Traffic effects on leaf macro- and micro-morphological traits

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Abstract
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The aim of this study was to evaluate the traffic-related effects on morphological and anatomical traits of *Fraxinus rotundifolia* and *Morus alba* along the Malayer-Hamedan road. In the study area, populations of two species of *F. rotundifolia* (30 individual trees) and *M. alba* (30 individual trees) were selected for sampling. The results showed that the leaf and stomata dimensions at distances near to the roadside were significantly lower compared to longer distances from the road. For *F. rotundifolia*, the number of veins showed the lowest plasticity, while in case of *M. alba,* the stomata length (P = 0.52) and the number of veins (P = 0.54) showed the lowest plasticity related to the environmental conditions. Results of discriminant analysis for population grouping for the two species of *F. rotundifolia* and *M. alba* confirmed the accuracy of grouping 74.8% and 79.5%, respectively. In case of trees that were farther away from the road, guard and epidermal cells were located at the same level. We found that the leaf stomata in *M. alba* were closed more than in *F. rotundifolia* at the same distance. Totally, the results of this research show that the air pollution stress impacted the tree morphological traits. From the two species, *F. rotundifolia* was more resistant in terms of pollution stress.

Keywords
leaf traits, pollution, roadside, stomata, traffic

Introduction

As a result of industrialization and population growth in cities over the latest decades, air pollution has become one of the most frequently discussed issues (Molashahi et al., 2014). The recent rapid economic growth, industrialization and urbanization notably enhanced air pollution. The most important air pollutants are, among others, lead, zinc, and cadmium which directly affect the fundamental activities in plants: such as photosynthesis, evaporation, transpiration, and respiration. Different plants show different sensitivities to varying levels of air pollution.

Air pollutants in urban and industrial areas may be adsorbed, accumulated or integrated into the plant bodies. In case of toxic pollutants, these may injure the plants to some degree. The level of injury will be high in sensitive species and low in tolerant ones. The sensitive species are useful as early warning indicators for pollution, the tolerant ones help in reducing the overall pollution load, making the air relatively free of pollutants.

The traffic-induced pollutants are deposed on the leaf surface and prevent the light to reach it, which impairs photosynthesis. As a natural filter, vegetation plays an important role in improving air quality by absorbing dust particles onto leaf surface. The use of plants has been recognized as an effective and appropriate method for purifying air. Although trees are effective in reducing air pollution, they are also negatively affected and damaged by the pol-

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lutants (Ghorbanli et al., 2009). This damage shows its adverse effects through various forms of anatomical, morphological and physiological changes and deformations in trees and plants (Rashidi et al., 2011).

The absorption of chemical pollutants through the stomata and deposition of dust and airborne particles on the leaf surface can adversely affect plant species (Sadeghi et al., 2015). Deposition of small pollutant particles on plant leaves may result in damaged leaf structure, reduced performance, interrupted light absorption and gase release, and, finally, disrupted photosynthetic process (Rahman, 2015). The leaf size and stomatal density in an urban area were observed lower than in a rural area and also the leaf area was heavily covered by dust particles, the leaf stomata were closed and the cuticle was thinner in the urban area (Pourkhabbaz et al., 2010). A study of the effects of air pollution on leaf anatomy of *Robinia pseudoacacia* and *Ailanthus altissima* trees around the Iranian Aluminum Company (northeast of Arak province) showed that the trichome density on the leaf lower surface, as well as trichome length on both leaf upper and lower surfaces of the two species, increased significantly due to air pollution (Amini et al., 2016). The results of studies carried out on the effects of atmospheric pollutants on an ornamental tree of *Acer negundo* in an urban forest showed that the leaf traits such as leaf length, leaf maximum width, and leaf surface area decreased under air pollution conditions (Babapour et al., 2014). A research focused on the ability of tree species of *Morus alba*, *Fraxinus rotundifilia* and *Pinus eldarica* to absorb pollutants indicated that among the three species discussed, *Morus alba* was most capable to sequester pollutants because of its wider leaf surface area compared to the two other species (Molashahi and Feizi, 2014). The use of morphological and epidermal characteristics of leaves of *Ficus benjamina* and *Fraxinus pennsylvanica* species as a pollution indicator in urban areas indicated that their stomata length and width in urban areas were significantly different from the rural ones so that the stomata length and width were higher in rural areas (Arriaga et al., 2014). Squires (2016) observed that air pollution resulted in decreased stomatal conductance of leaves in light conditions and increased stomatal conductance in dark conditions. Also, pollutant particles decreased the photosynthesis through shading the leaves. Plant morphology is very important in determining the tolerance of plants against air pollution. Therefore, some traits such as stomatal characteristics, cuticle thickness, cell density, and cell wall properties are of special importance for decreasing air pollution and preventing the entrance of pollutants into the plant.

