

Leaves anatomical and physiological adaptations of *Vinca major* 'Variegata' and *Hedera helix* L. to specific roof garden conditions

Sorina A. PETRA¹, Mihaela I. GEORGESCU^{1*}, Cristina R. MANESCU¹,
Florin TOMA¹, Monica L. BADEA², Elisabeta DOBRESCU¹,
Vlad I. POPA³

¹University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture, Department of Landscape Architecture, Biodiversity and Ornamental plants, 59 Marasti Boulevard, District 1, 011464, Bucharest, Romania; sorina.petra@gmail.com; mihaelaigeorgescu@yahoo.com (*corresponding author); xtina.ro@gmail.com; florin27toma@gmail.com; veradobrescu@gmail.com

²University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture, Department of Bioengineering of Horticultural Systems, 59 Marasti Boulevard, District 1, 011464, Bucharest, Romania; monalisa588@yahoo.com

³University of Agronomic Sciences and Veterinary Medicine of Bucharest, Research Centre for the Quality of Agrofood Products, 59 Marasti Boulevard, District 1, 011464, Bucharest, Romania; popa.ivlad@yahoo.com

Abstract

Urban agglomerations create extreme microclimates for plants, in which growth, development and survival means adaptation. Plantations expansions beyond the typical gardens to buildings, walls or other build structures were realized in many cities with a rigorous selection of plant species. Although the number of woody species well adapted to the urban environmental conditions is quite large, few species manage to grow and develop on the roofs. Two species - *Vinca major* 'Variegata' and *Hedera helix*, regularly used for this type of plantations in Bucharest, were selected to understand their mechanism of adaptation. A comparative study was conducted on these species, growing on a rooftop garden and at the ground level into a typical garden. Both species revealed considerable anatomical differences of the leaves. In addition, physiological determinations revealed a stronger intensity of photosynthesis, an intense transpiration and a lower respiration rate at plants grown in the roof garden.

Keywords: evergreen plants; extreme environment; leaf structure; rooftop plantation

Introduction

The extension of the vegetal carpet from the ground level to the roof of the buildings was imposed not only as a solution for increasing green areas in these extensive build and polluted environments, but also as a manner to insulate and cool the buildings (Galbrun and Scerri, 2017; Cao *et al.*, 2019). Long-term sustainability of rooftop plantations is based on a rigorous selection of species, considering not only the plants survival in hard microclimate (shallow soils, modest water, constant wind, extreme temperatures), but also the

increase of biodiversity, aesthetic of the plantations and benefits for human health (Snodgrass and Snodgrass, 2006; Butler *et al.*, 2012; Whittinghill and Rowe, 2012; Arabi *et al.*, 2015; Eksi *et al.*, 2017; Bădărau, 2018).

In cities with hot and dry summers and cold winters, as Bucharest, Romania, the number of species, that can be used for roof gardens are very limited (Nicolae and Petra, 2018). Many species, despite being chosen from appropriate habitats, failed on roof gardens (Rayner *et al.*, 2016). The complexity of the factors involved in the survival of species on -roof garden makes difficult to specialists to recommend certain plants - succulents, herbaceous perennials, grasses, shrubs, without testing plants in each climate (Tran *et al.*, 2019). Roof gardens usually combine all those types of vegetation. As result, it is assured not only enhanced efficiency of the cooling effect (Cao *et al.*, 2019; Wolf and Lundholm, 2008), but also an improved visual, a positive perception by people and economic benefits (Rahman *et al.*, 2015). Certain species, such as sedum plants, can improve the performance of other species in association by reducing the temperature of soil and preserving water (Butler and Orians, 2011). However, in some cases of mixed-species community, the associations and arrangement of species must be done with caution, for survival and coverage (Schindler *et al.*, 2019; Liu *et al.*, 2019).

