Aesculus hippocastanum, *Castanea* sativa and *Cydonia oblonga* as bioindicators of industrial and road traffic pollution: Morphological study

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Abstract. *Aesculus hippocastanum*, *Castanea sativa* and *Cydonia oblonga* have been studied under industrial (Copper Smelter Works – Pirdop, Gara Yana village, Botunets village) and urban conditions, mainly of road traffic pollution in Sofia (Tsarigradsko Shose Blvd., Tsar Boris III Blvd.), in the period 2008–2010. Three morphological parameters were investigated: linear growth of the annual twigs (cm), number of leaves on the twigs and leaf area. The results showed that the species *C. sativa* and *C. oblonga* can be used as bioindicators only of pollution presence. *A. hippocastanum* and *C. oblonga* were defined as specific indicators for industrial pollution, whereas *C. sativa* was found to indicate road traffic pollution. On the basis of the observed sensitivity of the species to air pollution, recommendations for their planting in the studied regions are made.

Key words: air pollution, bioindicators, Bulgaria, trees

Introduction

Bioindication occupies a distinctive place in the system of environmental monitoring. Plants as biomonitors or accumulators of the environment polluting substances are successfully used in economic and ecological aspect, according to Manning & Feder (1980) and Kovács (1992). Plant reactions to atmospheric pollution and the affected plant species show an early presence of pollutants in the air (Posthumus 1988; Sikora & Chappelka 2004). Plants were used for monitoring and determining the overall effect of all environmental factors and for initial diagnostics of the environmental status. Due to a number of plants advantages – integration of the biological effects of pollutants and simple collection of the samples – plant bioindication is suggested as a valuable source for characterization and monitoring of air quality (Garrecc 1999).

The most popular methods for biomonitoring of air polluted regions include measuring of morphological changes in high plants (Schubert 1985; Tingey 1989). Trees and shrubs are the most suitable objects for air pollution bioindication. They are characterized with relatively long life, high ability to accumulate polluting substances and to reflect local conditions (Nali & Lorenzini 2007). Trees exhibit greater tolerance to environmental changes than other plants (Berlizov & al. 2007). Therefore, trees and shrubs are the most popular bioindicators in the investigations of air quality monitoring. Gas-resistance of plants is one of their important characteristics and, therefore, can be used for air pollution bioindication too.

While *Aesculus hippocastanum* was already investigated in Bulgaria (Kurteva 1995), there are no data on the morphological parameters of *Castanea sativa* and *Cydonia oblonga* in the polluted regions of Bulgaria. Furthermore, there are no studies of the application of *Castanea sativa* for planting in the regions under anthropogenic environmental pollution. Insufficient data and the importance of knowledge of the environmental status of these tree species support the idea of conducting a morphological study of these species in regions with high level of industrial and road traffic pollution in Bulgaria.

The aim of the present study was to establish if some tree species under conditions of industrial and road traffic pollution could be used as air pollution indicators, as well as for planting them in the polluted areas. This required evaluation of changes in three morphological indexes which characterize the growth and development of the plants.

Material and methods

The following three tree species – *Aesculus hippocastanum* L. (Horse Chestnut), *Castanea sativa* Mill. (European Chestnut, Sweet Chestnut) and *Cydonia oblonga* Mill. (Quince) – were objects of the research. *Aesculus hippocastanum*, as an endemic species for the Balkan Peninsula, is distributed in the mountains of Albania, Greece and Former Jugoslavia (Delkov 1992). In Bulgaria, the Horse Chestnut grows only in Mt Preslavska, in the Dervisha Managed Reserve.

Castanea sativa is distributed in the West Caucasian region, at the Black Sea Coast, part of Asia Minor and the entire Mediterranean region (Delkov 1992). Presently, this tree species is cultivated across Europe (Fernández-López & Alía 2003) and up to the Baltic Coast (Urbisz & Urbisz 2007), North Scandinavia and Great Britain, as well as in North America and Japan (Gramatikov 1992). In Bulgaria, the species is spread in mesophytic and xeromezophytic deciduous forests in the Belasitsa, Slavyanka, Ograzhden, Berkovishki Balkan (Western Balkan Range), Pirin and Rhodopi (Western) mountains, and the Valley of Mesta River (Lyubenova & Bratanova 2012).

Cydonia oblonga originates from the Caucasus, Southwest and Central Asia, but owing to its wide-range cultivation as a fruit tree almost across the world, the species is classified as cosmopolitan (Delkov 1992).

The indicated tree species possess a different degree of gas resistance to SO_2, NO_x, NH_3, O_3 and organogenic compounds, according to the investigations and scales of gas resistance by various authors. For instance, *Aesculus hippocastanum* is defined from relatively resistant to SO_2 (Ilkun 1978) and O_3 (Byalobok 1988), low sensitive to HF (Schubert 1985) to poorly resistant (Antipov 1979), nonresistant (Prokopiev 1978; Delkov 1992), sensitive to the pollution from Devnya industrial region (Zhelyazkov & al. 1986), strongly affected by SO_2 (Treshow 1984), F_2 (Kurteva 1982) and very sensitive to Cl_2 (Sikora & Chappelka 2004). Most authors refer to *Castanea sativa* as medium resistant. However, according to Dässler (1981), the species is sensitive to HF. About *Cydonia oblonga*, Antipov (1979) reported this species as resistant to SO_2.

Concerning data on the availability of the studied species for planting, various authors have elaborated on that topic. According to Kondratjuk (1980), *Cydonia oblonga* and *Aesculus hippocastanum* are recommended for planting in regions with medium and lower-level pollution (II and III zones), while *Castanea sativa* is particularly suitable for planting in cities (Dässler 1981).

In the Pirdop town (referred to as Pirdop in the study), industrial pollution of the environment was found out near the Pirdop Copper Smelter Works [Union Minier Pirdop Copper (UMPC) – Pirdop], with the following geographic coordinates 42°42'39"N, 24°10'27"E, and was considered a region with the highest degree of pollution. The influence of the Kremikovtzi Metallurgical Works was studied in two areas heavily affected by industrial activity: Gara Yana village (Yana) (42°43'54"N, 23°33'24"E) and Botunets village (Botunets) (42°44'31"N, 23°30'59"E), both in Sofia district.