Researchers also reported that sensitive plants to air pollutants had showed changes in their morphology, anatomy, physiology and biochemistry (Silva et al., 2005). Various authors investigated the effects of pollution on different plant species (Shannigrahi et al., 2003; Machava et al., 2016; Rai et al., 2016). A strong correlation between the degree of contamination and concentrations in all plant leaves assessed provides evidence that plants like *Robinia pseudoacacia* reflect the environmental changes accurately, and that they appear as an effective biomonitoring tool for environmental quality (Celik et al., 2005).

Due to the different plasticity in resistance of trees on the roadsides to the traffic-related pollution, the present study has been aimed to investigate the effects of these pollutants on leaf morphological traits in *Morus alba* and *Fraxinus rotundifolia* growing at different distances from the road along the Malayer-Hamedan highway.

Materials and methods

Study area

The research was conducted in the end of July 2016, on the roadside green belt of Malayer-Hamedan road, section from 10 km to 50 km, the Hamedan province, Iran. The geographic coordinates of the study site are 34°22´25´´N and 48°40´11´´E, with an average altitude of 1,720 m (a.s.l.). The mean annual rainfall in the studied area is 290 mm, the mean maximum temperatures in the warmest month (August) is 24.8 °C and the mean minimum temperatures in the coldest month (February) is –4 °C (Hamedan Meteorological Station). The region is characterized by cold semi-arid climate and young soils without the fully developed profile. The soil is partially deep on low-slope terrains but very shallow at high elevations and on steep-slopes. The soil texture in the study area is sandy, containing a high proportion of gravel and rubble.

Sampling

The studied greenery was planted on both road sides in 2004 (with active participation of the local community and the local government under the Roadside Tree Planting Program as a green belt). Populations of the two species of *Fraxinus rotundifolia* and *Morus alba* were sampled at the distances of 10, 20 and 30 m from the roadside (in total, 30 individuals for *F. rotundifolia* and 30 individuals for *M. alba*). These species were planted as a green belt along the road, and the trees have the same age. The sampling was carried out for a period of ten days in the 20–30 July 2016. About 15 leaf samples were taken from each individual species from three different positions. The distance between two individuals from each other was 15 m and at each distance, 10 individuals were sampled and 30 leaves from different parts of the crown were collected from each individual tree.

Macro- and micro-morphological traits

Nine leaf traits were investigated in order to study the traffic effects on macro- and micro-morphological traits. These traits and their abbreviations are given in Table 1.

Table 1. Traits studied for leaf

Row	Morphological traits	Scale	Abbreviation
1	Leaf length	Cm	LL
2	Leaf teeth	Number	T
3	Leaf width	Cm	LW
4	Petiole length	Cm	PL
5	Number of veins	Number	V
6	Number of leaves	Number	N
7	Stomata length	Micrometer	SL
8	Stomata width	Micrometer	SW
9	Stomata area	Square micrometer	SA

The leaf dimensions representing leaf length, leaf width and petiole length were measured by photography and by applying of an Image Tools software. For assessing the stomatal traits, five leaves were sampled from each tree. To measure the dimensions of the stomata (length and width), the leaves were first placed in boiling water for 10 minutes, and very thin specimens were separated from lower epidermis layer, using a cutter, and then they were inserted between a slide and a cover slip. Stomatal parameters such as stomatal length and width were measured using an Image tools software. Stomata micrographs were also prepared by applying electron microscopy. To do this, sub-samples from the lower surface of the leaves were prepared with a cutter (Blade). The epidermal samples were assessed by light microscopy (LM) and photos were taken with 40× lens from stomata. An image Tools software (version 2.0) was used for stomata morphometric measurements (Paridari et al., 2012).

Leaf plasticity is the potential or capacity of a plant to respond to its environmental conditions. The total plasticity (Pl) was calculated using equation (1):

$$P_l = 1 - (x/X), \quad (1)$$

where x and X are the minimum and maximum values for each parameter (Bruschi, 2003).

Statistical analysis

The normality of the data was assessed according to Kolmogorov-Smirnov; to evaluate the homogeneity of variances the Leven test was applied. One-way ANOVA was used to compare the means, and Duncan test was applied to compare the means of the parameters at different distances from the roadside. Due to the large volume of data, multivariate analysis, principal component analysis (PCA) was applied to generate uncorrelated morphological axis. By applying principal component analysis, the most important influencing components and the maximum variance were extracted. Finally, discriminate analysis was used in order to confirm the accuracy of the grouping and to determine the relationship between the data as well as to identify the most important data creating differences in the groups.