Plants develop different morphological, anatomical and physiological adaptations of the leaves, stems and roots in order to survive in stressful growth conditions: drought (Mansoor *et al.*, 2019; Morales-Tapia *et al.*, 2019; Ashfaq *et al.*, 2019), salinity (Mansour, 2014; Albaladejo *et al.*, 2017; Bernstein, 2019) or pollution (Pollicelli *et al.*, 2018; Lu *et al.*, 2018). Anyway, the plant response at the leaf level is different depending on the species or age. Gray and Brady (2016) showed that in the case of increasing the CO₂ concentration, only young plants reacted, enlarging the mesophyll and increasing the palisade and spongy parenchyma. Also, drought diminished the rate of leaf expansion, while high temperatures accelerate the phenomenon. In some other plants, a number of changes in the anatomical structure of the leaves occur with the altitude. The epidermis and cuticle become thicker with increasing altitude (Körner *et al.*, 1983; Srivastava *et al.*, 2018).

The present research, a comparative study was conducted on *Vinca major* 'Variegata' and *Hedera helix*, growing on a rooftop garden and at the ground level into a typical garden. Species were selected for their comparable characteristics - both are woody plants, with persistent leaves. The main objective was to identify changes in the leaf's anatomy and physiology as a mechanism of adaptation to the challenging climate of the roof gardens. Understanding the reaction of plants and their level of adaptations in critical conditions, it is a necessary step before develop more roof gardens, especially when the future is threatened by climate changes.

Materials and Methods

In order to examine plants adaptation at roof garden conditions, for a comparative study, two identical units were installed, on University of Agronomical Sciences' campus in Bucharest (44°24'49"N and 26°05'48"E). The first experimental unit was placed into an existing roof garden on the top of the Research Centre for the Quality of Agrofood Products Building, a four storey high, which covers an area of 225 m². The garden, planted in 2014, was designed with a mixture of vegetation - shrubs, herbaceous perennial and grasses, which are well adapted to the climate of Bucharest. For the present study, only two species were selected: *Vinca major* 'Variegata' and *Hedera helix*, which were planted in full sun, into a garden soil mixed with compost with a depth of 60 cm. Plants were watered using the existing drip irrigation system, together with the rest of the garden. The second experimental unit was located on the ground level in the Botanical Garden, at 50 m distance from the building. Here, the plants were growing in similar conditions of light, humidity and soil with those placed on the roof.

Climatically, Bucharest has an extreme weather, with an annual average of temperature of 10.5 °C. The city experiences regularly very low temperatures in winter (below -10 °C) and two-four snow storms per winter and hot temperatures in summer (over 35 °C) and tropical nights (23-25 °C) in July and August. Precipitations occur especially in spring and late autumn, with a volume below 500 mm/year.

For anatomical investigations, ten leaves per experimental unit of each plant of *Vinca major* 'Variegata' and *Hedera helix* were collected in July, 2019 from the middle part of the annual shoots. Transverse sections were made through the mid-section of the laminae using free hand sectioning technique. The sections were clarified with chloral hydrate in saturated solution, for 24 hours and washed with tap water, according to the protocol of (Șerbănescu-Jitariu *et al.*, 1983). Observations and measurements were performed with the Leica DM 1000LED microscope, with the eye piece lens of 10 x and objective lenses 25 x, provided with LAS-CORE software. Photos were taken with the DFC 295 camcorder. Surface of leaves was investigated using FEI Inspect S50 SEM, in low vacuum mode, with a working pressure of 160 Pa and acceleration voltage of 5 kV. Measurements concerning stomatal size (guard cell length, in μm) and density of stomata (number mm^{-2}) were taken by ocular micrometer. Physiological measurements were made with LCpro+ (ADC BioScientific Ltd, UK), in July and December, 2019 directly on plants using each time two mature leaves, from the annual shoots, for each plant. Correlations were investigated using Pearson test at $P \leq 0.05$ and linear regression analyses.

Results

The anatomical studies on leaves of *Vinca major* 'Variegata' collected from plants growing on the two experimental units (roof garden and typical garden) revealed notable differences (Figure 1).