Road traffic pollution was monitored in Sofia city, the capital and the largest city of Bulgaria: along the Tsarigradsko Shose Blvd. (42°41'23"N, 23°20'21"E), and only for *Castanea sativa* – also along the Tsar Boris III Blvd. (Tsar Boris III Blvd.) (42°40'17"N, 23°16'22"E).

According to data of the National Automatic System for Ecological Monitoring (Newsletters for environmental status of SCSESD at MEW*), the type of pollution and the main pollutants in the regions during the period of investigation are presented in Table 1.

Table 1. Type of pollution in the studied settlements.

Site	Type of pollution	Main pollutants
1	**Copper Smelter Works (UMPC – Pirdop) in Pirdop town**	**Dust, SO_2,** H_2S, aerosols of H_2SO_4, **Cu, Pb, As,** Zn, **Cd** and NO_2
2	**Gara Yana, Sofia district**	**Dust, SO_2, phenol,** Pb-aerosols, H_2S
3	**Botunets village, Sofia district**	**Dust,** Pb-aerosols, SO_2, **Cd**
4	**Tsarigradsko Shose Blvd., Sofia**	**Dust,** benzene, **NO_2, SO_2, CO, Cd, Pb, Zn,** Ni, CO_2, hydrocarbons, aldehydes, aerosols
5	**Tsar Boris III Blvd., Sofia**	**Dust, phenol,** SO_2, NO_2, **CO, benzene,** Zn, Cd, Ni, aldehydes, hydrocarbons, aerosols

Legend: 1. UMPC – Pirdop; 2. Gara Yana; 3. Botunets; 4. Tsarigradsko Shose Blvd., Sofia; 5. Tsar Boris III Blvd., Sofia. Pollutants in bold are with concentrations exceeding LPC (limited permissible concentration). MoEW* – Ministry of Environment and Waters of Bulgaria.

Individuals of the species *Cydonia oblonga*, growing in the yards of Gara Thompson village were used as a control version (K). The controls for *Aesculus hippocastanum* and *Castanea sativa* in 2008 were located in the park of the Forestry Research Institute (Sofia) (*A. hippocastanum*); and for *Castanea sativa* in the forest above Lokorsko village. However, in 2009–2010, as control variants were used the trees in the natural habitats of the species: Dervisha Managed Reserve in Mt. Preslavska (for *A. hippocastanum*) (43°06'54" N 26°43'33"E) and Mt. Belasitsa (for *C. sativa)* (41°21'41"N, 23°11'08"E).

Morphological analysis required determination of the following three indexes: linear growth of annual twigs (cm), number of leaves developing on them, and leaf area (cm^2). The leaf area in *A. hippocastanum* could not be determined due to the peculiarities of the leaves – large, complex, palm-like, consisting of five to seven converse egg-resembling leaflets. Early defoliation of the tree crowns in the first ten days of August was another obstruction for fixing the leaf area of this species. The above-mentioned morphological parameters reflect the changes in growth, as far as a total drop in the growth of the tree species under the conditions of industrial pollution (Keller 1979; Manning & Feder 1980) is manifested by decreasing of radial growth, growth in height, linear growth of annual twigs, and size and number of needles and leaves. Alterations in growth by measuring the separate organs or the growth of annual twigs, according to Gluch (1980), are more sensitive indicators, as compared to measuring the dry mass of the whole plant. For this reason, the alterations in growth, even though in most cases unspecific, are a more sensitive indicator than necrosis (Jäger 1980). Therefore, they are widely used for fast and early diagnostics of the state of the tree species (Luchkov 1992), as well as for examination of the species ability to adapt to industrial pollution (Dorogan & Djukov 1990; Korshikov & Tarabrin 1990). According to many authors (Manning & Feder 1980; Halbwachs 1988; Volkova & Belyaeva 1990; Girs & Zubareva 1992; Gryshko 2002) and also confirmed by our studies (Kurteva 1999, 2008; Kurteva & Stambolieva 2007; Kurteva & al. 2004), these morphological parameters are useful for early detection of air pollution and for long-term biomonitoring of the impact of air pollution on the growth and development of plants, as well as for preparing a selection of trees suitable for planting in industrial and urban areas. According to Tingey (1989), plant growth, along with the changes in morphological indexes, can be used as an expression of adaptive response of the plants to environmental pollution.

The linear growth of annual twigs was measured linearly (cm), while the leaf area (cm^2) was determined after Roznjatovskiy (1954), with some modifications: instead on photosensitive paper, the imprints of leaves were taken on plotting paper and their area (cm^2) was determined by the weighting method. For leaf area determination, a leaf of the annual twig was taken, together with the petiole.

The investigation was carried out during a three-year period (2008–2010). The morphological measurements and collection of samples for leaf area determination were conducted in the first half of August, when linear growth of the annual twigs was completed, the number of leaves on them was final and the leaf blades were fully developed and had reached their final size. The modal individuals of the studied plant species were similar in regard to age, height and form of growth. The samples were collected from the sunny side, at 130–180 cm height from each species, in all regions of the study.

Data obtained from the morphological analysis was statistically compared by using "one-way ANOVA" test with Bonferroni's correction, by application of OriginPro 7.0 software. Statistical significance was evaluated at $P<0.05$, $P<0.01$ and $P<0.001$ (marked in the figures, respectively, with one, two and three asterisks). The sample sizes are presented in Table 2.

Table 2. Sample sizes involved in the present study and arranged according to years, sites and investigated morphological criteria.

