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Dry deposition of
ATMOSPHERIC
polycyclic aromatic hydrocarbons
IN *Plantago*
THREE
species

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Abstract

The concentrations of polycyclic aromatic hydrocarbons (PAHs) in the leaf wax of three *Plantago* species were determined weekly for three weeks. The almost glabrous, free-standing leaves of *Plantago major* and the sparsely hairy *Plantago lanceolata* leaves, were more heavily contaminated with low molecular weight (MW) PAHs (MW < 228) than the densely hairy, partly overlapping *Plantago media* leaves. This may be caused by the lower canopy roughness (higher aerodynamic resistance), the higher amount of leaf hairs (higher boundary resistance) and/or the higher leaf overlap (smaller accessible leaf area) of *P. media*. On the other hand, PAHs with MW ≥ 252 tended to show higher concentrations in *P. media* than in the other two species. This is likely caused by the dense layer of hairs on *P. media* leaves, which can efficiently intercept the largely particle-bound high MW PAHs. When the PAH concentrations were normalised to projected leaf surface area the differences between *P. media* and the other two species became significant ($P < 0.05$) for the high MW PAHs, while the differences for the low MW PAHs decreased. Although the differences in PAH concentrations between species are relatively small (factor 2-5), this study clearly shows that plant architecture and leaf hairs influence the dry deposition of PAHs.

Introduction

The uptake of compounds from the atmosphere in plant leaves involves three steps (see **Chapter 2**): the transportation from the (turbulent) atmosphere to the laminar air boundary layer surrounding the leaf (1), the crossing of the boundary layer (2) and the interaction of the molecule with the leaf surface (3). The dominant resistance to the uptake of SOCs is in the atmosphere (step 1 and 2) or in the plant (step 3), depending on environmental conditions, plant characteristics and the properties of the compound.

The main compound property determining the dominating resistance is the K_{oa} (the partition coefficient between octanol and air). For compounds with a low K_{oa} , the cuticle is relatively impermeable and the plant resistance is the main resistance. In this case, the concentration differences between plants may be explained by the amount (Simonich and Hites 1994b) and composition (Kömp and McLachlan 1997a) of the lipids. On the other hand, compounds with a high K_{oa} (e.g. PAHs, PCDD/Fs) are highly soluble in the cuticle and therefore, atmospheric resistance limits uptake (McLachlan *et al.* 1995). The atmospheric resistance, (consisting of the aerodynamic and the boundary resistance, corresponding to respectively step 1 and 2) is influenced by the plant architecture as well as the shape of the leaf surface. The surface roughness of the canopy influences aerodynamic transport (high roughness increases transport), while the roughness of the leaf surface is one of the factors that determines the thickness of the laminar boundary layer ($\delta_{b,l}$). The $\delta_{b,l}$ is also influenced by wind speed and irradiation.

PAHs are present in the atmosphere both in the gaseous phase and bound to particles. While PAHs with MW < 252 are predominantly present in the gaseous phase, large particle-bound fractions are found for PAHs with higher MW, due to their low vapour pressures and high K_{oa} values (Jones *et al.* 1992, Kaupp 1996). The deposition of particles is dependent on particle size, plant characteristics and environmental conditions (Chamberlain and Little 1981). Several plant characteristics have been related to particle deposition. As for gases, a high aerodynamic surface roughness leads to efficient turbulent transport of particles (Burkhardt *et al.* 1995). Particle deposition on different types of vegetation was found to increase with increasing leaf area index (Jonas and Heinemann 1985, Heil 1988). Wind tunnel experiments with radio-labelled particles with sizes ranging from 0.03–44 μm have shown that hairy leaves are better particle collectors than glabrous leaves (e.g. Romney *et al.* 1963, Chamberlain 1967, Wedding *et al.* 1975, Little and Wiffen 1977).

Plant architecture and leaf hairs may thus affect the deposition (rate) of gases and of compounds bound to particles. In this chapter, we compare the dry deposition of gaseous and particle-bound PAHs in three species of *Plantago*, which differ in the amount of leaf hairs and in the architecture of the plant, but have similar wax characteristics.