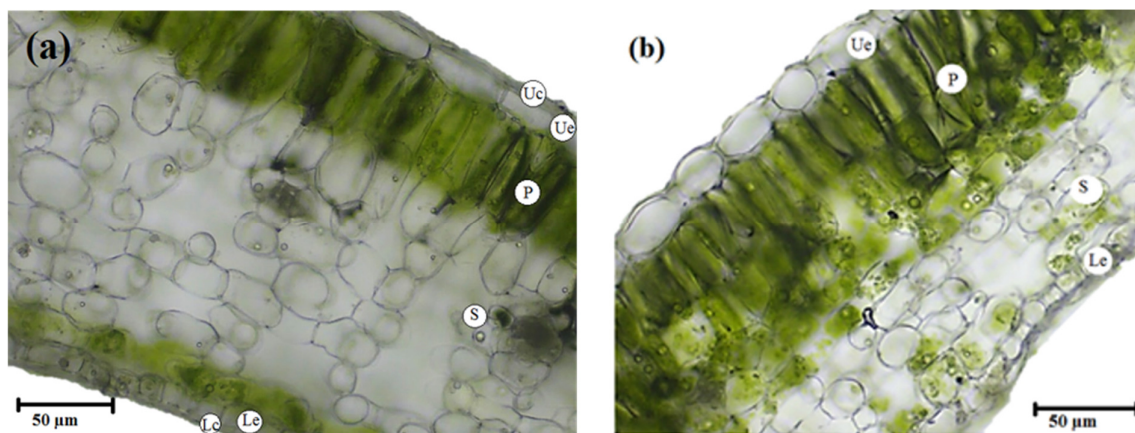


Figure 1. Transverse section through leaf of *Vinca major* 'Variegata', (a) roof garden plant, (b) typical garden plant. Uc = upper cuticle; Ue = upper epidermis; P = palisade parenchyma; S = spongy parenchyma; Le = lower epidermis; Lc = lower cuticle

Leaves upper cuticle was $4.9 \mu\text{m}$ in size at plants located on the roof garden and $3.5 \mu\text{m}$ at those on the ground level. In the roof conditions, the shape of upper epidermis cells was oblong, forming a layer of $16.68 \mu\text{m}$ in size and in ground level conditions, leaves have developed an upper epidermis from oblong-ovoidal cells of $17.89 \mu\text{m}$ in size.

Palisade parenchyma cells were cylindrical, single-layered with a thickness of $51.78 \mu\text{m}$ at roof garden plants and double-layered, $60.04 \mu\text{m}$ in size, at ground level plants. At roof garden plants, spongy parenchyma cells were found with 7-8 layers and $135.57 \mu\text{m}$ in size, while at plants growing at the ground level were 6-7 layered and $107.16 \mu\text{m}$ in size. For both locations, these cells were ovoid, oblong or circular shaped. Lower epidermis cells were round, oblong or irregular in shape, forming a layer of 13.39 and $11.90 \mu\text{m}$ in size at roof garden plants, respectively, at ground level plants. Leaves collected from roof garden presented a lower cuticle of $3.8 \mu\text{m}$ in size and those from the typical garden of $2.74 \mu\text{m}$ in size.

The surface of leaves was quite similar (Figure 2). Images revealed hypostomatous leaves, with a small decrease of stomatal density in the case of plants from the roof garden (88 stomata per mm², compared with 119, at plants from the typical garden). Stomatal size (guard cell length) was almost equal in value in the two experimental units (12.3 µm, roof garden plants and 12.9 µm, at typical garden plants).