Results and discussion

Leaf macromorphological traits

Trees acting as an absorbent of bio-contaminants can filter the toxic and harmful substances through the surface of their leaves and roots, which results in changes in the size of leaves during this process (Squires, 2016). The results of this research have confirmed that both investigated species, *F. rotundifolia* and *M. alba* show a significant increase in the size of their leaves (LL and LW) and stomata (SW, SL and SA) with increasing distance from the road (Table 2). These findings confirm the results presented by Seyyednezhad et al. (2013), Ianovici et al. (2011) and Laghari and Zaidi (2013), who suggest that air pollutants could reduce growth in plants by changing their morphological traits. Morphological changes occurring in plants are often accompanied by decreasing leaf area and increasing stomata number per unit area, which can lead to disruption in photosynthetic activity and thereby a decrease in growth (Ghorbanli et al., 2009). Many authors have reported that traffic-induced polluted conditions lead to leaf and stomatal damage, early aging, decrease in membrane permeability as well as reduced growth and yield in sensitive plant species (Tiwari et al., 2006). In some tree species, environmental factors can act more significantly by influencing their leaf morphology compared to genetic factors; for example, the number of leaf teeth increases with decreasing air temperature (Royer et al., 2009; Zarafshar et al., 2009; Paridari et al., 2012). In this study, the number of leaf teeth (T) in both species – *F. rotundifolia* and *M. alba* decreased with increasing distance from the roadside (Table 2). Ghorbanli et al. (2009) report that the number of leaf teeth (T) was significantly influenced by changes in temperature, and that traffic-induced pollution could affect the number of leaf teeth and veins, so that the trees near the roadside area experience more changes and stress, which is responded by more active defensive mechanism. This also follows from our observations, according to which the N and V number of two evaluated species were sensitive to environmental stresses such as pollution caused by machine traffic. In general, the findings of this study correspond to the results about the changes in the leaf area and the number of leaf serrations in *Acer saccharum*, leaf area in *Ginkgo biloba* (Sianping et al., 2009), petiole length (PL) in *Parrotia persica* (Yousefzadeh et al., 2009), petiole length (PL) and number of leaf teeth (T) in *Celtis australis* (Zarafshar et al., 2010) and leaf traits in *Tilia begonifolia* (Yousefzadeh et al., 2011; Akhondnezhad et al., 2010). According to the results, it was found that in both species the stomata dimensions (SL, AW) increased significantly with increasing distance from the road, indicating the negative impact of pollutants on the stomata dimensions. This finding was consistent with the results of Raina and Sharma (2006), Raina and Bala (2007), Akbarian et al. (2011), and Rai (2016); who report significantly reduced leaf stomata size (SL, SW) in plant species exposed to pollution. As Raina and Bala

(2011) stated, reduced SL and SW in polluted areas is a defensive mechanism against the effects of air pollutants on plant physiological activities such as photosynthesis. Stomata with smaller dimensions (SL, SW) are more adaptable to environmental conditions. Gostin (2009) reported that the number of epidermal cells increased significantly in polluted areas, while stomata size (SL, SW), as well as the size of epidermal cells, decreased at the same time. In general, environmental conditions and the associated microclimate affect the leaf quantitative traits.

In this study, the trees were classified in groups according their 10, 20 and 30 distance from the road, the discriminate analysis proved this grouping accuracy for *F. rotundifolia* at a percentage level of 74.8%. In the case of *M. alba,* 79.5% of trees were classified correctly according to their three distances from the road (Table 3). The separation and grouping of individuals trees from each other in terms of morphological traits was well performed. In general, we can conclude that the diversity of environmental conditions factors induces changes to the plant morphology (Ianovici et al., 2011).

The principal component analysis working with morphological traits of *F. rotundifolia* showed that the first and second principal components accounted for 50.12% and 22.73 % of the total variation, respectively (Table 4). All the characters contributed negatively to the first component. The number of veins (–0.4937), stomatal area (–0.4208) and stomatal length (–0.3877) contributed more to the variation. In the second component, the characters contributing to the component included the number of veins (V), number of leaves (N), stomatal length (SL), stomatal width (SW), and stomatal area (SA) contributed to the second component negatively, while LL and LW, PL and T were positively correlated with the second component. The highest correlation was observed for the leaf length (0.6078) (Table 4). The distribution of tree individuals based on the first two axes showed that the individuals in *F. rotundifolia* population at the three distances were well separated (Fig. 1).

