1 Introduction

Changes of environmental conditions in urban areas restrict the growth of many species (Gillner et al., 2013). Growth and vitality are considerably influenced by microclimatic conditions, especially changes in air humidity and air temperature caused by reradiation effects, higher surface temperatures, and higher winds pells (tunnel effects) in streets (Sieghardt et al., 2005; Huang et al., 2008). Lack and irregular distribution of water cause periods of drought. Drought is the most important environmental stress, severely impairing plant growth and development (Anjum et al., 2011). It is known that drought significantly influences photosynthesis by changes in metabolism and regulation of stomatal conductance (Bota et al., 2004). Stomatal responses to changes in environment are essential for the plants acclimation to environmental conditions (Heterington and Woodward, 2003; Berry et al., 2010). Stomatal closure is a protective strategy against waterloss and xylem hydraulic failure (Choat et al., 2007; Chen et al., 2010). In relation to plant strategy of stomatal control used to eliminate consequences of water losses, there are two known types of plants. Isohydric plants under drought conditions close their stomata gradually to reduce gas exchange and waterloss (Kumagai and Porporato, 2012; Sade et al., 2012). Anisohydric plants tolerate a decline in water potential by keeping stomata open to enable continuous gas exchange within certain levels of water stress (Tardieu and Simonneau, 1998). Stomatal closure is generally considered a protective mechanism against drought stress (Tyree et al., 1998).

Stomata have two main functions in plants. They participate in photosynthesis course and optimise water balance. Stomata open after sunrise (when there is light for photosynthesis) and close after sunset (Procházka et al., 1998). Stomata play key role in retaining water in plant. Plants are able create tolerance against water deficit by creation of tolerance to conditions of water deficit or by prevention of water loose (Bray, 2001). Transpiration is related to water content in leaves. Relative water content (RWC) indicates metabolic changes in plants. RWC represents the total amount of water needed by a plant at full saturation. The RWC expresses the water content in per cent at a given time as related to the water content at full turgor (González and González Vilar, 2001). Decrease of the RWC is accompanied with changes in physiological functions of plants, synthesis of growth and stock substances and metabolical changes. Relationship between the RWC, water content in soil and stomatal conductance could be an indicator of plants reactions to water stress.
The objective of the presented study was to clarify the interaction between stomatal closure and water content changes in the leaves in relation to dryness of soil substratum. *A. campestre* L, native maple from dry sites of Slovakia, was chosen as the subject of the study. We supposed that the relative water content and stomatal closure would be in direct relation and *A. campestre* L. would preserve water in leaves by stomatal closure after detection of water scarcity in the substratum.

### 2 Materials and methods

Plant material was produced from seeds. The plants were three-years old; a year grown in 2 litres containers. The height of plants was 700–800 mm, with trunk diameter 7–9 mm (measured 150 mm above ground). Donor parent trees were grown in the Arborétum Mlyňany in southern Slovakia. TS 3 standard substratum (pH 5.5 to 6.0 + fertilizer 1 kg/m³) enriched by clay fraction (0–25 mm/m clay 20 kg/m³) with 10% of additional sand was used. Up to end of June, the humidity of substratum was on the level of 70% of soil water content. From the mid-July (at the beginning of experiments), irrigation was stopped and the process of continual substratum desiccation began. Irrigation was stopped up to the end of the experiment (for next 13 days). Water content in the substratum was 25% in the end of the experiment. During the experiment the plants were stored in a greenhouse, protected from rain and direct sunlight.

Stomatal conductance, as an index of plant stress and indicator of photosynthetic activity, was measured with an AP4 Leaf Porometer. Measurements were taken in three days intervals on two fully expanded leaves of five plants between 7 and 10 a.m. Ten replications were made for each measurement.

During measurements (once in three days) there were defined soil water contents and relative water contents in the leaves. There were used two plants for analyses each weak. Water contents were set gravimetrically. The *RWC* was set as follows:

\[
RWC = \frac{100 \times (FW - DW)}{TW - DW}
\]

After the end of the experiment, the analysis of variance and the multiple range test were used for data evaluation. The software Statgraphics Centurion XVI was used for statistical evaluation.

### 3 Results and discussion

Substratum desiccation caused immediate decrease of stomatal conductance in the leaves of *A. campestre* L. Changes in the relative water content (*RWC*) in the leaves were observed after seven days without water. In relation to substratum desiccation, *A. campestre* L. plants reacted by linear decrease of stomatal conductance. After the first days, when water content of the substratum decreased from 70% to 62%, stomatal conductance decreased slowly by 8% (from 0.65 mm/s to 0.57 mm/s). After the next three days, when water content of the substratum decreased on 53%, stomatal conductance decreased by 14% (from 0.57 mm/s to 0.43 mm/s). The decrease in stomatal conductance was observed in all next days. After thirteen days, stomatal conductance decreased to 0.23 mm/s (Table 1).

During the first seven days of the experiment, the observed *RWC* value in the leaves was about 84%. The *RWC* decreased to 30.7% at the end of the experiment.

According to the study by Li et al. (2016) *A. campestre* L. belongs to anisohydric plants. It was found to tolerate a declining water potential with a slow reduction of

| Table 1 | Multiple range test of stomatal conductance in *A. campestre* L. leaves under water scarcity (*P* value <0.05) during experiment |
|---------|-------------------------------------------------------------------------------------------------------------|
| Experimental days | Count | Mean | Differences in groups |
| 0 | 10 | 0.65 | * |
| 3 | 10 | 0.57 | * |
| 7 | 10 | 0.43 | * |
| 10 | 10 | 0.30 | * * |
| 13 | 10 | 0.23 | * |
Plants in Urban Areas and Landscape

In the first week without water, plants maintained balanced RWC. During the second week, the RWC dramatically decreased. The RWC is closely related to photosynthesis limitation. A border of photosynthesis limitation in relation to the RWC could be very variable and reactions of plants are specific due to species differences (Bota et al., 2004; Flexas et al., 2006). Chaves et al. (2002) consider decrease of the RWC to 70–75% a limit for photosynthesis. Plants of field maple reached this limit after seven days of drought, and the use of stomatal closure was not more able to preserve water in the leaves.

4 Conclusions

As we supposed, relative water content and stomatal closure of *A. campestre* were in direct relation. The decrease of the RWC began three days after stomatal closure induction, when the water content in substratum decreased to 53% of soil water capacity. The preservation of water in the leaves of young plants by stomatal closure after detection of water scarcity in the substratum lasted 7 days. After decrease of water content in substratum under 50% of soil water capacity, the RWC decreased dramatically.

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Figure 1: Development of water content in the substratum, stomatal conductance, RWC content in the leaves of *A. campestre* L. under conditions of water scarcity.
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CONTENTS