c). The causal insects of leaf bites and miners can be diagnosed by the mine shape, gallery and blotch. Many *Lepidoptera* and *Coleoptera* species produce leaf bites. Oval or elongated mines between veins with green lower epidermis without reticulation were caused by larvae of the moth *Phyllonorycter maestingella*. Mines that meandered between two lateral veins towards the leaf margin were caused by the larvae of the leaf miner moth *Stigmella* sp. The miner tunnels were filled with granular faeces. Also, larvae of the leaf miner beetle *Orchestes fagi* mine the leaves of beech. The mine begins as a duct on the midrib of the leaf and ends in a blotch. Woody, ovoid, unilateral galls (S2g), 4-10 mm long, produced by the beech gall midge *Mikiola fagi*, were present on the upper surface of beech leaves. Anthracnose disease symptoms on leaves were caused by the fungus *Apiognomonina errabunda*. The symptoms, i.e. leaf spots, develop from the leaf veins and spread over the leaf (S2e). Canker was observed on branches and the trunk. Symptoms included necrosis, sunken tissue, orange to reddish bark coloration, open canker, and tissue dieback. Twig and branch canker were caused by *Nectria cinnabarina* (Tode); trunk canker (S2f) by *Neonectria ditissima* (Tul.). Thus, a total of four damage types were recorded on beech leaves, with three damage types caused by insects and one by a pathogenic fungus. Canker, recorded on the bark of the trunk and on branches, was caused by pathogenic fungi.

The analysis of types of damage per tree showed a prevalence of three damage types. However, in provenances DE47, RS68, RO64, BA6, HU42 and provenance HR27, two and four types of damage per tree were observed, respectively. Trees with five damage types were not observed in this study. In general, trees with one damage type were most common, ranging from 63.92% (HR27) to 91.43% (DE47) (Figure 1).

Intensity of Crown Damage

The survey of the intensity of the European beech crown damage is shown in Figure 2. Eleven provenances were not infested by aphids; ten provenances showed slight crown damage by aphids, except RS66, showing 0.83% of the trees had severe crown damage (Figure 2a). Although galls were present in each provenance (Figure 2b), the most moderate and severe crown damage was observed in DE48 and DE46 (15.04% and 46.72% of the trees, respectively). BA62 was the least affected by galls and showed slight crown damage (7.07% of the trees). Moderate chewing crown damage was present in the HU42, DE48, RS66, BA62, and BA30 ranging from 0.70% to 1.41% of the trees. Severe chewing crown damage occurred in RS66 (0.83% of the trees) and BA62 (3.03% of the trees) (Figure 2c). Slight anthracnose crown damage was present in all provenances up to 48.89% of the trees (RO63) (Figure 2d). The provenance with the highest percentage of moderate anthracnose crown damage was RO64 (9.32% of the trees). The most severe anthracnose crown damage was found in BA60 (4.26% of the trees). Taken together, the lowest and the highest average slight crown damages were related to aphids (0.59% of the trees) and anthracnose (31.53% of the trees), respectively. The lowest average moderate and severe crown damages were related to aphids (0.00% and 0.83% of the trees, respectively). The highest average moderate and severe crown damages were related to galls and anthracnose (5.63% and 31.53% of the trees, respectively). The significant statistical difference between intensities of crown damages was shown: aphids (sample size=2473, $\chi^2=26.430$; $p<0.0001$), galls (sample size=2473, $\chi^2=1110.780$; $p<0.0001$), chewing damages (sample size=2473, $\chi^2=243.950$; $p<0.0001$) and anthracnose (sample size=2473, $\chi^2=1.286$; $p<0.0001$). Thus, examination of the intensity of crown damage in the provenance trial showed the predominance of slight crown damage (less than 10%) in four types of damage (aphids, galls, chewing damages, and anthracnose).

Branch canker was the lowest on the provenance DE49 (0.78% of the trees), the highest on HR26 (6.98% of the trees), and was not present on RO64, RS66, and CH65. Trunk canker was the lowest on DE49 (0.78% of the trees) and the highest on BA60 (13.83% of the trees) (Figure 3). The significant statistical difference between intensity of canker on trunk and branch was shown (sample size=2473, $\chi^2=37.923$; $p<0.0001$).