The results of principal component analysis working with the leaf macro-morphological traits in *M. alba* indicated that the first two main PCAs were extracted from the complicated components, the total cumulative variance of these two factors amounted to 62.12% and these components had eigenvalues >1. The PCA grouped the estimated morphological traits variables into two main components: PCA1 accounting for about 43.6% of the variation and PCA2 for 18.52% (Table 5). All the traits contributed negatively to the first component, and LL, LW and PL contributed more to the variation.

The number of leaf teeth (T), was also positively correlated to the second component. The stomatal width contributed negatively to the second component. LW, T, V and SW contributed positively to the third component.

The distribution of individual trees along the coordinate axis based on the first two axes showed that in the *M. alba* population, the individuals were well separated according to the three related distances (Fig. 2).

The values of leaf plasticity were calculated for all of the macro-morphological traits. The highest and lowest mean plasticity values in *F. rotundifolia* were recorded for the T (P = 0.86) and V (P = 0.54), respectively. Leaf plasticity values of the number of leaf teeth in *F. rotundifolia* was not consistent with the results of Espahbodi et al. (2006). In contrast, the results of the variability of the number of veins were consistent with the results of Fallah et al. (2012).

Table 2. Morphological traits (Mean ± SE) *Fraxinus rotundifolia* and *Morus alba*

Traits	*Fraxinus rotundifolia*			*Morus alba*		
	Distance from road (m)			Distance from road (m)		
	10	20	30	10	20	30
LL	3.47 ± 0.7[c]	4.10 ± 0.71[a]	4.51 ± 0.95[a]	4.51 ± 0.95[c]	4.8 ± 0.88[b]	6.29 ± 0.93[a]
LW	1.43 ± 0.25[c]	1.64 ± 0.28[b]	1.87 ± 0.42[a]	1.87 ± 0.42[c]	3.53 ± 0.67[b]	4.24 ± 0.77[a]
PL	3.04 ± 0.51[b]	4.06 ± 0.78[a]	4.24 ± 0.9[a]	4.24 ± 0.9[b]	1.31 ± 0.30[b]	1.65 ± 0.24[a]
T	97.61 ± 29.21[a]	96.17 ± 37.39[a]	91.5 ± 41[c]	91.5 ± 41[a]	48.05 ± 13.53[b]	44.51 ± 16.51[c]
V	52.26 ± 12.02[a]	49.41 ± 13.39[a]	44.74 ± 13.09[b]	44.74 ± 13.09[a]	12.95 ± 2.23[b]	14.54 ± 3.21[a]
SL	22.74 ± 2.24[c]	27.99 ± 2.51[b]	29.83 ± 7.8[a]	29.83 ± 7.8[b]	21.39 ± 2.07[b]	27.01 ± 28.99[a]
SW	18.51 ± 3.23[b]	22.21 ± 2.54[a]	23.58 ± 2.56[a]	23.58 ± 2.56[b]	16.14 ± 1.70[b]	17.85 ± 1.68[a]
SA	384 ± 95[b]	575 ± 85[a]	624 ± 95[a]	624 ± 95[b]	281 ± 56[b]	357 ± 60[a]

Different letters: significantly different at 5%.

Table 3. Results of discriminant analysis in two species of *F. rotundifolia and M. alba*

Distance from road side	*Fraxinus rotundifolia*			*Morus alba*		
	1	2	3	1	2	3
10 m (group 1)	94.4	5.6	0	77.5	22.5	0
20 m (group 2)	7.2	65.2	27.5	14.5	76.8	8.7
30 m (group 3)	1.4	34.3	64.3	2.9	12.9	84.3
Grouping accuracy percentage of each species	94.4	65.2	64.3	77.5	76.8	84.3

Table 4. The first five factors extracted in the PCA based on macro-morphological traits of *Fraxinus rotundifolia*

Traits	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
LL	–0.3697	0.3270	0.2459	0.7028	0.4003
LW	–0.299	0.6078	–0.2825	–0.1032	–0.5085
PL	–0.3787	0.2424	0.1993	–0.6787	0.476
T	–0.0852	0.0513	0.2568	0.1181	–0.1738
V	–0.4937	–0.1871	0.4519	–0.1061	–0.0631
N	–0.4640	–0.0163	–0.7205	0.0599	0.4122
SL	–0.3877	–0.4791	–0.0683	0.0158	0.1546
SW	–0.41	–0.3632	–0.1071	0.0716	–0.3524
SA	–0.4208	–0.2560	–0.1212	–0.0237	–0.0497
Variance (%)	50.128	22.73	11.19	5.22	4.42

