MORPHO-ANATOMICAL ASPECTS OF FOLIAR LAMINA AND BEECH NUT QUALITY OF *Fagus sylvatica* L. FROM TWO NATURAL PROTECTED AREAS IN THE ROMANIAN CARPATHIANS

PETRUȘ-VANCEA Adriana, POP Daniela-Nicoleta, SUCEA Felicia Nicoleta, DUMBRAVĂ Amalia-Raluca, MOȘ Andreea-Selena, CICORT Abigail, CUPȘA Diana

Abstract. In 2022 we investigated the morphological and structural parameters of the foliar lamina and the quality of beech seeds (*Fagus sylvatica* L.) from two natural protected areas, the Jiu Gorge National Park, and the Iron Gates Natural Park, from individuals in unpolluted areas, compared to those from trees on European roadsides crossing the two natural protected areas: E 79 and E 70. In both natural protected areas, pollution had the same effect on the investigated features. Therefore, we found that there was an adaptation of the leaves to pollution caused by road traffic, by decreasing stomata size and increasing stomata density, but also decreasing leaf size. These changes were independent of altitude differences. On the other hand, harmful effects of air pollution near roads were the large areas of beech leaves with necrosis, decreased dry mass, and on a histological level, large lesions in the fundamental parenchyma, peri phloem sclerenchyma, and vascular bundle.

Keywords: epidermis, leaf structure, mass dry, pollutants, stomata, Jiu Gorge National Park, Iron Gates Natural Park.

Rezumat. Aspecte morfo-anatomice ale limbului foliar și calitatea jirului de fag (*Fagus sylvatica* L.) din două arii naturale protejate din Carpații României. În anul 2022 am investigat parametrii morfologici și structurali ai limburilor foliare, precum și calitatea semințelor de fag (*Fagus sylvatica* L.), din două arii naturale protejate Parcul Național Defileul Jiului și Parcul Natural Porțile de Fier, provenite de la indivizi aflați în zone nepoluate, comparativ cu cele de la arbori aflați la marginea drumurilor europene, care străbat cele două arii naturale protejate: E 79 și E 70. În ambele arii naturale, poluarea asemănătoare a avut același efect asupra caracteristicilor investigate. Prin urmare, am constatat că a existat o adaptare a frunzelor la poluarea cauzată de traficul rutier, prin scăderea dimensiunilor stomatelor și creșterea densității acestora, dar și scăderea dimensiunii frunzelor. Aceste schimbări au fost independente de diferența de altitudine. Pe de altă parte, ca efect nociv al poluării aerului din preajma drumurilor, la nivelul frunzelor de fag au fost identificate suprafețe mari cu necroze, masă uscată diminuată, iar histologic s-au evidențiat leziuni mari la nivelul nervurilor principale, atât în parenchimul fundamental, subepidermic, cât si la nivelul sclerenchimului perifloemic și al fascicolelor libero-lemnoase.

Cuvinte cheie: epiderma, structura frunzei, masa uscată, poluanti, stomate, Parcul Național Defileul Jiului, Parcul Natural Porțile de Fier.

INTRODUCTION

Common beech (*Fagus sylvatica* L., fam. *Fagaceae*) is a deciduous species found in temperate marine climate regions of Central and Western Europe (BOLTE et al., 2007). It prefers humid soils and has an ecological role, from the point of view of the study of climate change (ROIBU et al., 2022). The beech extends southward into the Mediterranean basin, where its distribution is limited to mountain regions with a suitable amount of precipitation (GARCÍA-PLAZAOLA & BECERRIL, 2000). Even in these sites, many beech forests can be considered relicts, as they are present at the limits of their ecological requirements (GARCÍA-PLAZAOLA & BECERRIL, 2000). The classification of Carpathian beech forests as a tertiary relict only refers to some herbaceous species in these forests (LUICK et al., 2021). Pollen analysis indicates that the beech had a discontinuous presence in Northern Romania since the late Preboreal Period (LUICK et al., 2021). It first appeared in Romania in the Subatlantic, probably from the proximity of the Southeastern Balkan Peninsula or Podolian Highlands (POP, 1945; DONIȚĂ, 1989). Due to its biological characteristics, especially the fact that it loves the shade, the beech led to the elimination of the sessile oak and oak, respectively, the local lowering of the upper limits of their areas (PAȘCOVSCHI, 1967). The expansion of the beech also had a character of phylogenetic expansion. So, the beech lived in the forests of the current Romanian territory in the Tertiary, then it was eliminated and returned to the Holocene (PAȘCOVSCHI, 1967). The beech bears the imprint of intense hybridizations from the past. After the establishment of climate, it began to expand intensively, especially the species *F. sylvatica* (PAȘCOVSCHI, 1967).

Large-scale models indicate increasing beech decline in recent decades (NEYCKEN et al., 2022). On the other hand, beech trees occupy areas with a wide range of ecological conditions, suggesting that they can adapt (PEUKE et al., 2002). Even though in Romania, beech is the dominant species in forests (CHIRIȚĂ et al., 1981), since the Medieval Period, due to anthropic activities, it went into decline, the present composition of forests in the Romanian Carpathians being different from the original one (GRINDEAN et al., 2019). Thus due to anthropic interventions, the amount of beech and other oaks in the forests of Romania decreased in favor of spruce (MUNTEANU et al., 2016).