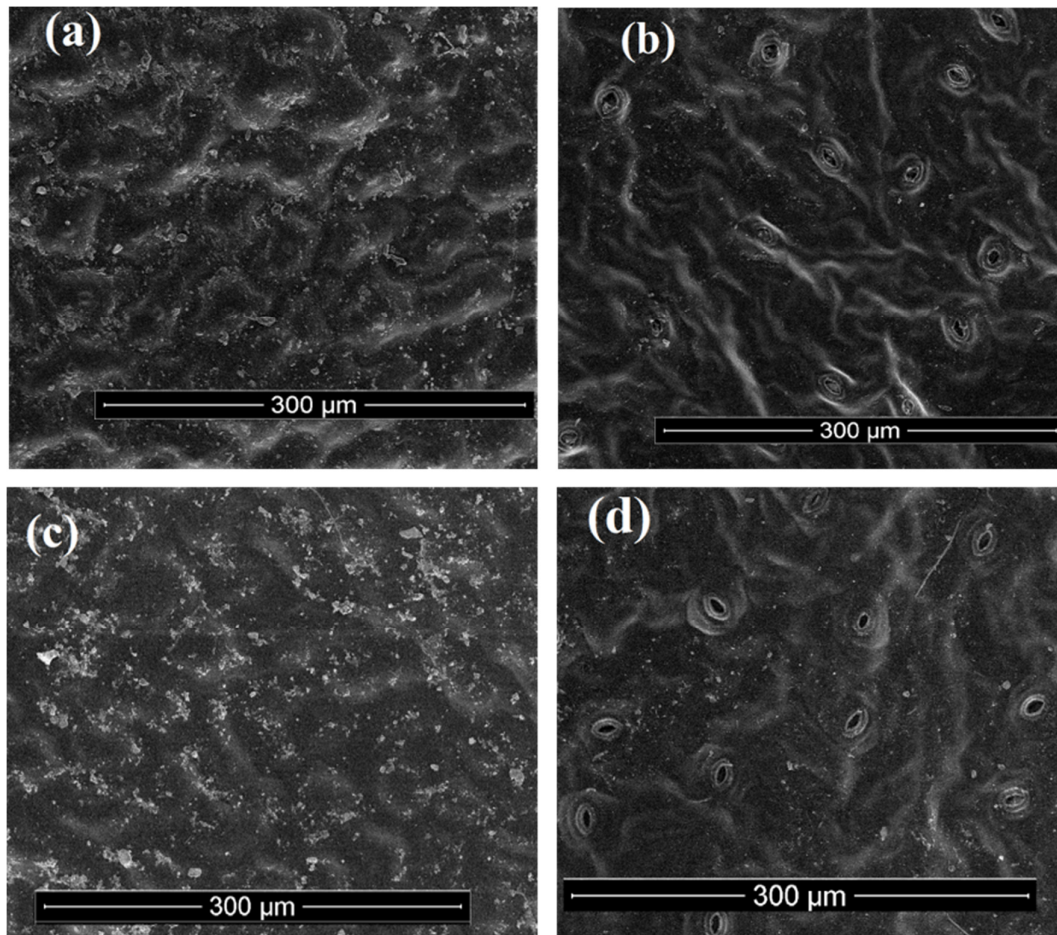


Figure 2. Microscopic images (photographed at 400x) of *Vinca* leaves surface. Top images are from the roof plants: (a) adaxial surface; (b) abaxial surface. Bottom images are from the typical garden plants: (c) adaxial surface; (d) abaxial surface

Comparative microscopic measurements made at *Hedera helix* plants growing on the roof garden and at ground level showed variation in anatomical structure (Figure 3). For both sites, the upper cuticle of leaves presented the same thickness (2.72 µm). The upper epidermis cells were oblong-ovoidal in shape, forming a layer of 6.26 µm in size at roof plants and 6.24 µm at ground level plants.

The palisade parenchyma in leaves collected from roof garden was formed from double-layered cells of cylindrical shape, with a total thickness of 73.04 µm. In the case of leaves developed by plants growing at ground level, the palisade parenchyma contained triple-layered cells of almost spherical shape, with a total size of 56.76 µm. For both locations, the spongy parenchyma cells were rounded or oblong in shape. The thickness of the spongy parenchyma was 125.08 µm and 111.14 µm, at roof garden plants and respectively ground level plants. Cells of the lower epidermis were rounded, ovoid or with irregular in shape, with a size of 4.8 µm, at the roof

garden plants and $6.4\ \mu\text{m}$, at the typical garden plants. Leaves collected from roof garden presented a lower cuticle of $2.12\ \mu\text{m}$ in size and those from the typical garden of $3.25\ \mu\text{m}$ in size.