Table 5. The first six factors extracted in the PCA based on macro-morphological traits of *Morus alba*

Traits	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
LL	–0.4844	0.2596	–0.0264	–0.1484	–0.0885	–0.4106
LW	–0.4803	0.2471	0.0373	–0.174	–0.2228	–0.3187
PL	–0.4512	0.3224	–0.0277	–0.0131	–0.0817	0.8190
T	–0.0545	0.2591	0.7706	0.0920	0.5501	–0.0658
V	–0.0011	–0.4341	0.5939	–0.3942	–0.5147	0.1398
N	–0.2071	–0.3546	–0.2228	–0.6814	0.5492	0.0513
SL	–0.3599	–0.4588	0.0323	0.4369	0.2377	0.1006
SW	–0.4009	–0.4193	–0.0014	0.3585	–0.1007	–0.1503
SA	3.488	1.482	1.238	0.986	0.476	0.194
Variance (%)	43.6	18.52	15.478	12.319	5.593	2.431

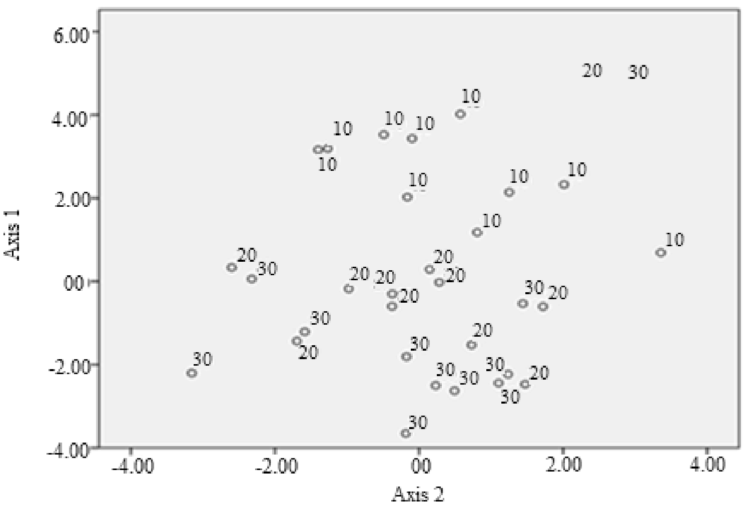

Fig. 1. Distribution of trees according to the two first components of the PCA in *Fraxinus rotundifolia*

In case of *M. alba*, PL and SL showed the highest (P = 0.80) and lowest (P = 0.52) plasticity, respectively (Fig. 3). The results of PL were different from Fallah et al. (2012), but these results confirm the results of Sack et al. (2003), Yousefzadeh et al. (2009), Ghorbanli et al. (2009) and Zhu et al. (2012). It seems that each plant has its own plasticity-related strategy because there have been presented different reports for different species. The leaves of trees with high adaptability and wide distribution can easily react to environmental conditions changes (e.g. light and temperature) (Markesteijn et al., 2007; Lusk et al., 2008). In general, the number of veins (V) and stomatal dimensions (SL, SW) has minimal plasticity, and stomatal density and leaf dimensions have the highest variability (Zhu et al., 2012). Zhu et al. (2012) found that the vein density was less sensitive to environmental changes, and these authors also report that the vein trait was a protected trait. In general, different environments, and consequently different microclimates can affect the variability in leaf quantitative traits. Bruschi et al. (2003) have confirmed that the leaf plasticity was low due to air dryness.

Leaf micromorphological traits (Stomatal traits)

Our results show that the *F. rotundifolia* guard cells are positioned at the same level as the epidermal surface (Fig. 4). This finding is consistent with the results presented by Yousefzadeh et al. (2011) and Paridari et al. (2012). In general, environmental factors impact the position of stomata, so that trees growing near motorways experience drier conditions than trees growing at farther distances. The stomata in pollution-stressed trees are situated at the same or lower level relative to their adjacent epidermal cells. One of the mechanisms the plant species develop in moist site conditions is forming their stomata at a higher level relative to their epidermal cells (Klimko and Wiland-Szymanska, 2008).