The territory of the Jiu Gorge National Park (JGNP) is 85% covered by the forest (MANAGEMENT PLAN, 2022). The beech forests shelter a great variety of herbaceous species, but also fauna (MANAGEMENT PLAN, 2022). TOMESCU et al. (2011), studying 10 beech forests in this park, identified that they shelter a high diversity of terrestrial isopods. Beech forests in Southern and Eastern Central Europe have been found to host distinct species assemblages, including larger contingents of saproxylic beetles and carabids in the Carpathians and narrow-range endemic gastropods

(WALENTOWSKI et al., 2014). Forests of the JGNP shelter a unique relict fauna in the case of saproxylic beetles, important at the European level (BUSSLER et al., 2005). These forests, dominated by beech, shelter a diverse leaf litter fauna, uniformly distributed in the park, represented by the native fauna of the region (CICORT-LUCACIU et al., 2020).

The Iron Gates Natural Park (IGNP) contains habitats specific to the European continental and alpine biogeographic zone, with a slight Mediterranean influence due to the movement of air masses along the Danube (ROZYLOWICZ et al., 2022). These features have contributed to the expansion of specific forests, shrubs, and grasslands towards Central Europe (for example, Middle-European and Dacian beech forests) and Balkan Europe (SANDA et al., 2008; HURDU et al., 2012). Beech is present in forests with woodruff (*Asperulo-Fagetum* beech forests), but also in areas of forest regeneration (MANAGEMENT PLAN, 2020). On the Danube Gorge beech is present at its lowest altitude in the country; other low-altitude populations, where the beech is considered relict are also present in other areas from south-western Romania (see in PAȘCOVSCHI, 1967).

Both parks are crossed by busy national roads. If invertebrate and vertebrate animals, due to their degree of mobility, become victims of road accidents (SEIBERT & CONOVER, 1991; ASHLEY & ROBINSON, 1996; BAXTER-GILBERT et al., 2015; CIOLAN et al., 2017; COVACIU-MARCOV et al., 2022), plants near roads are not left unaffected either (KHALID et al., 2020), as they absorb pollutants on their leaf surface (GOSTIN, 2009). Vehicle emissions create pathways of increased carbon and nitrogen concentrations near roads, which affect surrounding ecosystems. So, air pollutants from vehicle emissions have direct and indirect effects on roadside plant metabolism even before visible symptoms occur (VISKARI et al., 2000). Woody plants can be capable of taking up to 60% of nitric acid from polluted air (PADGETT et al., 2009), especially since their nitrogen content increases near heavily trafficked roads or industrial areas (AMMANN et al., 1999). Atmospheric nitrogen deposition is a major cause of global biodiversity loss due to air pollution (MARTÍNEZ et al., 2021). The difference in average C and N concentrations along roads depends on the distance between the trees and the road, traffic volume, and seasonal variations (KHALID et al., 2020). In 2005, over 500 journal articles and international conference papers were reviewed (Transportation Research Board and National Research Council, 2005), and the evaluation committee concluded that the environmental effects of roads are much larger than the roads themselves, and can extend far beyond the usual areas of quantification. To understand better their impact on ecological systems, information on the biodiversity components' resilience is needed. Leaf morphology is a fundamental functional characteristic of plants, expressing the balance between light capture and transpiration (BRUSCHI et al., 2003), therefore playing an important role in the plant's ability to adapt to ongoing environmental changes (McPHERSON et al., 2004; LIU et al., 2017). To reveal the variations depending on ecological gradients, some of the parameters frequently used are leaf weight and sizes (BUSSOTTI et al., 2005).

Interactions between plants and different types of pollutants from the perspective of vegetative organs structure have been studied by many authors. The response of different species to altered environmental conditions is related to their structural and functional characteristics. Studies show that plants develop a variety of morphological and anatomical changes under the influence of pollutants (VERMA et al., 2006; ALVES et al., 2008; GOSTIN, 2009; POURKHABBAZ et al., 2010; MITU et al., 2019; BIANCHINI, 2022).

On the other hand, seed quality directly impacts germination and successful seedling growth. The physical quality of the seed is related to characteristics such as size, colour, age, condition, and damage caused by diseases and pests (ELISOVETCAIA et al., 2018). Seed formation in beech is non-uniform over the years depending on annual climate variations (GAVRANOVIĆ et al., 2018). Climate changes and other environmental factors also have a significant impact on the seed viability and survival rate of beech forests. Therefore, environmental conditions have a greater influence on seed surface area than the age of trees (KALINIEWICZ et al., 2018).

The hypothesis from which we started our study was that air pollution caused by road traffic affects the morphology and anatomy of some beech vegetal organs. Thus, in the present study, we aimed to identify the particularities of some biological parameters in beech trees about the quality of the surrounding environment (polluted by road traffic and unpolluted) in two protected natural areas.

MATERIALS AND METHODS

Sampling. Vegetal material consisting of leaves and seeds with pericarp (nuts) of beech (*Fagus sylvatica* L.) were collected in August 2022, from 5 trees with similar ages, from four collection points in two natural protected areas, comparing the characteristics investigated in plants coming from unpolluted environment with those coming from a polluted environment affected by heavy traffic (Table 1). Leaves were collected from the shaded part of the plant, from the base of the crown of each tree, and analyzed as follows: about 100 g leaves for mass determination; 5 leaves each for macro- and micromorphology tests, all placed in polyethylene bags as soon as they were removed from the branches and transported in refrigerated thermo-insulated bags; 1 cm long fragments of 5 leaves were placed in 70º ethyl alcohol for subsequent anatomical studies. Morphological studies were carried out within 5 days of collection. Seeds that fell on the ground were collected, transported in plastic bags, and kept at 4 ºC during the winter. Although they can retain their viability for 5 years, their germination capacity decreases considerably if not kept in a cool environment (von WUEHLISCH, 2008).