Site \ Year	2008			2009			2010		
Morphological parameters	Growth of twigs	Number of leaves	Area of leaves	Growth of twigs	Number of leaves	Area of leaves	Growth of twigs	Number of leaves	Area of leaves
Aesculus hippocastanum									
Pirdop	48	48	–	50	50	–	58	58	–
Botunets	55	55	–	54	54	–	89	90	–
Tsarigradsko Shose Blvd.	91	91	–	83	83	–	126	126	–
K (Control)	61	61	–	60	60	–	115	115	–
Castanea sativa									
Gara Yana	62	62	62	57	57	57	80	80	80
Tsarigradsko Shose Blvd.	56	56	53	71	71	74	100	100	100
Tsar Boris III Blvd.	24	24	24	34	34	35	112	112	115
K (Control)	68	68	77	104	104	106	120	120	121
Cydonia oblonga									
Pirdop	54	54	54	78	78	81	89	89	75
Gara Yana	72	72	72	58	58	56	80	80	84
Botunets	52	52	51	66	66	68	100	100	101
Tsarigradsko Shose Blvd.	99	100	100	91	91	90	136	136	140
K (Control)	54	54	55	68	68	84	120	120	132

Results

The average data of the three parameters from the morphological analysis of the studied species are presented graphically in Figs. 1 to 3.

Variation of the linear growth of annual twigs in *Aesculus hippocastanum* was statistically proved in Pirdop for the entire period of study, but in Botunets and along Tsarigradsko Shose Blvd, only for 2010, there was statistical difference between probe and control. In terms of the number of leaves on the twigs, variation was statistically proved for the individuals in Pirdop and Botunets for the first year of investigation (2008) (Fig. 1A, B).

Our data showed the strongest drop in the Horse Chestnut twigs growth for Pirdop in 2008, for the entire period of research (Fig. 1A). The twigs showed the highest growth (3.03 ± 0.34 cm) in Pirdop in 2009 (Fig. 1A). On the contrary, the Horse Chestnut manifested reduced growth in all studied regions for 2010, as compared to the previous year. The lowest growth of twigs was manifested along the Tsarigradsko Shose Blvd. In the same year, an unusually low value of the control twigs (1.07 ± 0.08 cm) was observed (Fig. 1A). This explained why the samples from polluted regions exceeded the control measurements by 201.9 % (Pirdop) and 142.0 % (Tsarigradsko Shose Blvd.).

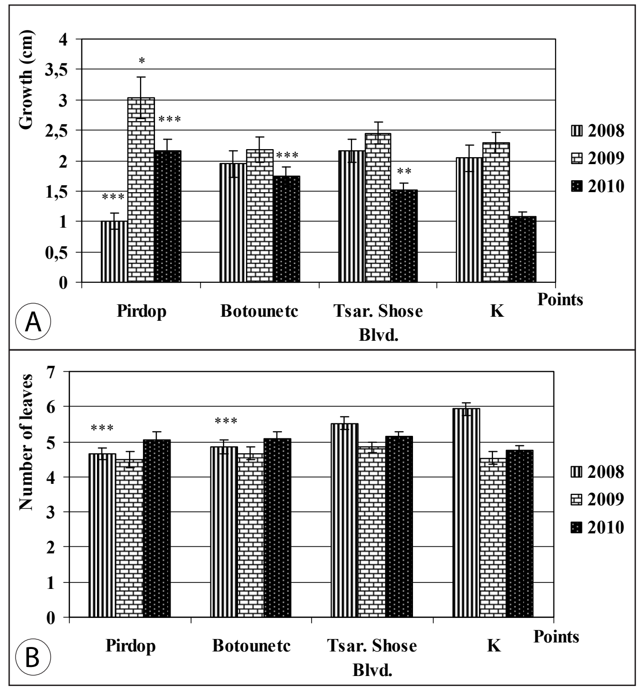

Fig. 1. Average values of the morphological parameters of *Aesculus hippocastanum*, 2008–2010.
A – Linear growth of the annual twigs (cm); **B** – Number of leaves on the twigs. Sites of study: Pirdop, Botunets, Tsarigradsko Shose Blvd., **K** – Control. The data are mean values ± SEM.

Mention deserves the fact that retarded growth of the control trees of Horse Chestnut (in the park of the Forestry Research Institute, Sofia) was observed in the first year of study. That was probably due to pollution in that region, even though there was no obvious local polluter. Therefore, we started to use as controls trees from the natural habitat of this species in Bulgaria – Dervisha Managed Reserve (Mt. Preslavska), for the years 2009 and 2010.

The number of leaves on the twigs parameter for *A. hippocastanum* (Fig. 1B) seemed more stable throughout the study. A tendency for reduction of the number of leaves during the second year was observed, especially along the Tsarigradsko Shose Blvd. and in the control trees (Fig.1B). With the smallest number of leaves (4.48 ± 0.23) were the twigs in Pirdop (2009), whereas the maximum degree of leafing was found along the Tsarigradsko Shose Blvd. (5.52 ± 0.18) in 2008 (Fig. 1B). All sites investigated during 2010 showed higher values than the controls

(104.8 % in Pirdop to 109.1 % along the Tsarigradsko Shose Blvd.).

An interesting phenomenon could be outlined: the reduced growth of twigs in all studied regions (2010) was partially compensated by a higher number of leaves, especially along the Tsarigradsko Shose Blvd. On the other hand, the high twigs growth was in agreement with the reduced number of leaves on them for the Chestnut individuals in Pirdop in 2009.

The average values for the morphological indexes of *Castanea sativa* in the studied sites are presented in Fig. 2, A-C.