Experimental

Chemicals

Phenanthrene (PHE, MW 178), anthracene (ANT, MW 178), benz[a]anthracene (BaA, MW 228), chrysene (CRY, MW 228), and benzo[a]pyrene (BaP, MW 252) were obtained from Sigma (St. Louis, MO, USA). Benzo[k]fluoranthene (BkF, MW 252) was obtained from Chem Service (West Chester, PA, USA) and benzo[g,h,i]perylene (BghiP, MW 276) from Fluka (Buchs, Switzerland). Fluoranthene, (FLUO, MW 202) and 5 α -cholestane were purchased from Aldrich (Steinheim, Germany).

Chloroform (p.a.) and diethylether (p.a.) were obtained from Merck (Darmstadt, Germany), methanol (HPLC gradient grade) and acetonitrile (ACN, HPLC gradient grade) from Baker (Deventer, The Netherlands). Octadecylsilica (C₁₈) was purchased from Baker and prewashed with methanol and ACN before use.

Plants

Plantago species are herbs with a short stem. The leaves usually arise from the base of the stem and are spirally arranged. The leaves of *Plantago major* L. (great plantain) and *Plantago lanceolata* L. (ribwort plantain) are relatively free-standing in the air, whereas the leaves of *Plantago media* L. (hoary plantain) are closely spreading on the ground and partly cover each other. *P. major*-leaves (length 10-15 cm) are broad and almost glabrous, while *P. lanceolata* has lanceolate leaves (length 10-15 cm), having more hairs, whereas *P. media* has ovate leaves (length 5-10 cm) with a dense layer of silky hairs.

P. major (seeds from wild origin), *P. lanceolata* (seeds from wild origin) and *P. media* (seeds from Meise, Belgium) were grown in a greenhouse (temp. 28°C). After 4 weeks they were put in separate pots and 5 weeks later they were transferred to a colder greenhouse (temp. 15°C). Fifteen weeks after sowing, the plants were fully grown and placed in an “open” greenhouse (missing the lower half of the walls) in the Botanical Gardens of Utrecht University. This site is considered an urban area. The distance to the nearest highway is approximately 400 m, and the distance to downtown Utrecht is approximately 4 km. The potted plants were placed close to each other, to minimize environmental variation. The plants were sprayed with collected rainwater twice a day. The temperature during the day was 29 \pm 5 °C and during the night 8 \pm 3

Samples

Leaf samples (8 g, quadruplicates) were taken on the last day in the greenhouse (Day 0) and on Day 6, 13 and 20. Each sample consisted of 1 leaf (*P. major*) or 3 to 4 leaves (*P. lanceolata* and *P. media*), originating from the same amount of individual plants. Leaf fresh wt. was determined, after which leaf wax was extracted and analyzed for PAHs. Finally leaf dry wt. was determined. Using plants which were not subjected to PAH analysis, the wax content, the specific leaf area (SLA, $\text{cm}^2 \text{ leaf area} \cdot \text{g}^{-1} \text{ dry wt}$) and the ratio of the projected surface area (A_{proj}) to total surface area (TSA) of the three species were determined ($f_{\text{proj}} = A_{\text{proj}}/\text{TSA}$). Wax content was determined on Day 13 and Day 20 ($n=1$), while SLA was measured on each sampling day ($n = 4$). The f_{proj} were determined for a different batch of plants ($n=3$).

Leaf wax and leaf area

To determine the amount and composition of the leaf wax, leaves were immersed in 2×25 ml chloroform, for respectively 30 s and 10 s, to extract leaf wax. Previous experiments have shown that in this time period the leaf wax is completely extracted (data not shown). Extracted leaves were dried in an oven (80°C, 24 h) to determine foliar dry weight. Extracts were filtered with a glass microfiber filter (pore diameter 2.7 μm , Whatman, Clifton, NJ, USA) to remove particles and after evaporation to dryness, wax weight was determined. The composition of the leaf wax (redissolved in diethylether) was determined as described in **Chapter 3**.