Morpho-anatomical analysis. Morphological and macroscopic observations were made on leaves and seeds, with or without pericarp (Fig. 1), using an Olympus SDF PLAPO 1XPF stereomicroscope with Olympus UC 50 camera, while epidermis morphology and foliar lamina structure were studied using an Olympus IX73 Inverted LED fluorescence microscope with Olympus UC50 camera, made in Japan by Olympus Optical Co. LTD.

Leaf length, leaf width, and dry mass were measured for each set of leaves after drying 2 g of fresh substance from each sample at 40ºC for 7 days (when they reached constant weight).

Table 1. The description of the sampling points of *Fagus sylvatica* L samples.

Sampling areas	Sampling sites	Geographical coordinates	Age of tree	Altitude (m)
Jiu Gorge National Park (JGNP)	Chitu Valley (unpolluted area)	45°16'16.0"N 23°22'54.0"E	80-100	400-450
	E 79, Meri (polluted area)	45°13'13.8"N 23°22'49.5"E	80-100	350-400
Iron Gates Natural Park (IGNP)	Trescovăț Massif (unpolluted area)	44°34'00.4"N 22°03'22.2"E	80-100	700-755
	E70, Vodița (polluted area)	44°42'57.4"N 22°28'46.7"E	80-100	70-80

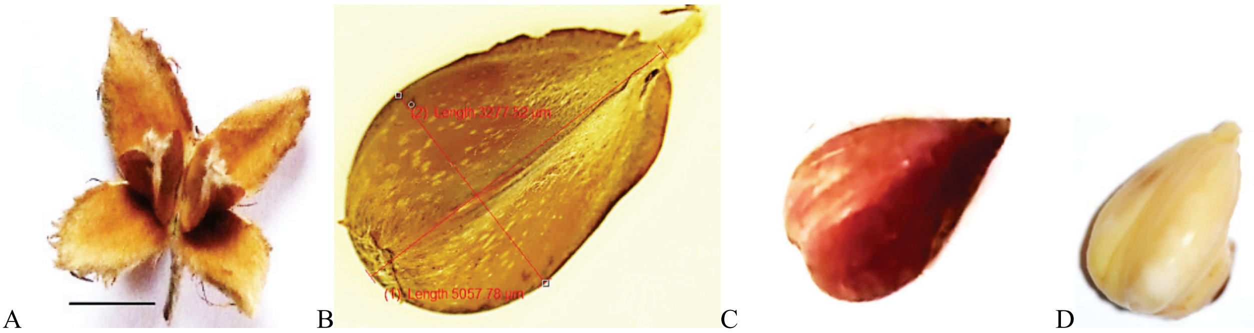

Figure 1. The beech fruit (A – bar = 2 cm); measurement types of nuts (seeds with pericarp) (B – 0.7X), seed with seed coat (C-0.7X); seed without seed coat (D – 0.7X).

The epidermis was imprinted by making replications of the leaf surfaces with a 2% collodion solution (dissolved in a mixture of equal parts ethyl alcohol and ether), according to the method described by ANDREI & PARASCHIVOIU (2003). The collodion solution was applied in a thin layer to the dry leaf surface on both the upper and lower half of the foliar limb from the middle zone. After 3 minutes, the solution solidified so it was skinned and examined under the microscope (ANDREI & PARASCHIVOIU, 2003). The mold represented the imprint of the epidermis surface. Evaluation of epidermal cell size, stomatal cell size, and stomatal number per microscopic field was performed with 20X objective and 10X (200X) ocular. Five measurements were taken for each sample.

Cross-sections were made by hand, using a razor blade, from the plant material preserved in 70% ethyl alcohol, in a transverse plan, at the level of the main vein and in the median area of the leaf, also following the ANDREI & PARASCHIVOIU (2003) method, to reveal the anatomical structure of the foliar lamina. The staining was done with Congo Red and Iodine Green.

Seed quality evaluation. The beech fruits were left to mature, in the cold, until February of the following year, then layered, and then 100 nuts with normal external appearance (no holes produced by insects and intact) were submerged in distilled water for 24 h, after which all traces of the pericarp were removed, identifying the presence or absence of the seed inside.

Statistical analyses. Descriptive statistics (mean and SD) and *t-test* for two different samples for each parameter to verify the significance of differences were made in Microsoft Excel 2019.

RESULTS AND DISCUSSION

Leaf morphology. On leaves of plants exposed to air pollution, both in JGNP (Fig. 2) and in IGNP (Fig. 3), macro-morphological changes were observed, such as necrotic areas and dust particles. Leaves near sources of air pollution accumulate particles, a fact reported as early as 1974 by RICKS & WILLIAMS.

Beech leaves from JGNP, from low altitudes were affected by aphids (*Phyllaphis fagi*) (Fig. 3 E si F). There can be numerous species of insects that affect beech, the entomofauna being represented by phytophages (STOIKOU & KARANIKOLA, 2014). Among the most common species are *Rhynchaenus fagi* (Coleoptera: Curculionidae), *Phyllobius pyri* (Coleoptera: Curculionidae), and *Anobium punctatum* (Coleoptera: Anobiidae). The largest population of insects can be seen in late spring, as the life cycle of leaf-feeding insects synchronizes with high rainfall (STOIKOU & KARANIKOLA, 2014).