Table 2. Types of damage on European beech trees in the international provenance trial.

Type of damage	Cause of the damage (insects and fungi)
Color changing and curling of leaves	<i>Phyllaphis fagi</i> (L.)
Chewing damages on leaves	<i>Orchestes fagi</i> (L.), <i>Phyllonorycter maestingella</i> (Müller), <i>Stigmella hemargyrel-la</i> (Kollar), <i>Operophtera brumata</i> L., <i>Erranis defoliaria</i> (Clerck)
Galls on leaves	<i>Mikiola fagi</i> (Hartig)
Anthracnose on leaves	<i>Apiognomonina errabunda</i> (Rob.) Hohn.
Canker on the branch	<i>Nectria cinnabarina</i> (Tode)
Canker on the trunk	<i>Neonectria ditissima</i> (Tul.)

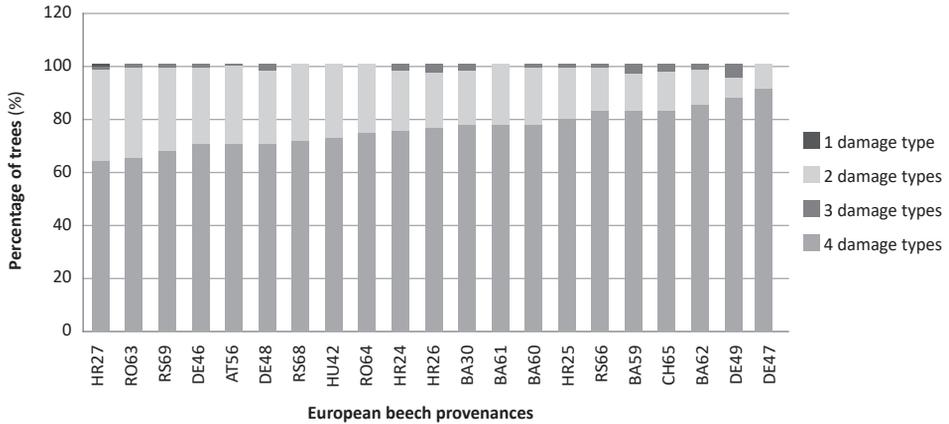


Figure 1. Percentage of trees of European beech provenances damaged by different damage types (Coding label of provenances: see Table 1).