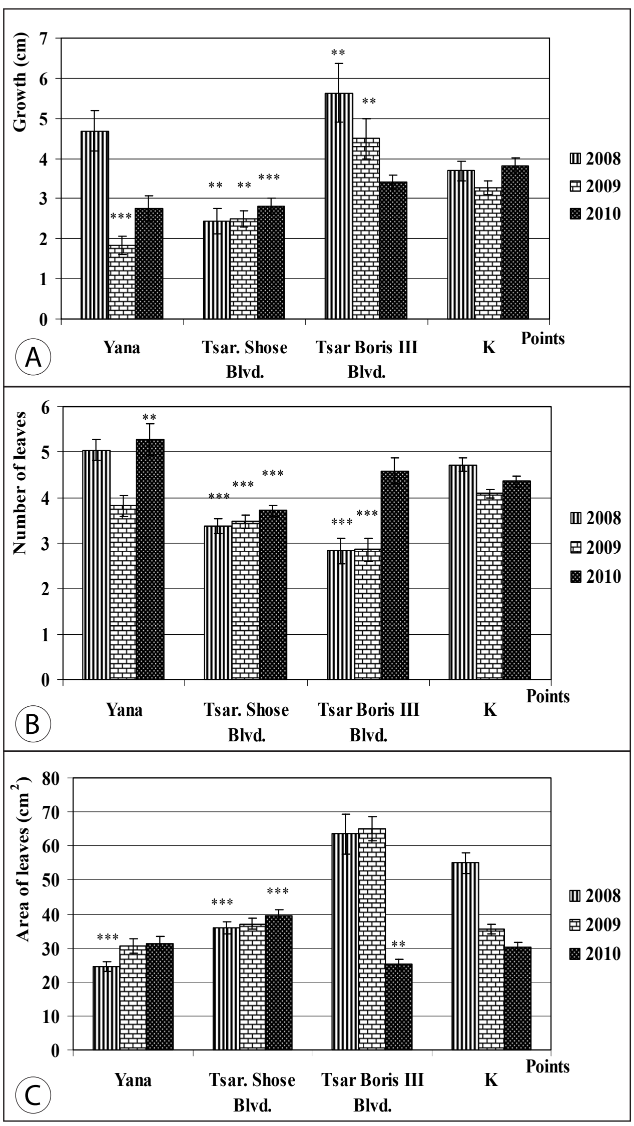

Fig. 2. Average values of the morphological parameters of *Castanea sativa*, 2008–2010.
A – Linear growth of the annual twigs (cm); **B** – Number of leaves on the twigs; **C** – Area of leaves (cm^2). Sites of study: Gara Yana, Tsarigradsko Shose Blvd., Tsar Boris III Blvd., **K** – Control. The data are mean values ± SEM.

According to the linear growth of annual twigs morphological parameter in *Castanea sativa*, variations were statistically proved in the regions under heavy traffic pollution (for the entire period of investigation) and in Gara Yana village (in 2009 and 2010). In respect to the number of leaves on the twigs, statistical differences were observed under the conditions of road traffic pollution (for the entire period of investigation) and for Gara Yana – only for 2010. Differences of the values concerning the leaf area were statistically confirmed in the regions with road traffic pollution (for the entire period of examination) and for Gara Yana – only in 2008 (Fig. 2).

The minimum growth of twigs in Gara Yana was registered for 2009–1.83 ± 0.22 cm (Fig. 2A), while the maximum linear growth of *Castanea sativa* twigs were found in the individuals along the Tsar Boris III Blvd. in 2008 (Fig. 2A; 5.63 ± 0.74 cm). With the strongest reduced number of leaves were the twigs under the road traffic pollution along the Tsar Boris III Blvd. (2008 and 2009; Fig. 2B). The minimum leaf area was measured in Gara Yana (2008), as well as along the Tsar Boris III Blvd. in 2010 (Fig. 2C).

Sweet Chestnut showed the most limited twigs growth under the road traffic pollution along the Tsarigradsko Shose Blvd. (for the entire period of study) (Fig. 2A). Moreover, visible injuries – dry-top trees, loss of foliage, some withered branches – were observed in 2010 along the Tsar Boris III Blvd.

A lower growth of the control individuals of Sweet Chestnut in the forest above Lokorsko village was observed at the beginning of the study (2008). For this reason, in the next two years the trees in the natural habitat of the species in Bulgaria, in Mt. Belasitsa, were used as controls. A comparison of data on the growth of twigs from the polluted regions with the controls showed that in 2010 the controls exceeded the values from polluted regions, more obviously in Gara Yana (1.4 times) (Fig. 2A). Data from the polluted areas indicated a lower growth than in the controls along the Tsarigradsko Shose Blvd. during the entire period of investigation, and in Gara Yana (2009, 2010), as shown on Fig. 2A. Measurements of the twig growth of this species exceeded the controls along the Tsar Boris III Blvd. in 2008 (152.6 %), although in the same site the growth has decreased at the end of study (89.5 %) (Fig. 2A).

It was found that the specific number of leaves on the twigs parameter depends on the type of pollution. Sweet Chestnut developed a limited number of leaves and, hence, had a lower decorative effect under the impact of road traffic pollution. Under the conditions of heavy road transport in Sofia, the twigs along the Tsar Boris III Blvd. demonstrated lower degree of leafing (Fig. 2B). *C. sativa* showed the highest values of this morphological parameter under industrial pollution (Gara Yana) (2010 and 2008), and especially in 2010: 5.28 ± 0.35 (Fig. 2B). A comparison of the values from Tsar Boris III Blvd and Tsarigradsko Shose Blvd with the controls showed a decrease in the number of leaves under the road traffic pollution for 2008 and 2009. On the contrary, the Chestnut individuals in Gara Yana showed better leafing than the controls in 2008 and 2010, and especially in 2010 (121.1 %). In 2010, the number of leaves on the trees along the Tsar Boris III Blvd. also exceeded that in the controls.