Leaf areas were measured with a leaf area meter (Licor Lincoln L1-3100). To determine f_{proj} , the projected surface area (A_{proj}) was divided by the total surface area of the leaves (= 2x measured leaf area). A_{proj} was determined by taking photographs of the plants from a vertical view, clipping out the projected plant and measuring the area of the clippings (correcting for the scale of the photos).

Sample clean-up and PAH analysis

Leaves were not washed before extraction, to make sure that particle-bound PAHs were not washed away. Extracts (made as described in the previous section) were evaporated to 10 mL under a gentle stream of nitrogen. An amount of 1.5 g C_{18} was added and the samples were further evaporated to dryness. Clean-up of the samples was performed by transferring the C_{18} to a 20 ml plastic syringe filled with a plug of quartzwool and 1 g C_{18} , followed by elution with 18 mL of ACN. Samples were evaporated to 0.5 mL under nitrogen, and injected into an HPLC-system with a Merck-Hitachi L-6200 Intelligent Pump (Merck, Darmstadt, Germany), which

was supplied with a Chrompack ChromSpher PAH-column (Chrompack, Middelburg, The Netherlands) connected to a Merck-Hitachi F-1050 Fluorescence Spectrometer (Merck, Darmstadt, Germany). The column was eluted with ACN/millipore water 55/45 with a linear gradient to 65/35 in 6 min. A ratio of 65/35 was used for 2 min, followed by a 6 min linear gradient to 100% ACN. Then, 100% ACN was flushed for 7 min, after which the ACN/millipore water ratio was reset instantaneously to 55/45. Used wavelengths were 255 nm (excitation)/ 405 nm (emission) for all compounds, except for fluoranthene, which was measured with 280 nm/450 nm.

Quantification

The PAHs were identified by comparing the retention times of the sample peaks to those of the standards. Signal collection and data processing was performed on a computer with Chromcard 1.17 (Fisons Instruments, Loughborough, UK). Recoveries of the PAHs were > 90%, except for BghiP, which was $77 \pm 9\%$. Procedural blanks ($n = 4$) were determined by extraction and cleanup with 50 ml chloroform. PAH concentrations in the samples were calculated by correcting the measured values for recovery and subtracting the average amount measured in the blanks.

Results and discussion

Cuticular waxes

The main components of the extractable cuticular waxes of *P. major* are linear alkanes ($C_{27}H_{56}$ - $C_{33}H_{68}$) and triterpene acids (oleanolic and ursolic acid), (**Chapter 3**). The other two plants have a similar wax composition (data not shown). The wax contents of the plants approximately doubled from Day 13 to Day 20. Nevertheless, the wax content relative to *P. major* remained fairly constant and differed not much from 1 (for *P. lanceolata* the data were 0.82 and 0.86, for *P. media* 1.12 and 1.31 for Day 13 and Day 20 respectively). The SLA of the plants was also similar and did not show a trend with time (144 ± 5 , 123 ± 5 and 165 ± 12 $cm^2 \cdot g^{-1}$ dry wt for *P. major*, *P. lanceolata* and *P. media* respectively). However, f_{proj} of *P. media* was significantly ($P < 0.01$) less than that of the other two species (0.22 ± 0.07 , in contrast with *P. major* and 0.48 ± 0.08 for *P. lanceolata*).

Time course of PAH concentrations and PAH-profiles

PAHs were already present in the wax of the leaves from the greenhouse (Day 0). The PAH concentrations in the plants (Figure 1) increased by a factor of 2-7 after they were transferred to the open greenhouse (with the exception of BghiP). This increase was likely caused by a higher supply of PAHs or smaller boundary layers around the leaves due to more wind in the open greenhouse. Concentrations on Day 6 and 13 were similar, but on Day 20 concentrations of most compounds decreased considerably (Figure1). The reason for this decrease remains unclear. The only change from Day 13 and Day 20 in the measured plant parameters was the twofold increase of the wax content, but this cannot explain a decrease in PAH-concentration expressed per g of leaf dry weight. Nevertheless, for the purposes of this study, namely the interpretation of differences between species, it is not of much relevance.