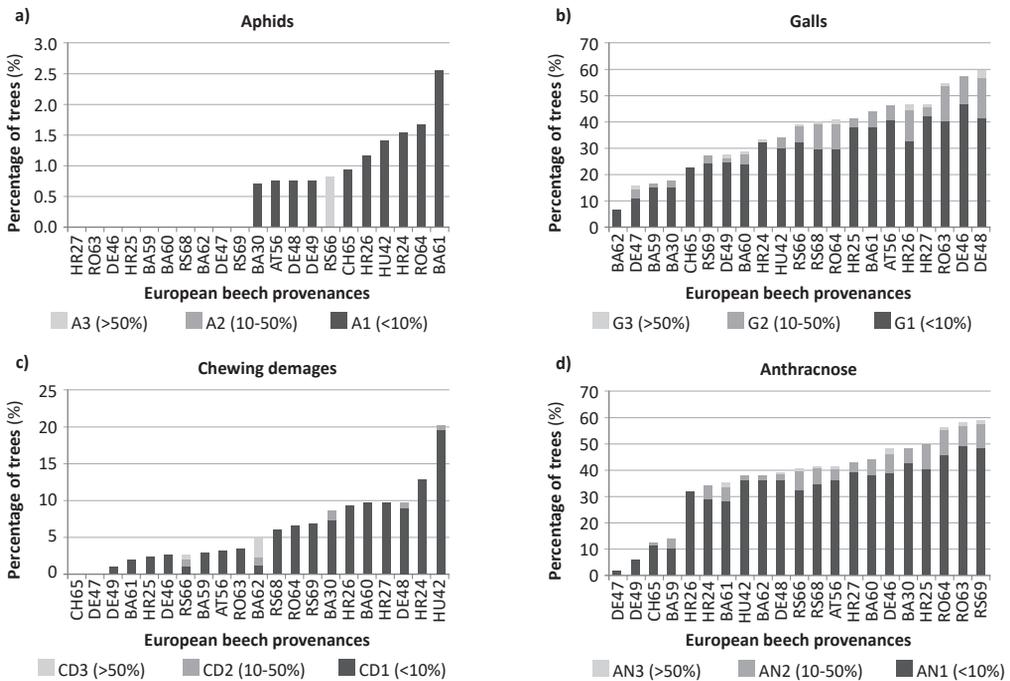


Figure 2. Intensity of crown damage by damage types: (a) aphids, (b) galls, (c) chewing damages, (d) anthracnose on European beech provenances (Coding label of provenances: see Table 1).

Frequency and Provenance Clustering Based on Damage Types

Frequency analyses of damage types are shown in frequency table (S3) and the mosaic map (Figure 4). According to damage frequency, anthracnose was the most common damage (42.62%), followed by galls (41.72%), canker (7.88%), chewing damages (7.02%), and aphids

(0.76%). Anthracnose, galls and canker were found in each provenance. Provenances DE47 (0.09%) and BA30 (3.23%) were the least, HR25 (3.23%) the most anthracnose-damaged provenances. The provenances that were the least and the most damaged by galls were BA62 (0.33%) and DE48 (3.75%), respectively. The lowest and the highest canker damages were observed on DE49 (0.09%) and DE47 (0.85%),

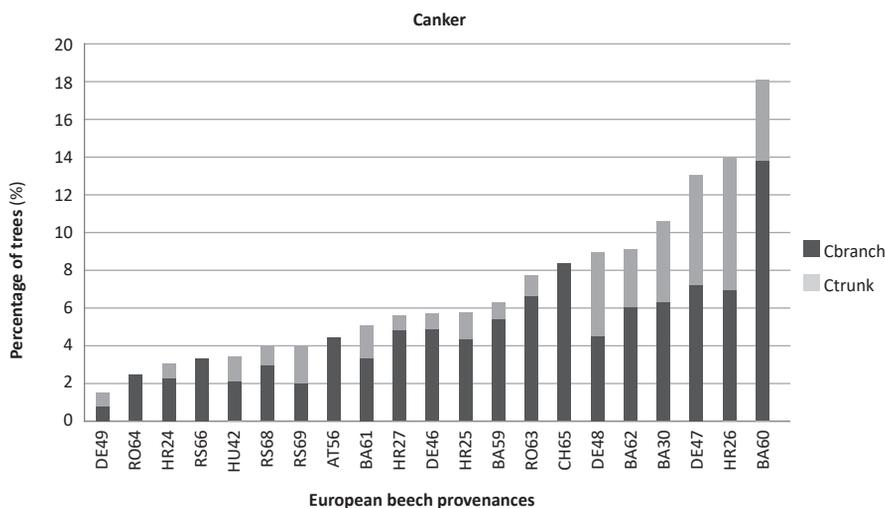


Figure 3. Percentage of canker per provenances (Coding label of provenances: see Table 1).

respectively. Chewing damages were detected in the range of 0.05% (DE49) to 1.38% (HU42) in most provenances, except for DE47 and CH65. Aphids were detected in the range from 0.05% to 0.14% in 11 provenances: AT56, BA30, HR26, DE48, DE49, CH65, RS66, HU42, HR24, RO64, and BA61. In the absence of aphid damage, the statistical chi-square test for damage to European beech provenances showed that there were significant statistical differences between provenances based on damage types (sample size=2091, $\chi^2=322.19$; $p<0.0001$). Thus, the dominant types of damage, anthracnose and galls, showed a total frequency of 84.34%, while canker, chewing damages, and aphids showed a total frequency of 15.66%.

