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Tree leaf wettability as passive bio-indicator of urban habitat quality

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ABSTRACT

This study evaluated the effect of urban habitat quality on the wettability of tree leaves. We measured leaf wettability of five common tree species, i.e., *Alnus glutinosa*, *Acer pseudoplatanus*, *Betula pendula*, *Quercus robur* and *Sambucus nigra*, in semi-natural and industrial urban habitats in the city of Gent (Belgium). Possible seasonal variation was taken into account by measuring in late spring and in late summer. Drop contact angle (DCA), and height over width ratio were measured on the abaxial and the adaxial leaf surface as proxies for leaf wettability. The relative standard deviation for the height over width ratio was higher than for the DCA, so that only the latter was considered further.

Habitat type significantly influenced leaf wettability: the DCA values of *Q. robur* leaves were significantly lower in the industrial than in the semi-natural areas, in both June and September while, for *S. nigra*, the DCA was in both sampling events significantly higher in the industrial areas. For the adaxial leaf side, the differences between the considered habitats were more pronounced in June than in September. The adaxial DCA of *A. pseudoplatanus* was significantly higher in June compared to September, while the opposite held for abaxial values of *A. glutinosa*. We conclude that leaf wettability is potentially a good indicator to point out differences in urban habitat quality, but selection of the most sensitive tree species and the appropriate time of measuring is an important prerequisite.

1. Introduction

The leaf surface of a plant is continuously exposed to atmospheric pollutants via dry and wet deposition. This leaf surface consists of a cuticle layer upon which almost all higher plants develop an epicuticular wax layer during leaf expansion, to protect the interior part of the leaf from damage (Cape and Percy, 1993). The epicuticular wax layer is important for leaf photosynthesis (Benzing and Renfrow, 1971), interception, transpiration, stomatal function, and susceptibility of leaves to infections by pathogens, especially fungi (Haines et al., 1985; Hollier, 1985; Reynolds et al., 1989; Smith and McClean, 1989).

The structure and chemical composition of the epicuticular wax layer can change due to the influence of atmospheric pollutants, climate and physical friction by wind (Baker and Hunt, 1986; Barnes et al., 1988; Cape and Percy, 1998; Shepherd and Griffiths, 2006; Burkhardt, 2010), and thereby influencing the leaf wettability or hydrophobicity. Besides these environmental factors, also leaf shape (Cape et al., 2003), epidermal characteristics (Neinhuis and Barthlott, 1997), and trichome height and density (Brewer et al., 1991; Haines et al., 1985) can affect leaf wettability.

Neinhuis and Barthlott (1998) observed the relation between accumulated particles and leaf wettability. They reported that leaves of *Ginkgo biloba*, having a low wettability, accumulate less particles compared to those of *Fagus sylvatica* and *Quercus robur*, having a high wettability. Besides, they found that the capacity of leaves of *Q. robur* to accumulate particles relates to its increasing wettability during the season. Neinhuis and Barthlott (1998) attributed this seasonal dynamics in wettability of oak to the epicuticular wax chemistry of this species, which is very susceptible to erosion.

The effect of individual pollutants such as O_3 on leaf wettability is reported by Barnes and Brown (1990) and Paoletti et al. (2007). Cape et al. (1995) reported a decrease in leaf wettability in older needles by ageing and increasing SO_2 for Norway spruce.

Most studies have measured the effect of air pollutants on leaf wettability of evergreen species (Percy et al., 1992; Cape et al., 1995; Barnes and Brown, 1990; Kupcinskiene and Huttunen, 2005) and less attention was paid to deciduous species (Neinhuis and

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Barthlott, 1998; Schreuder et al., 2001). Also, most of previous research evaluated leaf wettability as an indicator for simulated acid rain, a sole pollutant or at most a combination of some pollutants (Barnes and Brown, 1990; Cape et al., 1995; Neinhuis and Barthlott, 1998). Relatively less studies investigated the effect of ambient air pollution i.e., a complex mixture of different types and concentrations of particles and gaseous pollutants in non-controlled outdoor conditions on leaf wettability (Percy and Riding, 1978; Kupcinskiene and Huttunen, 2005).

Erosion, fusion, cracking or any other injury of the leaf surface including ageing, hereafter called 'damage', can be observed using scanning electron microscopy (Cape, 1983; Pal et al., 2000; Sant'Anna-Santos et al., 2006), but this is a rather expensive method. Alternatively, leaf surface damage can be estimated by measuring drop contact angles on the leaf surface as a measure of leaf wettability (Fogg, 1947; Percy and Riding, 1978; Paoletti et al., 1998a). Above all, air pollution may damage the leaf wax layer not homogeneously, which causes a heterogeneous damage of the leaf wax layer. We hypothesise that drop asymmetry can estimate this leaf surface heterogeneity. According to our knowledge no study was performed on biomonitoring of urban habitat quality (sensu Balasooriya et al., 2009; Kardel et al., 2010) or air quality in particular via leaf wettability and drop asymmetry of urban deciduous species.

The main aims of this study were (i) to evaluate the differences in tree leaf wettability between different urban habitats, (ii) to compare species susceptibility to leaf cuticle damage due to habitat quality, and (iii) to evaluate the seasonal variation in leaf wettability, and (iv) to assess the effect of habitat quality on drop asymmetry. Therefore, we measured leaf wettability at adaxial and abaxial leaf sides of five common tree species in two contrasting urban habitats in the city of Gent (Belgium) during early and late summer.