The profiles of the PAHs of *P. major* and *P. lanceolata* were very similar, while the profiles of *P. media* showed a slightly different pattern. This is mainly due to a consistently lower contribution of PHE (40-55% instead of 50-60%) and a higher contribution of higher MW PAHs to the total amount of PAHs taken up (data not shown). For example, BkF accounts for $\pm 4\%$ in *P. media* and for $\pm 1\%$ in the other two plants. FLUO also made up a major proportion of the total concentration (25-40%). The rest of the PAHs were only present in small amounts (0.5-10%).

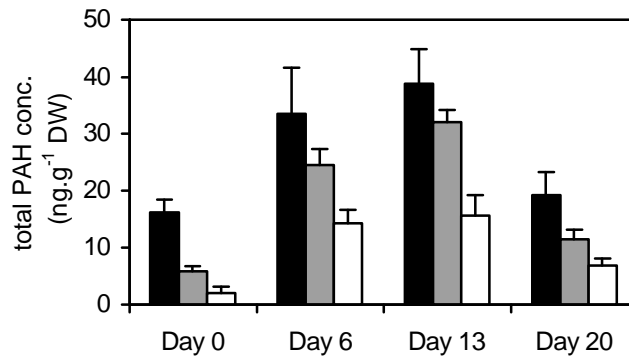


Figure 1. Total PAH concentrations (ng PAH.g^{-1} dry wt. of leaf) of eight PAHs in leaf wax of *P. major* (black bars), *P. lanceolata* (hatched bars) and *P. media* (white bars) on the different sampling days. Error bars represent standard deviations of the four replicates.

Species differences

To compare the concentrations of PAHs in the three *Plantago* species, the results of Day 13 are plotted in Figure 2. On the other sampling days, the species differences were similar, and therefore the results of these days are not shown. Concentrations are expressed per g dry weight, since this parameter was measured for all samples, in contrast to the leaf area and the

wax content, which were only determined for a number of control plants. However, the differences in the wax content and in the SLA between the species are relatively small and plots based on these units result in similar figures, showing the same significant differences as Figure 2.

The concentrations of PAHs with MW 178 and 202, which are largely present in the atmosphere as gases (Jones *et al.* 1992, Kaupp 1996), were 2-5 times higher in *P. major* and *P. lanceolata* than in *P. media* (Figure 2).

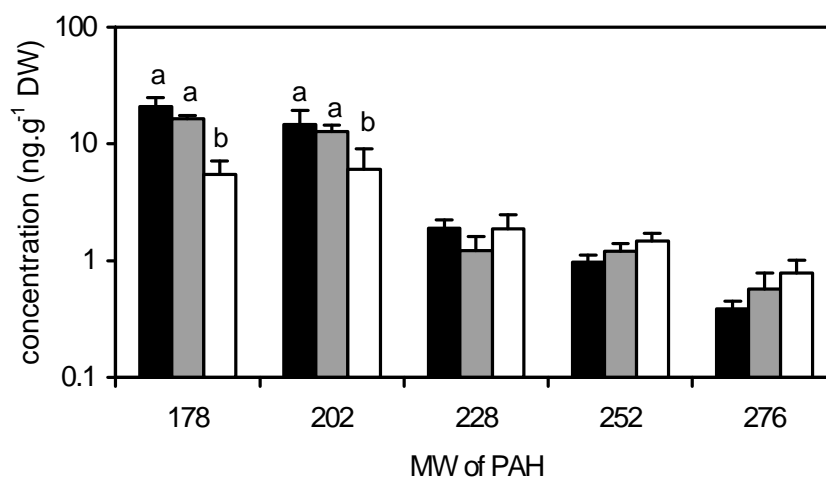


Figure 2. PAH concentrations (ng PAH·g⁻¹ dry wt of leaf) in leaf wax of the three *Plantago* species on Day 13. PAHs are grouped according to their molecular weight (MW). Differences between the average concentrations of the three species were tested with ANOVA (Prism 2.01, P<0.05) and when found significant, indicated in the figure with letters a and b.