The European beech provenances were grouped into four clusters based on damage type (Figure 5-7). Cluster 1 was characterized by the highest damage type of galls, followed in descending order by anthracnose, chewing damages, canker, and aphids. Cluster 1 included six provenances from Southeast Europe: RO63, BA61, HR24, HR26, RS68, and RS66, as well as one from Central Europe, HU42. Cluster 2 was characterized by the highest damage type by galls, followed in descending order by anthracnose, canker, chewing damages, and aphids. Cluster 2 included three Central European provenances (DE49, CH65, DE47) and Southeast European provenance BA59. Cluster 3 was characterized by the highest damage type due to galls, followed in descending order by anthracnose, chewing damages, canker, and aphids. Cluster 3 consisted of ten provenances from Central and Southeast European origins: DE48, HR27, AT56, DE46, HR25, RO64, BA30, BA60, BA62, and RS69. Cluster 4 was characterized by the highest anthracnose damage, followed in descending order by galls, canker, chewing damages, and aphids. Cluster 4 consisted of Southeast European provenances BA30, BA60, BA62 and RS69. Based on these data, aphids and galls caused the least and most damage, respectively, in all clusters except Cluster 4, where anthracnose caused the most damage. Provenances from Southeast Europe and Central Europe

predominated in Cluster 1 and Cluster 2, respectively. Cluster 3 contained provenances from both origins. Cluster 4 was the smallest and most homogeneous, consisting of Southeast European provenances at approximately the same latitude (44°06'N-44°33'N). Taken together, the provenances (DE46, HR25, HR27, AT56, RO64 and DE48) with the most damaged trees were grouped in Cluster 3 whereas the provenances (DE49, DE47, CH65 and BA59) with the least damaged trees were grouped in Cluster 2. The Central and Southeast European provenances did not form discrete clusters based on clustering analysis.

The international European beech provenance trial was set up with a different number of representative provenances from each country. Germany was represented by four provenances. Two of the German provenances, the southwestern (DE46) and the southeastern (DE48) provenances, were assigned to Cluster 3 and the other two provenances (DE49 and DE47) to Cluster 2. DE49 originated from the northern part of Germany and was the northernmost provenance in the entire provenance trial. The other three German provenances were from the southern part of the country. The southernmost provenance was DE47 with the lowest damage among the German provenances, but the highest canker damage among the 21 provenances. The German provenances showed the highest susceptibility to galls, followed in descending order by anthracnose, canker, chewing damage, and aphids. The Swiss provenance (CH65) originated from the northwest of the country, near the French border, and was grouped into Cluster 2. Interestingly, chewing damage was not observed on CH65. Canker was the highest damage type of CH65, followed in descending order by galls, anthracnose and aphids. The Austrian provenance (AT56) was from the central part of the country and was grouped to Cluster 3. The highest damage type of AT56 was galls, followed in descending order by anthracnose, canker, chewing damage and aphids. Hungarian provenance (HU42) from the country's southwestern region, close to the Croatian border,