2. Materials and methods

2.1. Description of study area and sampling sites

The city of Gent (Belgium; 51°00′N, 3°50′E, 233,000 inhabitants) was selected as study area. The city is influenced by a temperate maritime climate, with an average minimum and maximum temperature of 7.2 and 14.6 °C and rainfall being almost equally distributed throughout the year. In 2008, the year of sampling, annual rainfall was 862 mm and average air temperature, wind speed, and relative air humidity were 10.9 °C, 3.4 m s^{-1}, and 81%, respectively, at the centre of Belgium (Royal Meteorological Institute of Belgium, www.kmi.be). The average daily air temperature was 16.8 °C and 14.9 °C in June and September 2008, respectively. The prevailing wind direction was southwest.

The city suffers of high concentrations of atmospheric pollutants and particulate matter. In 2008 the average hourly concentrations of NO, NO_2, SO_2, O_3 and PM_{10} in the city centre amounted to 11, 34, 4, 35 and 35 $\mu g\ m^{-3}$, respectively (Flemish Environmental Agency, VMM). These values were comparable to the average concentrations of all monitoring stations in Flanders in that year (13, 30, 7, 38 and 31 $\mu g\ m^{-3}$, respectively). In 2008, the daily PM_{10} concentration for the city of Gent exceeded 50 times the European standard level of 50 $\mu g\ m^{-3}$, while, on average for all measuring stations in Flanders, the level was exceeded only 25 times.

Two contrasting urban habitat types were selected, i.e., a semi-natural area and an industrial area, since former biomonitoring studies indicated a clear distinction in urban habitat quality between these types based on stomatal characteristics of *Taraxacum officinalis* (Balasooriya et al., 2009), *Plantago lanceolata* (Kardel et al., 2010) and *Salix alba* (Wuytack et al., 2010). The semi-natural area encompasses (i) the nature reserve 'Bourgoyen-Ossemeersen' (230 ha), which mainly consists of wet meadows (Peters et al., 2009), and (ii) the recreational park 'Blaarmeersen' (105 ha). The semi-natural area is located north-west of the city centre. The industrial area consists of two locations, i.e., (i) a busy railway station ('Gent Sint-Pieters') and its close neighbourhood and (ii) the city's harbour area, located west and north of the city centre, respectively. The station copes with a high traffic flow of trains and is a busy junction of bus and tram lines. The harbour area encompasses metallurgic, car building, chemical and paper producing industries and a conventional thermal power plant, burning pulverised coal, fuel oil and residual industrial gases.

2.2. Species selection

Five common urban tree species were selected based on their distribution pattern in the study area: *Alnus glutinosa* (L.) Gaertn., *Acer pseudoplatanus* L., *Betula pendula* Roth, *Q. robur* L. and *Sambucus nigra* L. These tree species are hypostomatous (i.e., they only have stomata on the abaxial leaf surface) and differ in leaf shape, size and surface characteristics (Fig. 1 and Table 1).

2.3. Sampling and measurement of leaf surface characteristics

2.3.1. Sampling design

In both the semi-natural and industrial areas, five to eight isolated or dominated trees per species were selected. Only some *S. nigra* trees in the semi-natural areas were shaded by neighbouring trees. The height of sampled trees was for *Q. robur* between 4 and 5 m, *B. pendula* between 9 and 12 m, *A. glutinosa* between 4 and 6 m, *A. pseudoplatanus* between 4 and 6 m and for *S. nigra* between 3 and 4 m. Four to five apparently healthy leaves were sampled per tree, resulting in 20–32 leaf samples per species per habitat type. Fully expanded leaves were cut from the south side of the canopy at a height of 1.70–2.50 m above the ground surface in June and September 2008. Subsequent to cutting, leaf samples were immediately transported to the laboratory. During transport to the laboratory, and until the moment of analysis, samples were kept in a cool box. Measurements were performed on the mid part of both the adaxial and abaxial side of the leaves by avoiding the midrib and the leaf margin.

2.3.2. Leaf wettability

Leaf wettability was estimated from measurements of the drop contact angle (DCA), i.e., the angle between the perimeter of a droplet on a leaf surface, and the leaf surface. At room temperature (21 °C), a 7.5 μl droplet of distilled water was placed on the surface of the fresh leaf, which was fixed between wooden laths so that a horizontal, flat surface was obtained. Next, photographs were taken with a digital camera (Canon EOS 5D DS) fitted with a macro lens (Sony micro 105 mm F 2.8 EX DG). Finally, contact angles at the left and right margin of the droplet were measured with ImageJ software (http://rsb.info.nih.gov/ij) using a manual method and the plug-in 'Drop Snake Analysis'(Stalder et al., 2006). In the latter, the software draws a polynomial fit around the drop based on five to seven manually placed knots on the drop. Based on this fit, DCA values were automatically calculated. Although we found good correlation between the semi-automatic 'Drop Snake Analysis' and the manually derived values (correlation = 0.99; data not shown), we preferred to use the manual method for DCA determination. Reasons are that (i) a slightly higher variation (mean relative standard deviation = 3°) was observed for the semi-automatic method (data not shown) which might be due to the contrast between the droplet and the background is sometimes not good enough to make a good fit around the drop circumference and (ii) the manual method is rapid and allows an easy determination of drop height and width.