In respect of the area of leaves parameter, a specific reaction was observed in relation to the type of air pollution too. The leaves of *Castanea sativa* had the largest area under the hard motor traffic (Tsar Boris III Blvd.) in 2008 and 2009, with a maximum value of 65.08 ± 3.56 cm^2 in 2009, while the leaves under industrial pollution had the minimum size (Gara Yana) in 2008 and 2009, and especially in 2008: 24.56 ± 1.50 cm^2 (Fig. 2C). The leaves on trees in Gara Yana in 2009 were also smaller in size (30.58 ± 2.06 cm^2) than the ones along the Tsar Boris III Blvd. (Fig. 2C). The difference between the values in the two observation sites under road traffic pollution in the same year is smaller (1.7 times; Fig. 2C). The values of the leaf area showed an increase in 2009 in all studied sites, particularly in Gara Yana. In the same year, the Sweet Chestnut along the Tsar Boris III Blvd. developed leaves with the maximum area for the entire period of research (Fig. 2C). At the end of the study (2010), a decreased leaf area (2.3 times), as compared to the previous year, was registered in the area with road traffic pollution (Tsar Boris III Blvd.). A sharp drop in the leaf area at the above-mentioned site was due to the development of secondary leafing after premature fall of the primary formed leaves at the beginning of vegetation. These leaves were considerably smaller and narrower. The controls also registered a decrease in leaf area was, but to a more limited degree (1.4 times).

A tendency for partial compensation between the studied parameters in C*astanea sativa* could be outlined. Thus, the minimum leaf area in Gara Yana (2008) was compensated by higher growth of the twigs, whereas in 2010 a lower growth of the twigs was compensated by better leafing and greater area of the leaves. For the individuals along the Tsar Boris III Blvd., the higher growth of twigs, as well as the larger leaf area compensated for lower leafing (2008, 2009), while by 2010, on the contrary, development of a greater number of leaves was a partial compensation for their sharply reduced area, as well as for the more limited twigs growth.

The mean values of the morphological parameters for C*ydonia oblonga* at the sites of investigation are presented in Fig. 3, A-C.

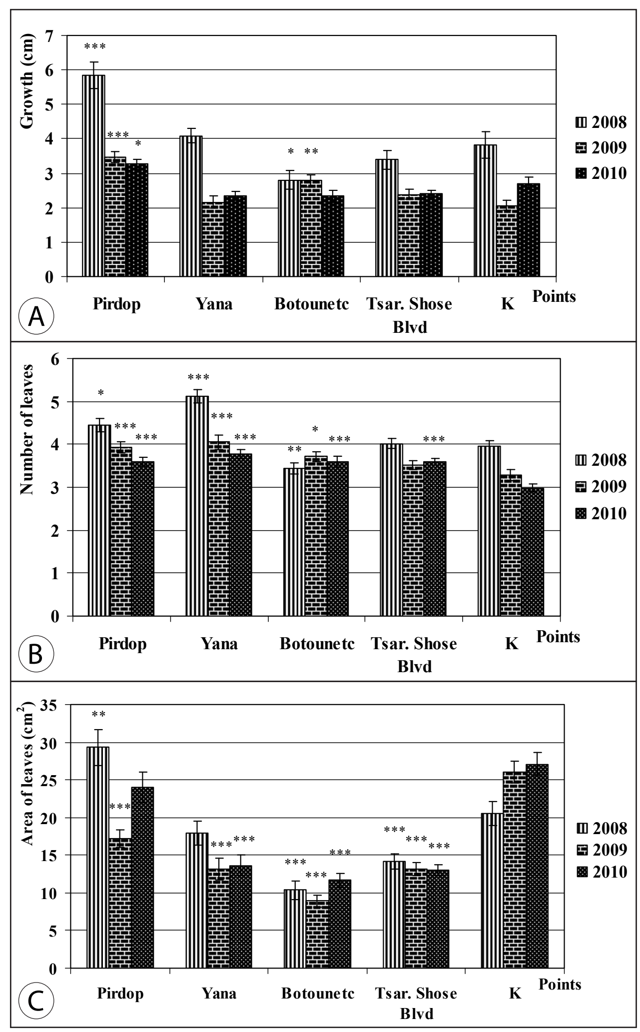

Fig. 3. Average values of the morphological parameters of *Cydonia oblonga*, 2008–2010.
A – Linear growth of the annual twigs (cm); **B** – Number of leaves on the twigs; **C** – Area of leaves (cm^2). Sites of study: Pirdop, Gara Yana, Botunets, Tsarigradsko Shose Blvd., **K** – Control. The data are mean values ± SEM.

Statistical differences between the mean values of linear growth of the annual twigs of *Cydonia oblonga* trees from the polluted regions and their controls were found in Pirdop for the entire period of study, and in Botunets for years 2008 and 2009 (Fig. 3A). Concerning the number of leaves on the twigs morphological index, there were statistically significant differences between the studied species and their controls in all sites with industrial pollution: in Gara Yana for the entire period of study, and along the Tsarigradsko Shose Blvd. in 2010 (Fig. 3B). Variations in the area of leaves were observed in Botunets and along the Tsarigradsko Shose Blvd. for the entire period, whereas in Gara Yana and in Pirdop there were statistical differences only during two years: 2009 and 2010 in Gara Yana, and 2008 and 2009 in Pirdop (Fig. 3C).

The specific influence on the investigated morphological parameters depended on the type of air pollution. The minimum values of the twigs growth depended on industrial (Gara Yana, 2009 and 2010) and transport (Tsarigradsko Shose Blvd., 2010) pollution. Presumably, the minimum mean values of the number of leaves were due to the air pollution in Botunets in 2008 and along Tsarigradsko Shose Blvd. (2009) (Fig. 3B). The leaf area has decreased significantly in the regions with predominant industrial pollution: Botunets (2009, 2008) and Gara Yana (2009 and 2010) (Fig. 3C). The lowest mean level of linear growth of the annual twigs for *Cydonia oblonga* for the entire period of study was observed in Botunets and along Tsarigradsko Shose Blvd. The minimum value was found in Gara Yana (2.17 ± 0.17 cm) in 2009, while the twig growth reached its maximum in Pirdop (5.83 ± 0.38 cm) in 2008 (Fig. 3A). Furthermore, the three-year dynamics of growth of the annual twigs of C*ydonia oblonga* showed its maximal level in 2008, especially in Pirdop and Gara Yana, followed by a sharp decrease in the values in 2009.