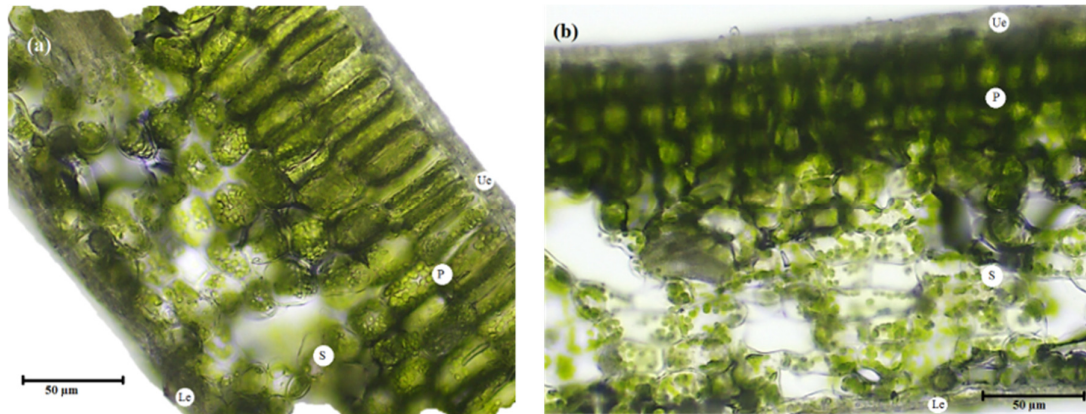


Figure 3. Transverse section through leaf of *Hedera helix*, (a) roof garden plant, (b) typical garden plant. Ue = upper epidermis; P = palisade parenchyma; S = spongy parenchyma; Le = lower epidermis

Microscopy images of leaves surface of *Hedera* showed differences between the two experimental sites (Figure 4). Leaves were hypostomatous, with a higher stomatal density in the case of roof garden plants ($268\ \text{stomata per mm}^2$ compared with 150, at plants from the typical garden). Leaves of roof garden plants presented increased stomatal size ($12.6\ \mu\text{m}$, compared with $10.3\ \mu\text{m}$, at plants from the typical garden).