Foliar lamina recorded longer lengths at samples from unpolluted areas, both in JGNP and IGNP, the differences being statistically significant (Table 2). The biggest foliar lamina, both by size and dry mass, were reported at beeches from Trescovăț (at 700 m altitude) and smaller at beeches from the edge of the road E70, near Meri (altitude 400 m). It is worth mentioning that beech trees located at the lowest altitude (70 m altitude) from our study (Vodița

Valley) present leaves of similar size to those on Trescovăț, their width being insignificantly smaller, but stomata density is significantly higher (Table 3), a fact reported in air polluted areas (GOSTIN, 2009).

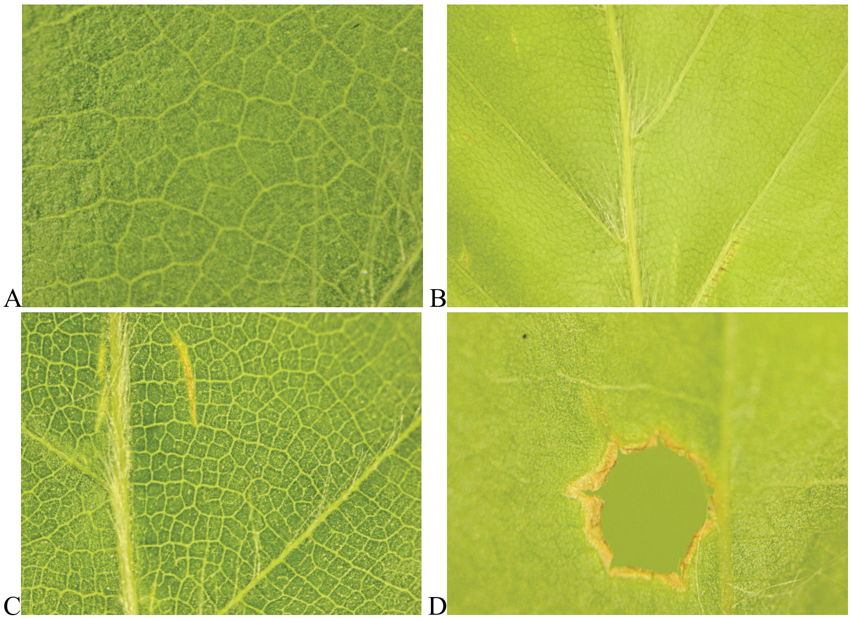

Figure 2. Aspects observed at stereomicroscope of beech leaves surfaces (*Fagus sylvatica* L.) sampled from Jiu Gorge National Park: Chitu Valley, adaxial (A– 4X) and abaxial epidermis (B – 1X) and E79 Meri, abaxial epidermis (C – 2X), adaxial epidermis (D – 4X).

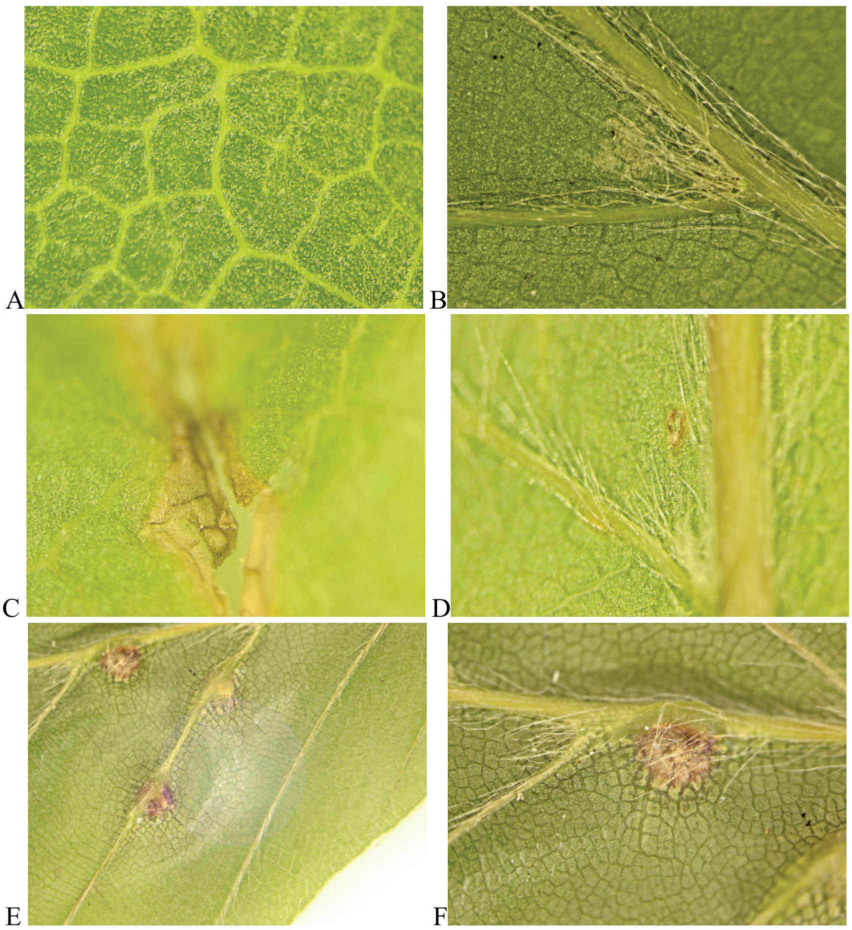

Figure 3. Aspects observed at stereomicroscope of beech leaves surfaces (*Fagus sylvatica* L.) sampled from Iron Gates Natural Park: Trescovăț, adaxial (A– 10X) and abaxial epidermis (B – 4X) and E70 Vodița, the adaxial epidermis(C – 8X), abaxial epidermis (D – 4X); abaxial epidermis of the beech leaves from E70 Vodița, attacked by aphids (E – 1X si F – 4X).