Curiously, a drastic drop in the control values of *Cydonia oblonga* was registered in 2009 – from 3.83 ± 0.38 cm (2008) to 2.07 ± 0.13 cm, and the last measurement marked the lowest value of the annual twigs growth of Quince not only for the control, but also for all individuals of this species for 2009 (Fig. 3A).

Considering the number of leaves, a variation was observed between the Quince individuals from regions with different types of pollution**.** The lowest measurements were obtained for the trees in Botunets (3.44 ± 0.13, 2008); very low were also the values along the Tsarigradsko Shose Blvd. (3.52 ± 0.11 for 2009 and 3.60 ± 0.09 for 2010) (Fig. 3B). Similar results for the same regions showed the twigs growth. The highest value of the number of leaves was recorded in 2008 in Gara Yana (5.13 ± 0.15; Fig. 3B).

The measurements of the leaf area demonstrated that in the regions under pollution, Botunets and Tsarigradsko Shose Blvd., there was a reduction in the leaf area (Fig. 3C). On the contrary, in Pirdop, which was also a region under pollution, the mean leaf area exceeded in size the measurements in Botunets. The leaf area reached its maximum value in the controls in 2010 and was also high in 2009. Mention deserves the fact that all measurements obtained in the polluted regions were lower than in the controls during the entire period of study.

A partial compensation was observed among the studied parameters in *Cydonia oblonga*. Thus, the minimum linear growth of the twigs was partially compensated by an increase in the leaf number (Yana 2009), or by a larger leaves area (Yana 2010), up to the maximum value (control 2009 and 2010 years). Another example of such type of compensation could be the compensation between the leaf area increase and an attenuation of the twigs growth observed in Pirdop and Botunets in 2010.

Discussion

Resistance of plants to environmental pollution is determined by both the biological characteristics of the species, and the ecological conditions. Atmospheric pollution and climatic factors are of crucial significance among overall ecological factors. The joint impact of a high pollution level and extreme climatic factors affects negatively the resistance of tree species.

It is well known that 2010 was a rather extreme year, with late and cool spring, hailstorms and flood rains, followed by powerful and continued drought, and all these factors influenced the results obtained during it. In 2010, *A. hippocastanum* showed a decrease in twigs growth in all studied areas, as compared to the previous year, and what is more, the decrease of this parameter was particularly pronounced under the road traffic pollution. The powerful and lengthy drought was responsible for a drastic drop in the growth of Horse Chestnut, as well as the high temperatures, to which the Horse Chestnut was highly sensitive (Delkov 1992;

Wilczynski & Podlarski 2007). Decreased growth of annual twigs in *A. hippocastanum* due to extreme climatic factors was reported earlier for the region of the T. Kostov Thermoelectric Power Plant – Iskar Railway Station, Sofia district (Kurteva 1995).

Many researchers have conducted morphological investigations of the tree species in regions with high level of environmental pollution. Alterations in the trees growth are among the major symptoms of the trees stress (Schütt 1989), while stunted growth, premature loss of foliage, or total defoliation are the types of visible injury of plants under air pollution (Sikora & Chappelka 2004). Decreasing twigs growth in deciduous species under the impact of environmental pollution was registered by many researchers (Kulagin 1974; Ninova & al. 1986; Halbwachs 1988; Volkova & Belyaeva 1990; Girs & Zubareva 1992; Kovács 1992; Gryshko 2002). This phenomenon is an adaptive response of the tree species during their adaptation to technogenic biotops, according to Korshikov & Tarabrin (1990). Reduction in the twigs growth of *Betula pendula* Roth. under industrial pollution was observed by Kulagin (1974), Ninova & al. (1986) and Volkova & Belyaeva (1990). *Tilia cordata* Mill. showed retarded twigs growth in industrial (Volkova & Belyaeva 1990) and in urban environment (Protopopova 1980; Frolov 1981).

In the present study, the growth of annual twigs in *A. hippocastanum* was defined as a sensitive index and this observation was confirmed by the results from earlier investigations of the author into this species in Kiev (Kurteva & Kalchev 1984) and in Bulgaria (T. Kostov Thermoelectric Power Plant) (Kurteva 1995). A decrease in the growth was observed in the above-mentioned studies. Because of the very low values of twigs growth and the number of leaves on them, especially of the twigs growth, the author referred *A. hippocastanum* to the species unsuitable for planting close to the Thermoelectric Power Plant (Kurteva 1995), as well as under pollution in Kiev (Kurteva & Kalchev 1984). A decrease of the twigs length of *A. hippocastanum* by approximately 2.5 times, as compared to the controls, in an environment with high pollution was also reported by Gryshko (2002). Using the earlier results and the results obtained in the present study, it could be assumed that this plant species is not suitable for industrially polluted regions (i.e. Pirdop). The observed decrease of the twigs growth of Horse Chestnut along the Tsarigradsko Shose Blvd. in 2010 was similar to the mentioned measurements for *Tilia tomentosa* Moench, *Acer saccharinum* L. and *Juglans regia* L. in the same area (Kurteva & Merakchiyska-Nikolova 2004).

A comparison of the data for *Cydonia oblonga* with the results for other fruit tree species, for example, *Cerasus vulgaris* Mill. *Pyrus communis* L. and *Juglans regia* L. in the region of the Kostov Thermoelectric Power Plant has shown higher, even though lower than in the controls, growth of the Quince twigs in Gara Yana, Botunets and along the Tsarigradsko Shose Blvd. (Kurteva 1994). The lower twigs growth of *Castanea sativa* and *Cydonia oblonga* in the region of Gara Yana during the last year of study (2010) coincided with the decreased growth in *Tilia cordata* in the same area (Kurteva 1999). Reduced twigs growth in the region of the Kremikovtzi Metallurgical Works was also registered by the author for *Acer pseudoplatanus* L. (Kurteva & al. 1997), *Acer saccharinum* and *Juglans regia* (Kurteva & Merakchiyska-Nikolova 2004).