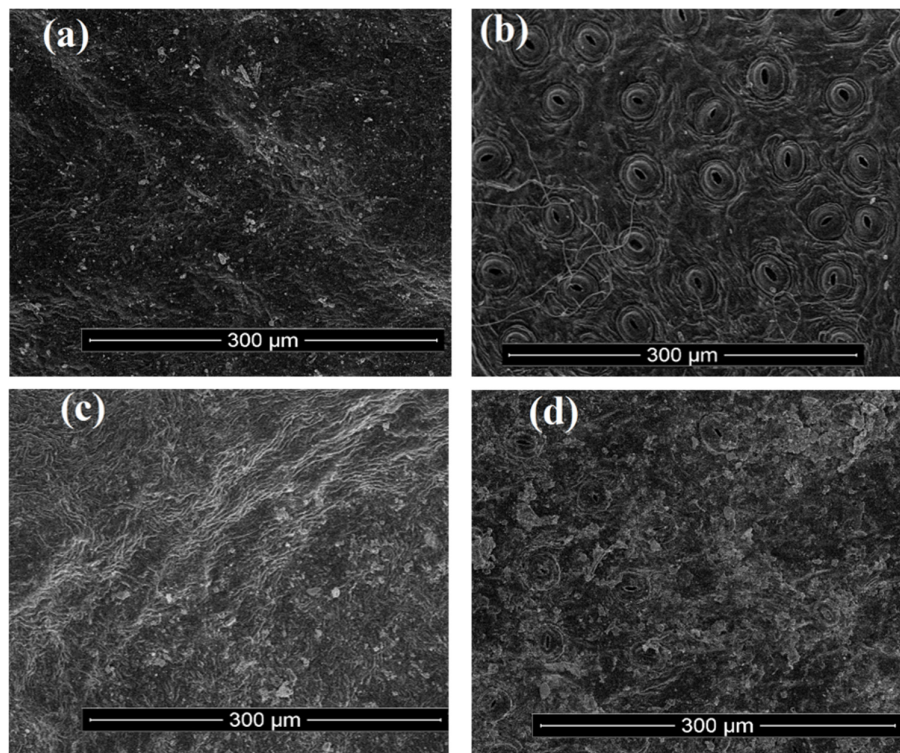


Figure 4. Microscopic images (photographed at 400x) of *Hedera* leaves surface. Top images are from the roof plants: (a) adaxial surface; (b) abaxial surface. Bottom images are from the typical garden plants: (c) adaxial surface; (d) abaxial surface

For the two species examined, data regarding the anatomical measurements of the leaves showed a significant positive correlation ($r = 0.9505$; $r^2 = 0.903$; $p = 0.0002$) between stomatal density and the thickness of the palisade parenchyma (Figure 5). No significant relationship was found between stomatal density and stomata size ($r = 0.089$; $r^2 = 0.008$; $p = 0.90$), stomatal density and leaf thickness ($r = 0.054$; $r^2 = 0.003$; $p = 0.93$) or stomata size and leaf thickness ($r = 0.712$; $r^2 = 0.508$; $p = 0.28$).

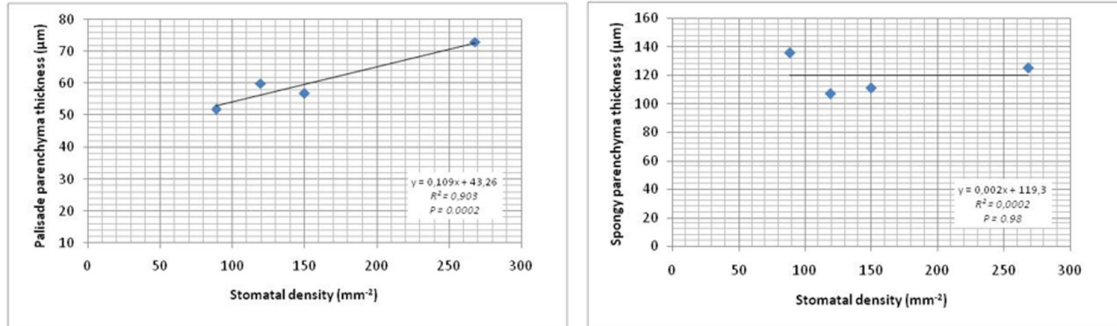


Figure 5. Correlations of stomatal density with thickness of parenchyma layers

Physiological processes measured at *Vinca major* 'Variegata', indicated dissimilarities among plants growing on the roof garden and those at the ground level (Table 1).

Table 1. Gas exchange parameters measured on leaves of *Vinca major* 'Variegata'

Parameters	July		December	
	Roof garden	Typical garden	Roof garden	Typical garden
Stomatal conductance (mmol H ₂ O m ⁻² s ⁻¹)	0.46	0.19	0.03	0.06
Photosynthesis rate (µmol CO ₂ m ⁻² s ⁻¹)	28.42	11.67	0.93	5.25
Transpiration rate (mmol H ₂ O m ⁻² s ⁻¹)	9.81	5.51	0.43	0.90
Respiration rate (µmol CO ₂ m ⁻² s ⁻¹)	9.10	10.34	0.16	1.72

At comparable light intensity (1,282.0 µmol m⁻² s⁻¹ on the roof and 1,280.0 µmol m⁻² s⁻¹ at ground level), the stomatal conductance was 0.46 mmol m⁻² s⁻¹ and 0.19 mmol m⁻² s⁻¹ in plants on the roof garden, and respectively, the typical garden. At an average air temperature of 43.6 °C measured in July at the leaves level of *Vinca* plants, growing on the roof, the photosynthesis was a rate of 28.42 µmol m⁻² s⁻¹ and transpiration of 9.81 mmol m⁻² s⁻¹. For the plants growing in the typical garden, the average air temperature was of 39.4 °C and the photosynthesis rate recorded mean values of 11.67 µmol m⁻² s⁻¹ and 5.51 mmol m⁻² s⁻¹ for transpiration rate. Respiration rate was slightly higher at plants growing at ground level. In December, at an air temperature of 9.1 °C, all the gas exchange parameters were lower at roof plants, compared with the typical garden plants.