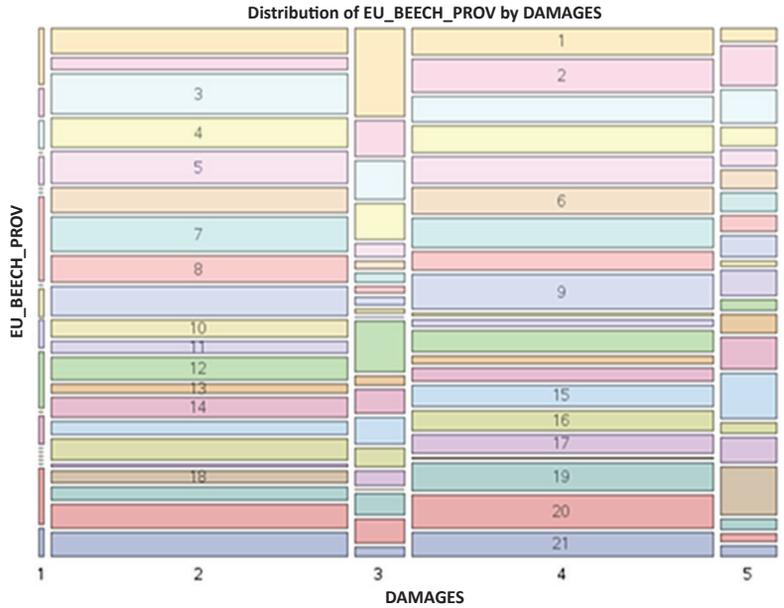


Figure 4. Mosaic plot of the distribution of damage types in European beech provenances based on S3. Axis X: 1. Aphids, 2. Galls, 3. Chewing damages, 4. Anthracnose, 5. Canker. Axis Y European beech provenances 1. HU42, 2. BA30, 3. DE48, 4. HR27, 5. AT56, 6. RO63, 7. DE46, 8. BA61, 9. HR25, 10. DE49, 11. CH65, 12. HR24, 13. BA59, 14. HR26, 15. BA60, 16. RS68, 17. BA62, 18. DE47, 19. RS69, 20. RO64, 21. RS66 (Coding label of provenances: see Table 1).

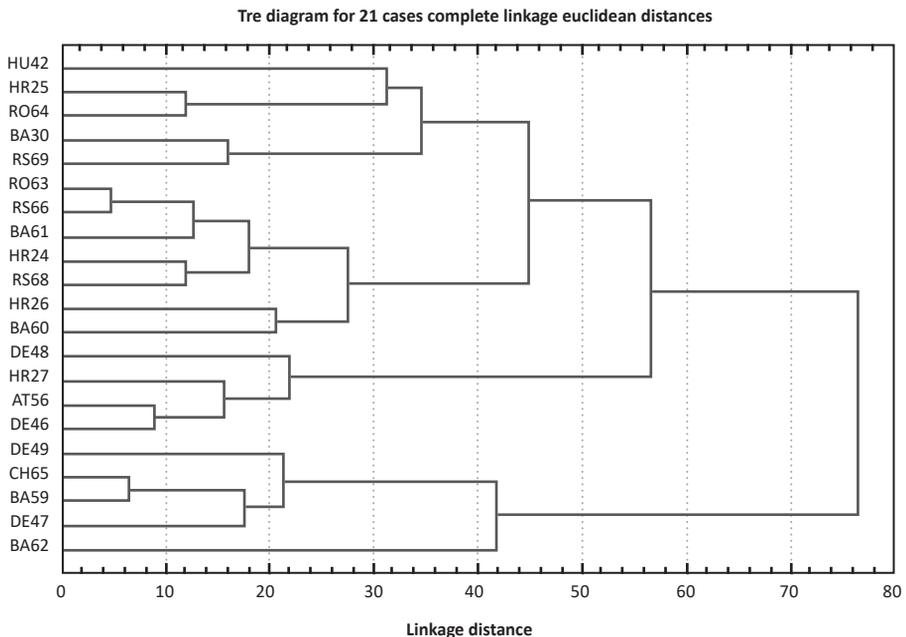


Figure 5. Dendrogram of 21 European beech provenances using five types of damage (aphids, galls, chewing damages, anthracnose and canker) produced by cluster analysis (complete linkage, Euclidean distance) using a cut point with four clusters (Coding label of provenances: see Table 1).

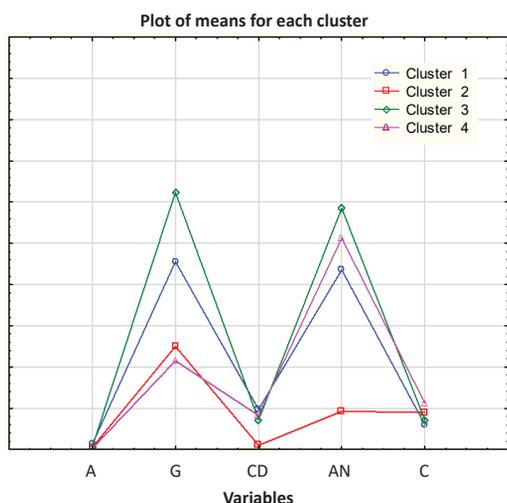


Figure 6. The plot of means for clusters of damage (X axis: A–Aphids, G–Galls, CD–Chewing damages, AN–Anthracnose, C–Canker, Y axis: % of damaged trees) of European beech provenances using the non-hierarchical k-means procedure.