Data concerning the strongly decreased twigs growth under pollution in Pirdop for *A. hippocastanum* (2008) and for *Cydonia oblonga* (2009, 2010) have confirmed the observed diminished twigs growth in some other tree species from the same region: *Tilia cordata* (Kurteva 1999; Kurteva & al. 2002) and *Acer pseudoplatanus* (Kurteva & al. 2002).

An increase in the twigs growth and leaf area found in *Castanea sativa* along the Tsar Boris III Blvd. in the first two years of study (2008, 2009) could be explained by the anticipated mode of action of nitrogen from the exhaust gases, which can act as fertilizer, enhancing the growth of the twigs (Laffray & al. 2010). Another explanation for the increased twigs growth and leaf area of the studied species could be found in a paper by Gratani & al. (2000). The authors suggested that the accelerated growth of twigs and leaf area in *Quercus ilex* L. may appear as compensation for the brief life of leaves under transport pollution.

Alterations in leaf morphology are widely applied for monitoring the levels of air pollution (Gratani & al. 2000; Balasooriya & al. 2009). The impact of pollution on plants is connected with morphology of the visual injury area of leaves, including reduction of leaf area, changes in morphology, and presence of chlorosis and necrosis (Heath 1980). In the present work, the authors observed visual chlorotic and necrotic spots, drying and premature loss of foliage, second forming of leaves, and second flowering in *A. hippocastanum* (Fig. 4, in both polluted regions and in the controls).

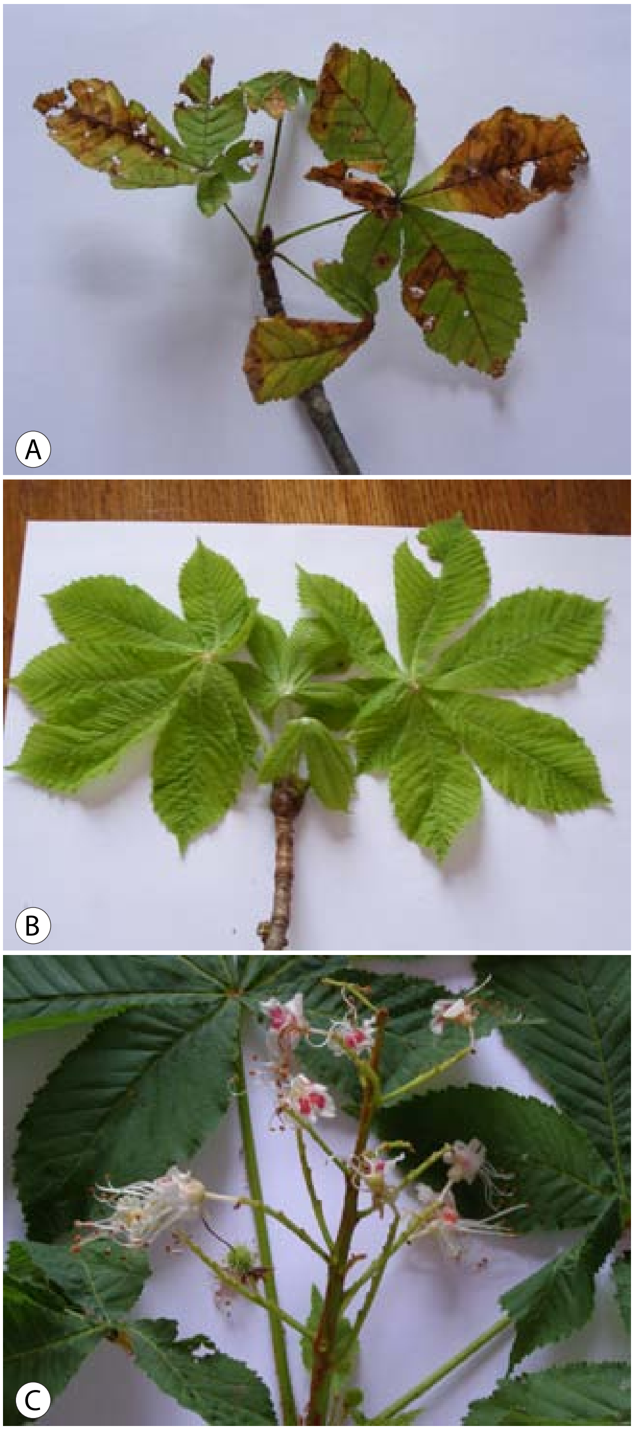

Fig. 4. Abnormal alterations in the development of *Aesculus hipocastanum* under air pollution and climatic factors, 2008–2010. **A** – Leaf injuries as a result of *Cameraria ohridella* activity; **B** – Secondary formed leaves; **C** – Secondary flowering.

In addition to the leaf area reduction, similar morphological deviation was observed during the present study in *Castanea sativa*, but only along the Tsar Boris III Blvd. (2010). An irreversible injury of *A. hippocastanum* results not only from pollution, but also from high temperatures, drought, and damaging activity of the parasite *Cameraria ohridella* Descka & Dimic on the leaves. In the control site, such injuries resulted only from the *Cameraria ohridella* activity. In our opinion, the registered minimum values of the morphological parameters in the controls of *Aesculus hippocastanum* in 2010 were due to the cumulative effect of the parasite and the extreme climatic factors.

A high degree of defoliation in the Horse Chestnut was observed under the influence of air pollution in Kaunas city (Stravinskiene 2010). The ability of plants to produce leaves again after premature loss of foliage under air pollution was registered by Kulagin (1974) and Kurteva (1982). It could be assumed that the secondary forming of leaves in *Castanea sativa* only along the Tsar Boris III Blvd. in 2010 might be related not only to traffic pollution but also to the poor health of the plants. The present results demonstrating decrease in the twigs growth, drastic drop in leaf area and visual injury were in accordance with the observation made by Ciordia & al. (2012). The authors reported a depressed growth, leaf area reduction and changes in leaf morphology after restrictions of water supply for *Castanea sativa*. The poor state of this humidity-sensitive species (Delkov 1992; Urbisz & Urbisz 2007) in the region of Tsar Boris III Blvd. could be associated not only with the pollution impact, but also with the poor conditions for growing. The prolonged drought in 2010 contributed also to the observed negative effects. Bad local conditions resulted in a high degree of injury of the Sweet Chestnut's leaves too (Antipov 1979). On the basis of the present data showing high values of twigs growth and leaf area of the Sweet Chestnut during the first two years in the area with vehicle exhaust pollution (along Tsar Boris III Blvd.), it can be assumed that this plant is suitable for planting in regions with such pollution. Furthermore, a planting area without shadow, with good soil quality, and high air and soil humidity levels should be recommended for this species.