Hedera helix had a physiological response at the microclimate created by roof garden (Table 2). In July, comparative with plants growing at the ground level into a typical garden, the leaves of the plants growing on the roof garden presented a greater stomatal conductance of 0.29 mmol m⁻² s⁻¹, at a light intensity of 1,281.0 µmol m⁻² s⁻¹.

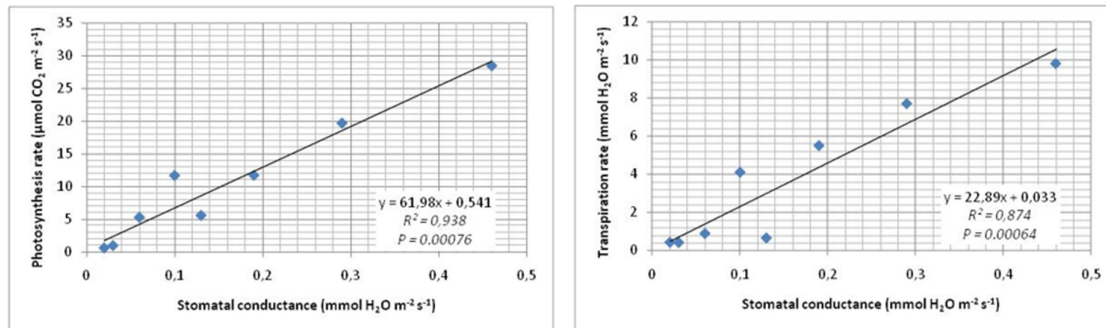
Table 2. Gas exchange parameters measured on leaves of *Hedera helix*

Parameters	July		December	
	Roof garden	Typical garden	Roof garden	Typical garden
Stomatal conductance ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$)	0.29	0.10	0.02	0.13
Photosynthesis rate ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$)	19.66	11.64	0.56	5.54
Transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$)	7.71	4.11	0.44	0.67
Respiration rate ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$)	4.53	6.46	2.31	1.38

The photosynthesis rate recorded at roof garden plants was a value of $19.66 \mu\text{mol m}^{-2} \text{s}^{-1}$ and the transpiration rate of $7.71 \text{mmol m}^{-2} \text{s}^{-1}$. These parameters were both at lower intensity of light ($1,238.0 \mu\text{mol m}^{-2} \text{s}^{-1}$) in the case of *Hedera* plants growing at the ground level. The respiration process was more intense in plants on the typical garden ($6.46 \mu\text{mol m}^{-2} \text{s}^{-1}$).

In December, at an air temperature of 9.1°C , all gas exchange parameters, except respiration rate, were lower at the roof plants.

For both species, stomatal conductance (Figure 6) was found strongly positive correlated with photosynthesis rate ($r = 0.9686$; $r^2 = 0.9382$; $p = 0.00076$) and transpiration rate ($r = 0.9353$; $r^2 = 0.8748$; $p = 0.00064$). No significant relationship was established between stomatal conductance and respiration rate.

**Figure 6.** Relationship among gas exchange parameters

Discussion

Both species, *Vinca major* 'Variegata' and *Hedera helix* proved their capacity to survive in challenging conditions created by the roof garden microclimate, developing leaves anatomical and physiological adaptations. Although all plants were grown in full sun, due to an excessive sunlight and high rate of transpiration, the upper cuticle of *Vinca* leaves was much thicker in case of plants growing on roof garden. Also, the cuticle was thicker on the adaxial surface and thin on the abaxial surface. This particular relationship between cuticle thickness and increased light intensity was observed also in other woody species (Ashton and Berlyn, 1994; Gratani *et al.*, 2006; Rossatto and Kolb, 2010).