Two different types of leaf stomata were identified in case of *M. alba* at the distance of 10 m from the road. In the first type, the guard cells are located at the same level as the epidermal surface (Fig. 5a), while in the other type, the guard cells are located at a higher level compared to the epidermal cells (Fig. 5b). At a distance of 20 m from the road, most stomata were located at lower levels relative to the epidermal cells (Fig. 5c). Finally, most of the stomata were at the same level relative to the epidermal cells at a distance of 30 m from the road (Fig. 5d). In plants exposed to a variety of environmental stresses, the plant stomata are covered with a cuticle layer and they occur at a lower level compared to the epidermal cells. The cuticle layer in plants acts as the main barrier against water loss, insects, and fungal diseases and it also serves as the first protective layer of plants against harmful solar radiation. The physical and chemical properties of the cuticle layer are influenced by climatic conditions; correspondingly, this layer controls the plant response to the environmental stress (Dodd and Poveda, 2003).

Unexpectedly, it was observed that the status of the stomata (pores) opening in *M. alba* was the same – closed at all the three studied distances from the road (Fig. 6). In case of *F. rotundifolia,* the result was different, with the stomata pores more open with increasing distance from the road (Fig. 7). The stomata density and size and their phenotypic plasticity are influenced by various factors, such as water availability and temperature (Luomala

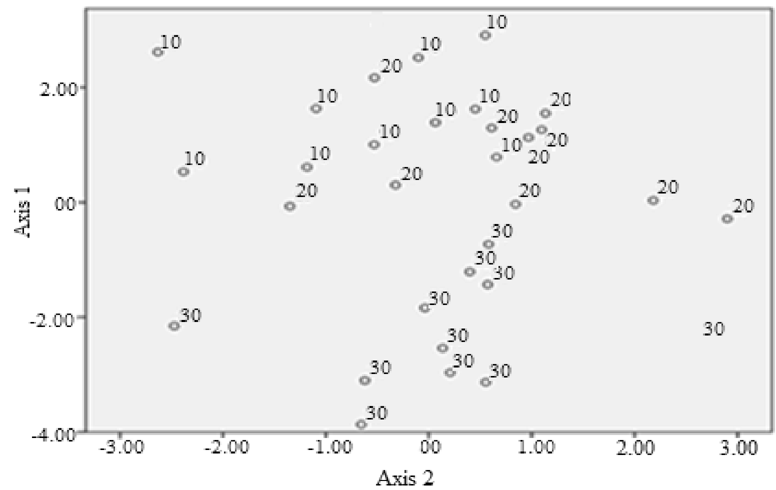

Fig. 2. Distribution of trees according to the two first components of the PCA in *Morus alba*.

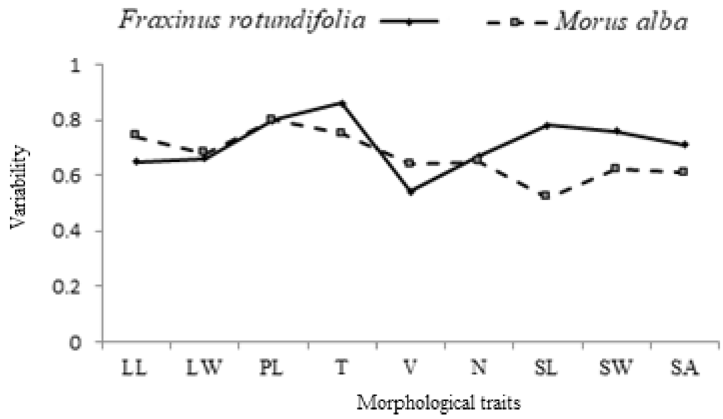

Fig. 3. The variability of leaf morphological traits in *Fraxinus rotundifolia* and *Morus alba.*