was grouped into Cluster 1. The highest damage type of HU42 was anthracnose, followed in descending order by galls, chewing damages, canker and aphids. Two of the four Croatian provenances (HR26 and HR27) originated from the southwestern part of the country. HR26 originated from the Istrian peninsula. The remaining two provenances (HR24

and HR25) originated from the eastern part of the country, Slavonia. Interestingly, HR 24 and HR26 were grouped in Cluster 1, HR25 and HR27 were grouped in Cluster 2. HR25 was the most damaged. HR26 was the least damaged provenance. Galls were the highest damage type of the Croatian provenances, followed in descending order by anthracnose, chewing damage, canker and aphids. Bosnia and Herzegovina had the highest number of provenances. They were divided into three clusters: Cluster 1 (BA 61), Cluster 2 (BA59), and Cluster 4 (BA 62, BA60 and BA30). BA61 was the most damaged while BA62 was the least damaged. The highest damage type was anthracnose, followed in descending order by galls, canker, chewing damages and aphids. Serbia was represented by three provenances originating from the centre of the country. Two of the provenances were grouped into Cluster 1 (RS68 and RS66) and one provenance (RS69) was grouped into Cluster 4. RS66 was the most damaged provenance and RS68 was the least damaged provenance. Canker was present in all three provenances to the same extent. The highest damage type was anthracnose, followed in descending order by galls, chewing damages, canker and aphids. Thus, anthracnose and galls were the dominant damage types in all countries except for the Swiss provenance (CH65) where canker was the dominant damage type. Furthermore, the provenances of one particular country were generally classified into several clusters.

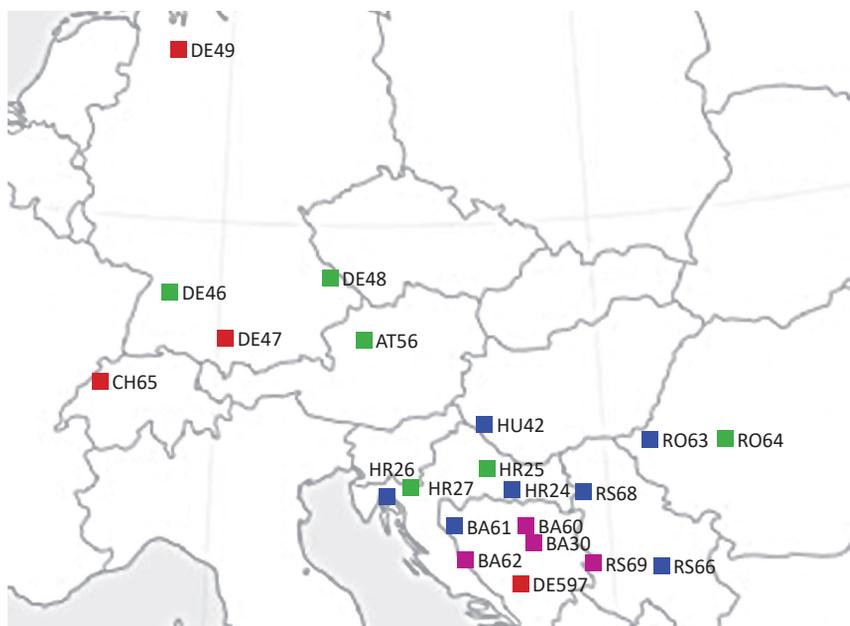


Figure 7. Map of origin of European beech provenances (coloured rectangles) of the provenance trial located in Croatia, Medvednica Mountain (black triangle). The colours represent different clusters of provenances analysed by the non-hierarchical k-means procedure: Cluster 1, blue; Cluster 2, red; Cluster 3, green; and Cluster 4, violet (Coding label of provenances: see Table 1).

DISCUSSION

The health condition of the European beech is concerning due to climate change. We investigated the health condition of European beech provenances in field trial located on Medvednica Mountain in Croatia. Medvednica Mountain is a natural environment for European beech covering 63.6% of the forest area. Thus, the provenance trial grows under the influence of abiotic and biotic environmental factors of the natural distribution area of this tree species. In addition, the international character of the provenance trial provided the opportunity to compare European beech provenances from Central and Southeast Europe.