The size of epidermal cells, both on the adaxial face and on the abaxial one was in all cases smaller at beech trees on the side of the road than ones from unpolluted areas, in most of the cases, the negative differences being statistically significant (Table 3). The beech has no stomata on the adaxial epidermis, and the leaves have a hypostomatic arrangement (Table 3). The density of stomata of the abaxial epidermis was significantly higher in the leaves of beech trees exposed to pollution, in both cases protected areas, compared to non-polluted areas.

In our case, all leaves came from the shaded parts of the trees; this fact can also be found in their structure (Fig. 4). The difference between leaves exposed to the sun and shaded leaves is the number of layers of palisade tissue. Leaves with a single layer of palisade cells are considered shade leaves (GAUMANN, 1935; ESCHRICH et al., 1989).

Table 2. Means ± standard deviation and *t-test* for each measured parameter of foliar lamina of beech (*Fagus sylvatica* L.) from JGNP and IGNP, situated in unpolluted areas (Chitu Valley and Trescovăţ Massif), or car traffic polluted areas (E 79 Meri and E 70 Vodiţa) (P-value <0.05).

Parameter	**Dry mass (g)**		**Foliar lamina lenght (cm)**		**Foliar lamina width (cm)**		**Necrotic foliar surface**	
JGNP								
Sample	Chitu Valley	E 79 Meri	Chitu Valley	E 79 Meri	Chitu Valley	E79 Meri	Chitu Valley	E79 Meri
s±sd	0.86±0.01	0.53±0.01	7.83±0.85	6.1±0.53	4.10±0.36	3.63±0.61	+	+++
t-test	0.000		0.025		0.166		-	
IGNP								
Sample	Trescovăţ	E70 Vodiţa	Trescovăţ	E70 Vodiţa	Trescovăţ	E 70 Vodiţa	Trescovăţ	E 70 Vodiţa
s±sd	0.89±0.01	0.79±0.01	7.90±0.46	6.43±0.4	4.37±0.21	3.93±0.51	-	+++
t-test	0.000		0.007		0.140		-	

Table 3. Means ± standard deviation and *t-test* for each measured parameter of the epidermis of beech (*Fagus sylvatica* L.) folia lamina, from JGNP and IGNP, located in unpolluted areas (Chitu Valley and Trescovăţ Massif) or car traffic polluted areas (E 79 Meri and E 70 Vodiţa) (P-value <0.05).

Sample	**Chitu Valley**	**E 79 Meri**	***t-test***	**Trescovăţ**	**E 70 Vodiţa**	***t-test***
adaxial epidermis cell length (µm)	35.19±1.22	34.80±1.04	*0.606*	38.80±1.63	35.76±1.94	*0.029*
adaxial epidermis cell width (µm)	25.44±2.98	18.99±2.66	*0.007*	17.49±1.55	15.21±1.30	*0.037*
adaxial epidermis stomata number	0.00±0.00	0.00±0.00	-	0.00±0.00	0.00±0.00	-
abaxial epidermis cell length (µm)	39.77±2.55	36.48±1.76	*0.049*	46.86±1.30	44.4±1.05	*0.013*
abaxial epidermis cell width (µm)	21.97±3.68	21.80±2.15	*0.932*	29.74±1.99	26.5±1.00	*0.017*
abaxial epidermis stomata number	16.60±1.14	19.80±1.48	*0.006*	18.20±1.10	20.6±1.52	*0.023*
abaxial epidermis stomata length (µm)	22.60±3.33	22.36±2.22	*0.896*	26.70±3.08	24.06±1.19	*0.132*
abaxial epidermis stomata width (µm)	19.88±0.66	18.16±1.43	*0.053*	20.84±0.82	18.06±1.51	*0.011*
stomatal opening (µm)	5.93±0.14	5.20±1.30	*0.280*	0.00±0.00	0.00±0.00	-

Figure 4. Unistratificated palisade tissue under the adaxial epidermis (arrow).

The histology of foliar lamina from unpolluted areas was normally structured, specific to a dicotyledonous plant (Fig. 5 A-C). Instead, both foliar lamina of beech trees on road E 79 Meri (Fig. 5 B-C), and those on road E 70 Vodiţa (Fig. 5 D), showed cell destruction at the level of the subepidermal parenchyma in the abaxial part (Fig. 5 B-D), major lesions in the vascular bundle and perivascular sclerenchyma (Fig. 5 C). Pollution stress has altered the structure of beech leaves near national roads. However, the species is quite resistant to the action of air pollutants and, despite the changes observed, continues its development, producing, as we will see below, viable seeds.

Nut and seed morphology. Beech nuts have three edges and bristles (Fig. 6 A). The quality of beech nuts was affected in both parks (Fig. 6 B; Table 4). We assume that physical damage occurred only to nuts without seeds (because of the empty inside space).

The insects' presence (Fig. 6 C-F) was remarked on the beech fruits, but only in the Iron Gates Natural Park, the fruits on Trescovăţ being less affected by the holes produced throughout the pericarp by these insects, but at low altitudes, close to the national road E70, 25,6% of the nuts were punched (Table 4). The sizes of the holes in the nuts from Trescovăţ were, on average, 1336/804 µm, and those near the E70 road were 1309/631 µm. We have not identified the insects that caused the damage, and their attack could be in the months of June-July. Secondary metabolites such as polyphenols would be the key components of the trees' defense mechanisms against these insects (OSZMIANSKI et al., 2015).