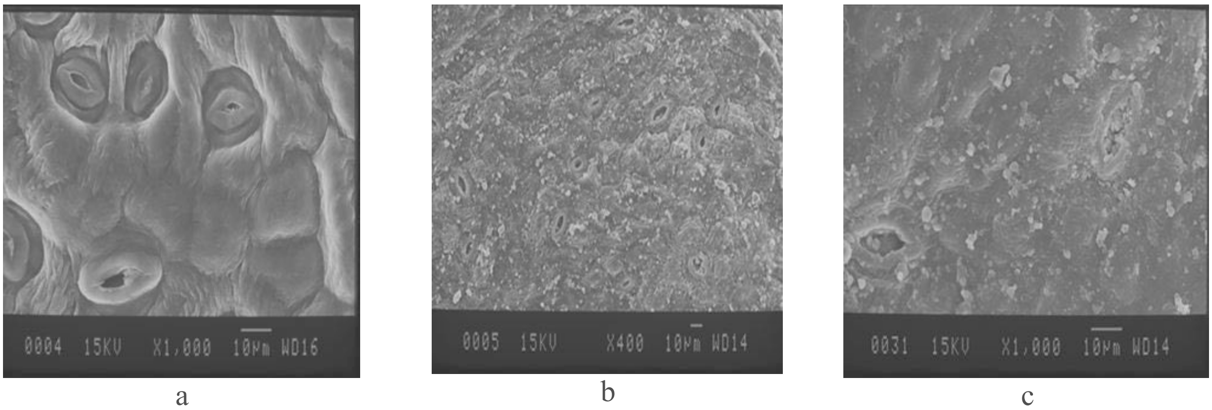

Fig. 4. SEM photomicrographs of the position of *Fraxinus rotundifolia* stomata relative to epidermal cells: a) distance of 10 m, (magnification 1,000×), b) distance of 20 m (magnification 400×), c) distance of 30 m (magnification 1,000×).

et al., 2005); leaf position (Woodruff et al., 2008); air pollution (Riikonen et al., 2010); light and UV radiation (Gitz et al., 2004); oxygen (Ramonell et al., 2001); plant hormones (Davies and Mansfield, 1987) and atmospheric humidity (Schulze et al., 1987). When plants are exposed to stressful conditions, they choose different ways to withstand these conditions. Exposing to heavy metals resulting from traffic pollution leads to symptoms similar to those of drought stress, which leads to stomatal closure under stress (Dineva, 2006; Kulshreshtha, 2009; Cai and Shi, 2009). The sensitivity of the *M. alba* to the traffic-pollutants leads to stomatal blockage which would reduce photosynthesis and growth rates. Stomata opening and closure are of vital importance for gas exchange, in some species, however, the pollution stress leads to stomatal blockage, which reduces carbon stabilization in plants. The lack of change in stomata opening relative to the air pollution in this area, as a mechanism in some plants, indicates the relative resistance of these plants, as this was clearly evident in case of *F. rotundifolia* (Fig. 7).

Conclusion

In general, the results of this study show that pollutants

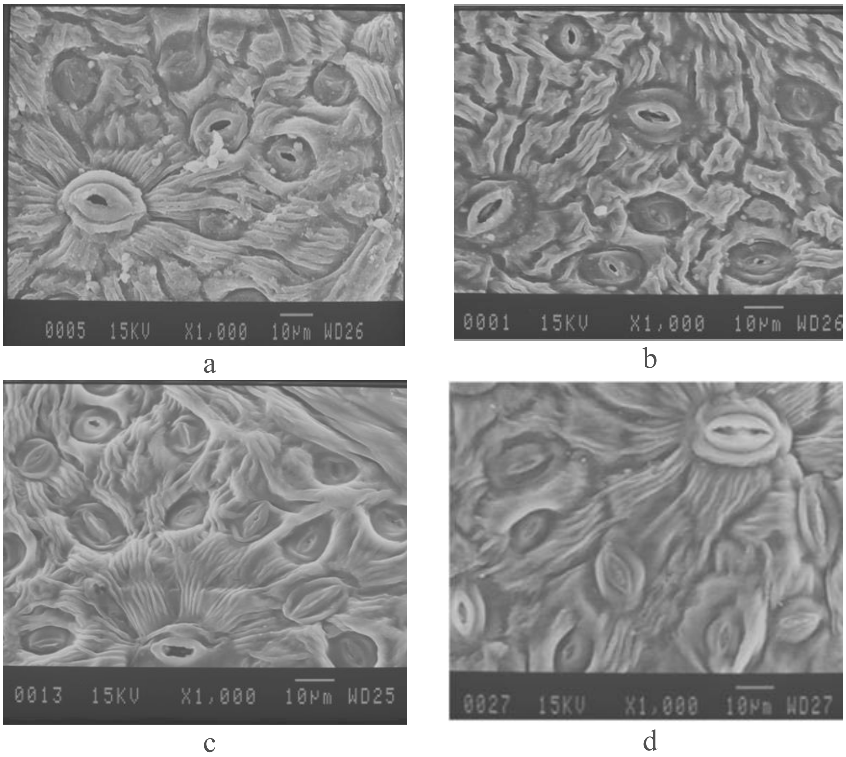

Fig. 5. SEM photomicrographs of position of *Morus alba* stomata relative to epidermal cells (magnification 1,000×): a) same level, b) higher level, c) lower level, d) same level.