The insect damages (aphids, galls and chewing damages) and damages from the fungal diseases (anthracnose and canker) of European beech provenances were almost equally represented (S3). Similar types of damage have already been reported (Gossner et al. 2014). Frequency analysis revealed that the presence of aphids was very low, less than 1%, while chewing damages were in the range of 10% and galls in the range of 50% (S3). Notably, aphids have a complicated life cycle with multiple developmental stages and morphology, and about ten generations of aphids of wingless and winged forms emerge annually in the ascending season (Van Driesche 2012). Sexual reproduction occurs in the autumn when eggs are produced for overwintering. In addition, aphid reproduction is more temperature-dependent than aphid generation (Ivrsen and Hardig 2007). The development of galls is promoted by light, warm temperatures and dry periods (Kelch 2016). Adult *M. fagi* leave the galls and mate in spring. The female lays eggs in April. The galls are well-developed by June. In autumn, the galls fall from the leaves with the larvae and overwinter on the ground. Considering the analysed meteorological data of Medvednica Mountain, the winter of 2015 was cold, with mean temperature between -0.2°C and 2.1°C from January to March. During the winter of 2015, most snowfalls occurred in January, February, and March (S4b). Thus, the high mortality of insects was favoured by low winter temperatures, as confirmed by the low percentage of trees affected by aphids and chewing damages. Galls affected a higher percentage of trees than aphids, indicating better overwintering of *M. fagi* larvae compared to aphid eggs. However, the milder winters and less snowpack will favour propagation and survival of the beech scale, as Stephenson and Ribarik Coe (2017) stated. Fungi are spread by wind and insects. They invade plants through wounded tissue, although they also enter through natural openings such as stomata, hydathodes, and lenticels. For infection, fungi require humidity, and fungal diseases are most commonly reported during rainy weather events (Bahnweg et al. 2005). *Apiognomonia errabunda* causes disease symptoms in leaves and twigs, resulting in premature leaf loss and the dieback of young shoots (Butin 1995). It occurs in buds, bud scales, and the bark of twigs and stems during the growing season (Sieber and Hugentobler 1987, Kowalski and Kehr 1996), sometimes without any disease symptoms (Petrini 1991, Sinclair and Cernauskas 1996, Stone et al. 2000). The fungus overwinters in leaf and branch canker tissue, and infection of emerging leaves occurs in the following spring by spores. However,

the degree of destruction is highly dependent on climatic factors. During budburst and early leaf formation, constant temperatures of 10°C - 14°C are essential for spore growth and infection. During this period, higher or lower average temperatures reduce disease severity. Thus, the average temperatures on Medvednica Mountain were below 10°C in March and April 2015, indicating unfavourable conditions for spore development. In May, the average temperature was above 10°C (S4a), favouring spore development. In addition, *A. errabunda* requires water to spread and infect; it does not spread under dry conditions. During the period of budburst and spore production in the early spring in April, the aridity index was semi-humid. The aridity indices ranged from extremely humid in May to humid in June (S4c). As a result, anthracnose was found in more than 40% of the damaged trees (S3) because of the high humidity.

Canker diseases are caused by the fungi *Nectria cinnabarina* and *Nectria ditissima*. *N. cinnabarina* is indicated by branch necrosis and dieback of young stems. In contrast, *N. ditissima* causes cankers on the trunk. These pathogens attack weakened trees under stressful conditions such as frost, drought, physical damage and fungal influence. They are saprophytes and weak parasites. Sites of infection include frost-damaged beech bark, bits of rodents or game, and openings such as lenticels (Montecchio 2011). Once the canker has infected the branch, dieback cannot be prevented. In beech forest stands, canker affects beech more frequently at lower altitudes and warm and dry areas (Metzler and Erffa 2000). In the present study, a low percentage of trees, ranging from 0.09% (DE 49) to 0.81% (BA60), were infested at low intensity (less than 10%) by *N. cinnabarina* and *N. ditissima* (Figure 3).