It seems that beech is a species resilient to pollution, because, on DN 79 Meri, although the leaf lamina showed structural damage, the seeds were of superior quality, 90% of the embryos being present in the nuts.

The mass of healthy seeds was higher in nuts taken from the trees on the side of the road, both in JGNP and IGNP, compared with the unpolluted control area. These differences were not statistically significant (Table 4). This phenomenon could have different causes, depending on the park. Thus, in JGNP, nuts from the Chitu Valley were lighter but not smaller in size, while in IGNP, this phenomenon was not present in the unpolluted area. It seems that the lack of seeds in nuts does not depend on atmospheric pollution generated by road traffic because on E79 Meri, fruits with seeds were the best represented in number. In the IGNP, we registered the most numerous destroyed nuts in the beech trees on the side of road E70 (24%) (Table 4). Nuts from the Chitu Valley did not show traces of insects, and they were not punctured, but this could be influenced by the fact that they did not contain seeds.

On the Chitu Valley, nuts were large (Table 5) but empty, without perforations, and destroyed just like those near the E79 road. Even if the nuts were larger in JGNP, after removing the pericarp, they had small-sized seeds with black seed coats (Fig. 7 A). In IGNP, the seed coats were brown (Fig. 7 B). After the removal of the seed coat, all seeds were white (Fig. 7 C).

The seeds in JGNP were much smaller compared to those in IGNP (Table 5).

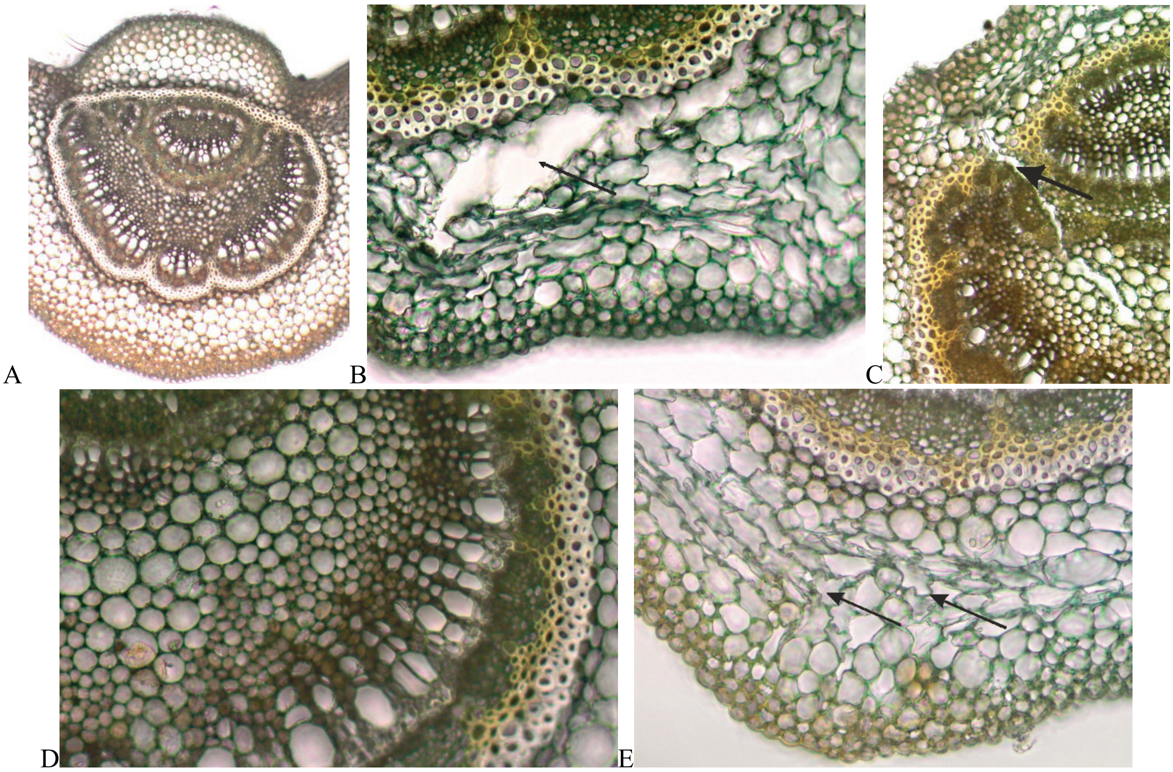

Figure 5. The foliar lamina structure of the beech leaves (*Fagus sylvatica* L.) sampled from: Jiu Gorge National Park: normal aspects of main vein – Chitu Valley (A– 100X); abaxial parenchyma destructions - E79 Meri (B – 200X); lesions in the vascular bundle and perivascular sclerenchyma – E 79 Meri (C – 200X), same as in Iron Gates Natural Park: normal structure - Trescovăț (D – 200X); parenchyma destructions - E 70 Vodița (E - 200X) (arrows indicated lesions at the level of the principal vein).

Broad-leaved tree species typically demonstrate high levels of diversity in leaf morphology in response to a variety of environmental conditions associated with changes in altitude (THOMAS, 2011; ADAMIDIS et al., 2021). This situation occurs as a result of the plasticity due to the decrease in temperature, but also due to the stress conditions specific to higher altitude habitats: low water availability, nutrient supply, exposure to wind, and intense solar radiation (RODERICK et al., 2000; PICKUP et al., 2005). Leaves usually decrease in size with increasing altitude (a fact not reported in our case) and the explanation comes from the fact that there is no simple and direct correlation with altitude, as plants often demonstrate variations in leaf traits as part of more complex adaptations (PEGUERO-PINA et al., 2020).