from motor vehicles are an important cause of morphological and anatomical variations in tree leaves in the roadside area. At distances close to the road, leaf and stomata dimensions (width and length) showed a significant decrease compared to more remote distances (away from the road). The results also show that in both species, *Morus alba* and *Fraxinus rotundifolia*, the petiole length (PL) and number of leaf teeth (T) manifested the highest plasticity, and the number of veins (V) showed the lowest plasticity due to environmental conditions. Consequently, it can be concluded that this trait is related to genetic features. The results of the discriminant analysis carried out on the populations of *F. rotundifolia* and *M. alba* were also indicative for the grouping accuracy in both species. In consistence with these results, the principal component analysis and distribution of tree individuals showed that this distribution well corresponded to the leaf morphological traits. The different position of guard cells relative to the epidermal cells dependent on environmental conditions is another mechanism developed in plants under stressed conditions, as in stress-free conditions, guard cells and epidermis are situated at the same level. Stomata closing in *M. alba* leaves at all three distances from the roadside indicates the sensitivity of this species to pollution and perhaps its lower resistance compared to *F. rotundifolia*.

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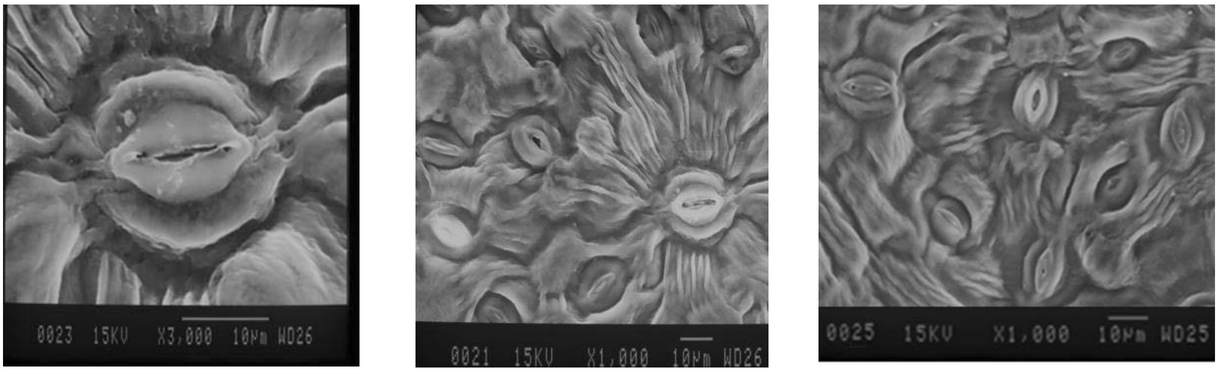
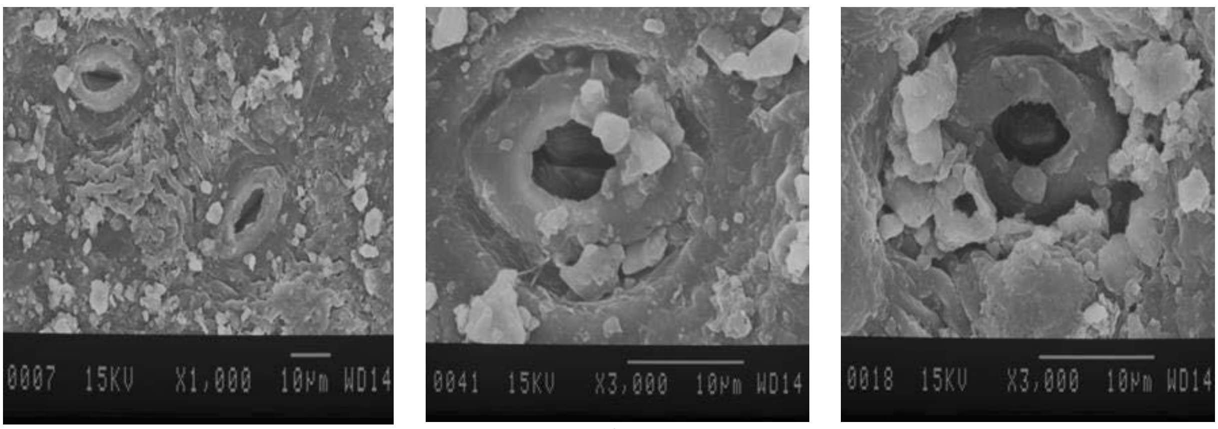


a b c

Fig. 6. Status of stomata opening (pore) in *Morus alba* leaves: a) distance of 10 m from the road (magnification 3,000×), b) distance of 20 m from the road (magnification 1,000×), c) distance of 30 m from the road (magnification 1,000×).



a b c

Fig. 7. Status of stomata opening (pore) in *Fraxinus rotundifolia* leaves: a) distance of 10 m from the road (magnification 1,000×), b) distance of 20 m from the road (magnification 3,000×), c) distance of 30 m from the road (magnification, 3000×).

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