In both species, palisade parenchyma was reduced with one layer at roof garden plants. In a previous study, *Vinca major* 'Variegata' was described having two layers of palisade cells (Cui *et al.*, 2011), so, this modification meant for the cultivar a return to the leaf anatomy of the species (*Vinca major*), which have a single layer of palisade cells. The anatomical response to stress was observed only at tissue level, without color variations of leaves. In case of *Hedera helix*, although palisade parenchyma loss one row of cells, it increased in thickness (on average 23% higher in roof plants than typical garden plants) due to the shape of cells. At this species palisade cells have changed their shape, from almost spherical (characteristic to shade-tolerant species),

as they were described in other studies (Săvulescu and Luchian, 2009), in cylindrical. Such an adaptation was necessary at intense light conditions of the roof garden, because a palisade round cells may create an optically dense layer, which limit the penetration of light into deeper cell layers (Terashima and Hikosaka, 1995). Consequently, the cylindrical cells could contribute to the higher photosynthesis performance per unit leaf area (Gotoh *et al.*, 2018).

Leaf thickness was increased in size at the roof garden plants, in both species. Mesophyll of leaves was greater at roof garden plants with an average of 10% at *Vinca* and 15% at *Hedera*. Spongy parenchyma contributed considerably more than other layers, in case of *Vinca* on an average with 60% to the total leaf thickness and in the case of *Hedera*, with 58%. This important leaf adaptation of both species at roof garden conditions had increased the photosynthetic rate by the improvement of internal CO₂ diffusion, due to lose of intercellular spaces in the spongy parenchyma, as in other woody species (Marchi *et al.*, 2008). A laxer spongy parenchyma allows air accumulation into intercellular spaces, which can be used by plant as a shock absorber to sudden temperature changes (Jiménez-Noriega *et al.*, 2015). Thicker spongy parenchymas were found at some woody plants living in stressful environments: high light (Jackson, 1967; Catoni *et al.*, 2015; Kong *et al.*, 2016), excess humidity (Pearce *et al.*, 2005; Rossatto and Kolb, 2010), water temporary deficit (Ennajeh *et al.*, 2010) or high altitude (Jiménez-Noriega *et al.*, 2015; Taneda *et al.*, 2016).

Stomatal density was notable increased in the case of *Hedera* plants growing in the roof garden. Also, at roof garden plants, stomatal size was found bigger only at *Hedera*. Nevertheless, the stomatal conductance was greater in the case of all roof garden plants. An increase of stomatal conductance was explained at other woody plants as a possible adaptation of leaves at high temperatures to enhance photosynthesis (Pearce *et al.*, 2005; Teskey *et al.*, 2015; Urban *et al.*, 2017). In fact, photosynthesis rate was in close relationship with the stomatal conductance and recorded increased values during summer at roof garden plants. Also, measured in July, during a heat wave, the transpiration rate was higher to the roof garden plants. In contrast, the respiration rate decreases at roof garden plants in both species.

Conclusions

This study revealed a series of adaptations of leaves at both *Vinca major* 'Variegata' and *Hedera helix* plants, which were growing in the roof garden. Plants reaction at the roof conditions was comparable with those of plants growing at altitude. The microclimate created by the four-level high building, determined the plants to adapt their leaf anatomy and physiological processes in order to survive at a greater light intensity, hot temperatures in summer and constant wind. Studied species revealed that the key for survival on roof conditions is linked with the capacity of increase the thicknesses of some tissue (cuticle and spongy parenchyma), decrease the layers of palisade cells, change the cells shape (from the epidermal and palisade parenchyma) or increase the stomatal conductance. Both species demonstrated a great capacity of adaptation, but further studies are necessary to confirm these results and maybe find other adaptations that woody species can develop in roof conditions.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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