Clustering analysis allowed the grouping of provenances into four clusters, showing no grouping of Central and Southeast European provenances into separate clusters. Cluster 2 consisted of three Central European provenances (DE49, DE47 and CH5) and Southeast Europa provenance BA59, while Cluster 3 consisted of three Central European provenances (DE46, DE48 and AT56) and Southeast European provenances (HR25, HR27 and RO64). Cluster 4 was composed of Southeast European provenances, mostly from Bosnia and Herzegovina (BA62, BA60 and BA39), and including one provenance from Serbia (RS69). Central and Southeast European provenances, as well as the provenances of each country, could not be clearly separated according to damages by cluster analysis.

Three factors should be present to support diseases, constituting a disease triangle: one side of the triangle is the host plant, the other side is the disease-causing organism, and the third side are the environmental conditions (Stevens 1960, Francl 2001). Considering the environmental conditions at the provenance trial location, European beech grows in an optimal habitat that ensures good physiological conditions for tree growth and development. The local climate on Medvednica Mountain is continental, typical for the Central European mountains, with high precipitation and low temperatures. The maximum precipitation occurs during the warm season. This type of climate has been called "the climate of beech" (Šegota and Filipčić 1996). Thus, damaging insects and pathogenic fungi were presented at low intensity

in this provenance trial due to the significant number of days with low temperatures, snow and frost in 2014 and 2015.

The limitations of this study should be acknowledged. Data collection was conducted on an unequal number of provenances from each country. Bosnia and Herzegovina was represented by five provenances, Germany and Croatia by four provenances. Serbia and Romania were represented by three and two provenances, respectively. Switzerland, Austria, and Hungary were represented by one provenance. Therefore, the results refer only to these provenances and cannot be generalized. In addition, this study provided an overview of damage types occurring in the middle of the vegetation period (July 2015). Future multi-seasonal and multi-annual studies will obtain a broader picture of damage types and intensity.

CONCLUSIONS

The results of this study provide information on the health condition of the International European beech provenance trial on Medvednica Mountain in Croatia. Tree survival was very high, with a predominance of healthy trees and a dominance of one disease per damaged tree. The five types of damages analysed were mostly present at low intensity (less than 10%). Anthracnose and galls were the most common damages, followed in descending order by chewing damage, canker, and aphids. Statistically significant differences in damage were found among provenances ($\chi^2=322.19$, $p<0.0001$). Cluster analysis of provenances according to damage showed four clusters. However, clustering did not separate Central European from Southeast European provenances, since both were found in the less and the most damaged cluster. Besides, the provenances of individual countries were clustered into multiple clusters. The favourable climatic and environmental conditions at the location of the provenance trials resulted in the good health of the European beech trees. These data are important for the projection of European beech forest survival and maintenance of the good quality of the forest stand. Considering climate change, continuous monitoring of the occurrence of European beech damage is necessary, drawing particular attention to extreme events.

Author Contributions

MI, SNA, NC and MGP conceived and designed the research, SNA, NC, ML and SB carried out the field measurements, NC processed the data, NC and AJ performed the statistical analysis, MI secured the research funding, supervised the research, MI, MGP, AD, and HR helped to draft the manuscript, NC, SNA, and AJ wrote the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

Supplementary Materials

Supplementary File 1. Percentage of surviving and healthy trees of the international European beech provenance trial.

Supplementary File 2. Damage types observed on the international European beech provenances trial: (a) *Phyllaphis fagi* on the leaf, (b) chewing damages - bitten parts of leaf, (c) leaf-mining, (d) galls of *Mikiola fagi* on leaf, (e) Anthracnose on leaf, (f) Canker on beech stem.

Supplementary File 3. Frequency table data of European beech provenances in the international European beech provenance trial.

Supplementary File 4. Meteorological data obtained from the Croatian Meteorological and Hydrological Service weather station Puntijarka in 2014–2015: (a) Monthly precipitation (MP), maximum (Max), average (AMT), and minimum (Min) monthly temperatures, (b) Snow and frost data, (c) Monthly de Martonne aridity index.

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