At beech, traits expressing lamina shape in shaded leaves were more related to altitude, while leaf size appeared to be more influenced by habitat (ADAMIDIS et al., 2021). In a study on beech trees from southern and northern Italy (ADAMIDIS et al., 2021), leaf morphology was the most sensitive to climate stress in the southern lots and environmental pollution (nitrogen deposition and tropospheric ozone concentration) in the northern ones. The morphological variability among trees indicates a high potential for adaptation to environmental extremes (ADAMIDIS et al., 2021).

Our study was consistent with the results reported by other authors regarding the higher density of stomata in the leaves of individuals on the roadside, a situation explained by the fact that stomatal density is related to the capacity to regulate gas exchange (GUTSCHICK, 1999). On one hand, the presence of numerous small stomata (high stomatal density) in combination with an increased LMA (leaf mass per area) is considered a xeromorphic adaptation, related to the origin of the specimen from a drier environment (ABRAMS, 1994). On the other hand, BUSSOTTI et al., (2005), in Italian beech trees, found that stomatal density was lower in the southern stands than in the northern ones; moreover, stomatal density was related to atmospheric nitrogen concentration and between nutrients rate (N/K, N/P, N/C, Mg/Ca). High stomatal density can also be induced by high ozone concentrations (PÄÄKKÖNEN et al., 1998).

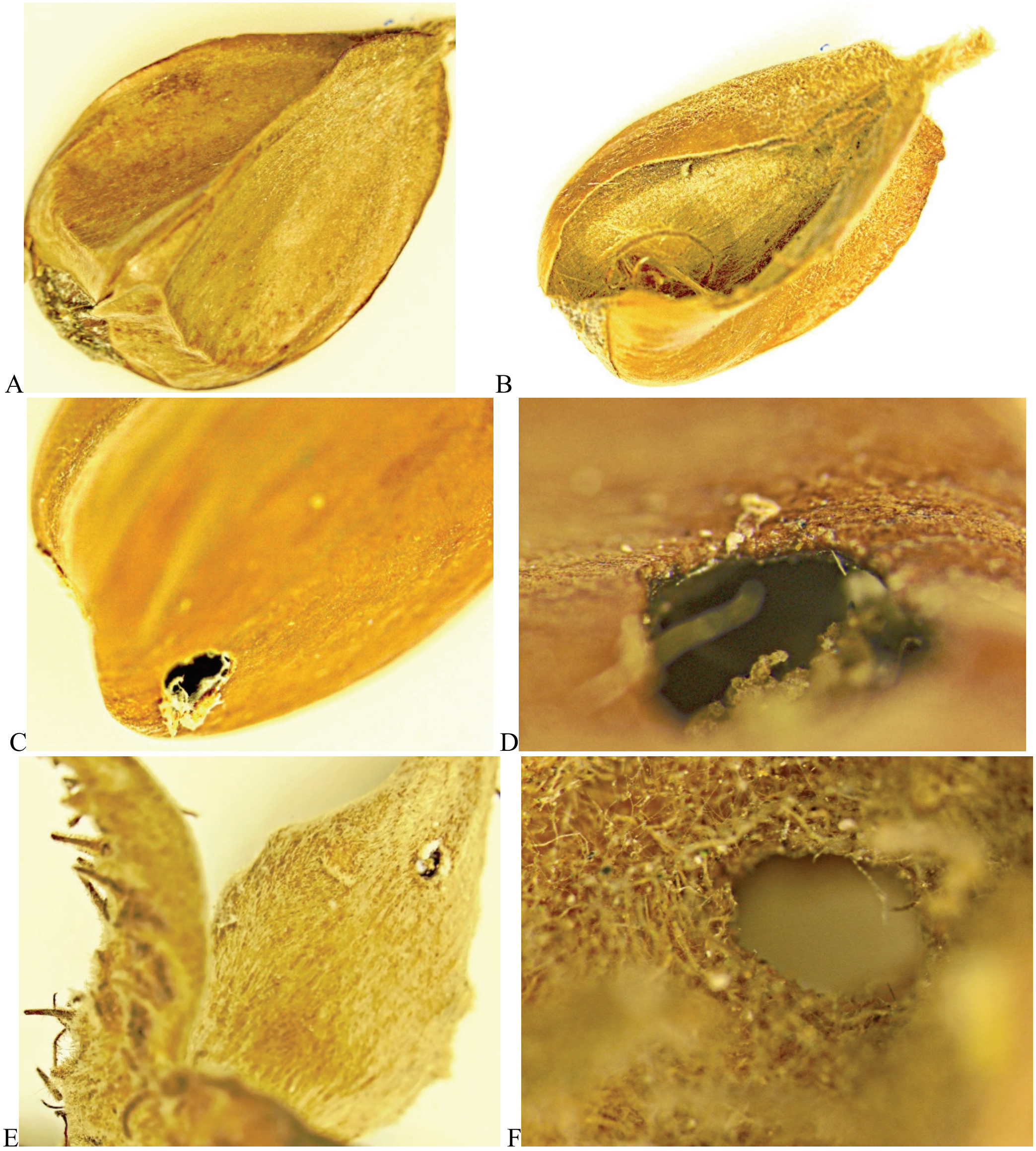

Figure 6. Morphology of beech seeds at stereomicroscope: normal (A – 2X), destroyed (B - 1X), punctured by insects (C – 2X și D – 8X); exocarp damaged by insects (E – 0.7X; F – 5X).