Most tree species often demonstrated strong reduction of the leaf area under industrial and urban pollution. Reduction of the leaf area under industrial pollution was found in *Tilia cordata* (Volkova & Belyaeva 1990) and *Betula pendula* (Ninova & al. 1986; Ninova & Dushkova 1987; Volkova & Belyaeva 1990). Under road traffic pollution, reduction of the leaf area was observed in *Tilia cordata* (Frolov 1981), *Albizia lebbeck* (L.) Benth. (Seyyednejad & al. 2009) and in the hybrid clones of *Populus tremula* L. x *Populus tremuloides* Michx. located 15 meters away from a highway (Nikula & al. 2011).

In the present study, the most apparent alterations in the area of leaves were found in *Cydonia oblonga,* and thus the morphological parameter of leaf area was defined as the most sensitive index for the species in the investigated regions. A noteworthy reduction of the leaf area in Quince individuals from Gara Yana, Tsarigradsko Shose Blvd. and Botunets during the extreme drought in 2010 can be due to a fact observed by Hale & al. (1987). The authors suggested that a tolerance of water content of issue in drought stress is possible by decrease of the leaf area. The extremely low values of the area of Quince leaves in Botunets and Gara Yana in 2010 year were in accordance with the minimum area of leaves observed in another species in the region of the Kremikovtzi Metallurgical Works: *Tilia tomentosa*, *Juglans regia* and *Acer saccharinum* (Kurteva & Merakchiyska-Nikolova 2004), as well as in *Tilia cordata*, *Acer pseudoplatanus* and *Platanus* x *acerifolia* (Aiton) Willd. (Kurteva & al. 1997). A very strong reduction of the leaf area in *Acer pseudoplatanus*, *Acer platanoides* and *Betula pendula* under the impact of the Kremikovtzi Works activity was registered by Dineva (2005). The very small leaf area of *Cydonia oblonga* along the Tsarigradsko Shose Blvd. (2010, 2009) corresponded to the documented very low values in *Acer saccharinum* and *Juglans regia*, as well as in *Tilia cordata* in the same region in earlier studies (Kurteva 1999; Kurteva & Merakchiyska-Nikolova 2004). The decreased area of Quince leaves in Pirdop, especially in 2009, is consistent with the observations of Ninova & Dushkova (1987) in *Betula pendula,* as well as in *Robinia pseudoacacia* L. registered in an earlier study of the author (Kurteva 2008).

Leaf area reduction is an adaptive response of the plants under extreme conditions of technogenic environmental pollution (Dorogan & Djukov 1990; Korshikov & Tarabrin 1990). Decrease in the size of leaves, change in their shape and low leaf numbers are common symptoms of a worsened state of *Betula pendula, Fagus sylvatica* L. and *Carpinus betulus* L. in the forests of Central Europe (Schūtt 1989).

The leaves of *Castanea sativa* along the Tsar Boris III Blvd. were larger in area than the controls in the first two years of study. An increase of the leaf area under industrial and urban pollution was found by some authors (Dorogan & Djukov 1990; Carreras & al. 1996; Gratani & al. 2000; Balasooriya & al. 2009). A larger area of leaves above the controls was registered in *Tilia cordata* and *Platanus* x *acerifolia,* again under the conditions of high traffic (Tsarigradsko Shose Blvd.) in an earlier study of the author (Kurteva & al. 1997).

Conclusions

The results obtained in the present investigation indicated that the studied tree species could be used as bioindicators of industrial and road traffic pollution in the monitored regions.

The studied species and the morphological indexes can be used to detect pollution as well as its types. Presence of unspecific pollution can be detected by the plants of *Castanea sativa* and *Cydonia oblonga* by means of the leaf area parameter, whereas specificity of pollution is determined by the linear growth of the twigs parameter in *Aesculus hippocastanum* in Pirdop, and the number of leaves parameter in Botunets. The twigs growth index of *Cydonia oblonga* can be used as an indicator of industrial pollution in Pirdop and Botunets, while the number of leaves on the twigs parameter in Gara Yana can be also used as an indicator of industrial pollution. The morphological parameters of linear growth of the twigs and number of leaves on the twigs in the plant species *Castanea sativa* are valuable bioindicators of intensive road traffic pollution.

The low values of twigs growth and the number of leaves in the controls of *Aesculus hippocastanum* (2010), as well as the lower twigs growth in *Cydonia oblonga* observed in 2009, and the number of leaves of the same species in 2009 and 2010, have confirmed the impact of other environmental factors, especially climate, on the growth and development of the investigated species.

Data presented in this paper could be used to determine which species are suitable for planting in the monitored regions. Of the studied species, *Cydonia oblonga* was defined as relatively resistant, with high twigs growth and good decorative effect, suitable for planting in the region of Pirdop. Because of the recorded low values of the measured indexes in the species of *Aesculus hippocastanum* in Pirdop and along the Tsarigradsko Shose Blvd. and of *Castanea sativa* in Gara Yana (i.e. these species were proved as more sensitive), they can be recommended for moderate application in the above-mentioned or in similar regions with high level of technogenic pollution. *Aesculus hippocastanum* (Botunets), *Cydonia oblonga* (Botunets, Gara Yana) and *Castanea sativa* and *Cydonia oblonga* (Tsarigradsko Shose Blvd.), which registered the lowest values of the studied morphological indexes, are considered very sensitive and, hence, unsuitable for planting in the investigated regions.

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