For the higher MW PAHs, which are almost completely bound to particles (Jones *et al.* 1992, Nakajima *et al.* 1995, Kaupp 1996), significant differences between the species could not be proved statistically (Figure 2). The absence of statistical significance could be caused by the limited sample size that could be cleaned up efficiently. Hence, the amount of high MW PAHs that was extracted is relatively low (down to approximately 1 ng) resulting in low precision. Nevertheless, when looking at the trends, concentrations of PAHs with MW 252 and 276 in *P. media* are highest and those in *P. major* lowest. This was also the case for the individual PAHs with MW 252, namely BaP and BkF. The same trend was found on the other sampling days as well.

The differences in PAH concentrations can be explained by the differences in plant characteristics of the three *Plantago* species. These can be divided in differences in surface roughness, leaf hairs and leaf overlap.

Surface roughness

Because *P. media* is a low growing plant with its leaves spreading close to the ground, the aerodynamic surface roughness of the canopy will be lower than that of the other two plants. This will result in less aerodynamic turbulence and therefore, to a lower supply of compounds from the bulk air. However, this will only lead to lower uptake if the aerodynamic component of the atmospheric resistance is the rate-limiting step.

For particle-bound PAHs it is expected that the lower surface roughness of *P. media* will have a similar effect on the deposition. Since the pattern of high MW PAHs in Figure 2 points in the opposite direction, the surface roughness cannot explain this finding and is probably over-compensated for by another factor, such as leaf hairs and leaf overlap.

Leaf hairs

Surface irregularities on the leaf, such as veins and hairs can induce turbulence and hence, decrease $\delta_{b,l}$. On the other hand, a dense mat of hairs is likely to increase $\delta_{b,l}$ by up to the thickness of the hair mat (Jones 1983). The effect of hairs on $\delta_{b,l}$ was demonstrated by Woolley (Woolley 1965), who showed that the wind speed 0.5 mm above a soybean leaf increased by 40% after the hairs had been removed. In contrast with the other two plants, *P. media* leaves are densely hairy and therefore the $\delta_{b,l}$ will be increased. This may cause a lower uptake rate for the gaseous PAHs, if the boundary resistance is the main atmospheric resistance.

It is not clear from this experiment whether the turbulent or the laminar component of the resistance determined gas transport to the leaf surface. Therefore, no conclusions can be drawn about the actual cause of the lower uptake of the low MW PAHs in *P. media*. Both the lower surface roughness and the higher density of leaf hairs of *P. media* are possible explanations for this phenomenon.

The trend of *P. media* having the highest concentrations of high MW PAHs (Figure 2), can be explained by its hairy leaves. PAHs are largely bound to particles $< 2 \mu\text{m}$ (Poster *et al.* 1995, Schnelle *et al.* 1995, Allen *et al.* 1996, Kaupp 1996). It is known that in the size range 1-5 μm , the deposition by impaction (inertial motion) is inefficient and the presence of fine hairs may be of major importance in intercepting particles (Chamberlain 1967). For particles smaller than 1 μm , diffusion becomes the dominant means of transport to the leaf surface, and the nature of the surface is not so important as for larger particles. However, once the particles have been deposited on the leaf surface, the hairy leaves act as an efficient particle trap. Because of the thick boundary layer, wind eddies cannot penetrate down to blow off the particles from the leaf surface (Gregory 1961). Another explanation for the increased particle collection efficiency of hairy leaves is that leaf hairs may cushion the impact and therefore reduce the bounce-off of particles (Chamberlain and Little 1981).