Table 4. Quality of beech nuts (*Fagus sylvatica* L.) from Jiu Gorge National Park and Iron Gates Natural Park, located in unpolluted areas (Chitu Valley and Trescovăț) or polluted areas (E 79 Meri and E 70 Vodița) (media ± standard deviation = s±sd; P-value <0.05).

Parameter	**100 healthy nuts mass (g)**		**Nuts punched by insects number (from 100)**		**Nuts destroyed number (from 100)**		**Seeds present in nuts number (from 100)**	
Jiu Gorge National Park								
Sample	Chitu Valley	E79 Meri	Chitu Valley	E79 Meri	Chitu Valley	E79 Meri	Chitu Valley	E79 Meri
s±sd	14.05± 0.58	13.54± 0.01	0.00± 0.00	0.00± 0.00	14.00± 1.00	14.33± 0.58	92.67± 2.52	90.00± 2.00
t-test	0.27		-		0.2113		0.07	

Iron Gates Natural Park								
Sample	Trescovăț	E70 Vodița	Trescovăț	E70 Vodița	Trescovăț	E70 Vodița	Trescovăț	E70 Vodița
s±sd	23.60± 0.01	24.07± 0.08	13.3± 1.53	25.67± 2.08	7.33± 2.08	24.00± 1.73	81.67± 1.53	54.33± 1.15
t-test	0.14		0.0015		0.0000		0.0014	

Table 5. Morphology of beech nuts and seeds (*Fagus sylvatica* L.) from Jiu Gorge National Park and Iron Gates Natural Park, located in unpolluted areas (Chitu Valley and Trescovăț) or polluted areas (E 79 Meri and E 70 Vodița) (media ± standard deviation = s±sd; P-value <0.05).

Parameter	**Nuts length (mm)**		**Nuts width (mm)**		**Seeds length (mm)**		**Seeds width (mm)**	
Jiu Gorge National Park								
Sample	Chitu Valley	E79 Meri	Chitu Valley	E79 Meri	Chitu Valley	E79 Meri	Chitu Valley	E79 Meri
s±sd	18.33± 2.08	16.0± 1.73	10.67± 1.53	10.33± 0.58	10.21± 4.58	11.24±4.16	3.07± 2.08	3.76± 2.52
t-test	0.6874		0.7507		0.675		0.598	
Iron Gates Natural Park								
Sample	Trescovăț	E70 Vodița	Trescovăț	E70 Vodița	Trescovăț	E70 Vodița	Trescovăț	E70 Vodița
s±sd	15.00± 1.00	14.0± 1.73	8.67± 1.53	8.33± 2.52	12.7± 3.61	11.6± 2.75	5.56± 3.79	5.07± 3.21
t-test	0.4465		0.8560		0.832		0.903	

Figure 7. Aspects of beech seeds (*Fagus sylvatica* L.) with black seed coat, from Jiu Gorge National Park (A) and brown, from IGNP (B) and without seed coat, similar from both parks (C) (the bars represent 1 cm).

VERMA et al. (2006) consider that changing the frequency and size of stomata in response to environmental stress is an important way of adapting to control the absorption of pollutants by plants. The decrease in stomatal size may be a mechanism to avoid the inhibitory effect of a pollutant on physiological activities such as photosynthesis. The authors reported a significant decrease in stomatal density in *Ipomea pestigridis* grown under different degrees of coal smoke pollutants. Gaseous pollutants that enter the leaves through the stomata can also interact strongly with the surface of the mesophyll cells, producing changes in them (VERMA et al., 2006).

The seeds had a black integument in the beeches from Meri, many were broken, punctured by insects, and small from the Vodița national road area with high car traffic. It remains to be seen what the germination rate of the healthy seeds is and the survival of the young seedlings over time, in the natural environment.

CONCLUSIONS

Beech leaves were affected by road traffic in both parks. The morphological characteristics of the investigated leaves indicated significant potential for resilience to air pollution, the same is not the case at the structural level, where lesions affect the integrity of the leaf, resulting in a decrease in their dry mass. The morpho-histological changes that occurred can potentially be used as biological markers for the presence of polluted air. In the case of seeds, significant differences between the polluted and non-polluted areas were reported only in the Iron Gate Natural Park, where the beech trees in the polluted area had numerous seeds broken or punctured by insects.

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Petruş-Vancea Adriana, Moş Andreea-Selena, Cicort Abigail, Cupsa Diana
University of Oradea, Faculty of Informatics and Sciences, Department of Biology, 1 Universității str., Oradea, Romania.
E-mails: adrianavan@yahoo.com; seles422@gmail.com; abigailcicort@gmail.com; cupsa2007@yahoo.com

Pop Daniela-Nicoleta
University of Oradea, Doctoral School of Biomedical Sciences, 1 Universității str., Oradea, Romania.
E-mail: dordea.daniela95@yahoo.com

Sucea Felicia Nicoleta
N.F.A. Romsilva, Jiu Gorge National Park, Str. Lt. Col. Dumitru Petrescu, No 3, Târgu-Jiu, Romania.
E-mail: feliciasucea@yahoo.ro

Dumbravă Amalia-Raluca
N.F.A. Romsilva, Iron Gates Natural Park Administration R.A., No 2, Civic Center, Orșova, Mehedinți, Romania.
E-mail: amalia.dumbrava@pnportiledefier.ro

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