Leaf overlap

The lower concentrations of gaseous PAHs in *P. media* may also be caused by the higher overlap of leaves of this plant. Because of the overlap, the leaves may be less accessible for exchange of air, thus preventing the uptake of gaseous SOCs in the covered leaves. This factor is not taken into account when expressing PAH concentrations per g dry wt, per g leaf wax or per m² surface area. Therefore, we normalised the PAH concentrations (Day 13) on the projected surface area, A_{proj} , which could be calculated for each sample from the measured total surface area and f_{proj} , which was determined for the control plants.

PAH concentrations based on A_{proj} are plotted in Figure 3. Concentrations of PAHs with MW 178 were again lowest in *P. media*, while the concentrations of FLUO (MW 202) were similar in the three plants. This suggests that A_{proj} may at least partially explain the observed differences for the gaseous compounds. After normalisation to A_{proj} , the high MW PAHs showed significantly higher concentrations in *P. media* than in the other two plants (Figure 3). This means that per unit “accessible” surface area, the concentrations of particle-bound PAHs in *P. media* are significantly higher than those of the other two plants.

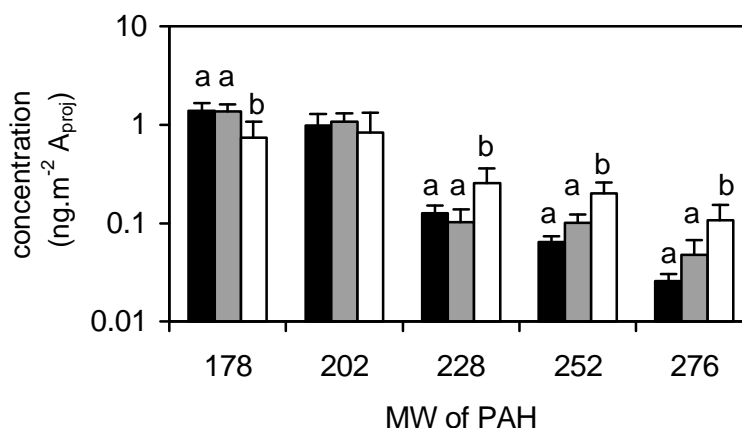


Figure 3. PAH concentrations (ng PAH · m⁻² projected area) in the leaf wax of the three *Plantago* species on Day 13. PAHs are grouped according to their molecular weight (MW). Error bars represent standard deviations of the four replicates (taking into account the error in the determination of A_{proj}). Differences between the average concentrations of the three species were tested with ANOVA (Prism 2.01, $P < 0.05$) and when found significant, indicated in the figure with letters a and b.

The projected surface area (A_{proj}) represents the surface area that is not covered by other leaves. Since in this approximation the vertical dimension is lost, the use of A_{proj} is only acceptable when using plants with highly similar morphology. Although the plants used in the present study belong to the same genus, they differ in height, which complicates the interpretation. The greater height of *P. major* and *P. lanceolata* may increase the interception of

PAHs. However, in spite of the possibly extra deposition caused by their height, high MW PAH concentrations in *P. major* and *P. lanceolata* expressed per projected area (A_{proj}) were lower than those of *P. media*, which emphasizes the effective particle collection of hairy leaves.

Since A_{proj} probably reflects a “minimal accessible leaf area”, only a rough idea of the influence of the plant architecture on the deposition of PAHs can be obtained in this way. Besides, since gases are able to diffuse much faster than particles, due to their higher diffusion coefficients, the use of A_{proj} as a measure of accessibility will likely be of more relevance for particle-bound compounds than for gaseous compounds.

Kinetics

The explanations given in the previous sections consider the atmospheric resistance. The atmospheric resistance can only determine the measured concentrations if an equilibrium between air and plant has not been reached. This is because in an equilibrium situation, the concentrations in the plant are independent of the aerodynamic turbulence and boundary layer thickness. Because of the lack of data for the kinetic behaviour of PAHs in *Plantago*, the time needed to reach equilibrium was estimated from data measured for other plants. Since the elimination rate decreases with increasing K_{oa} (Paterson *et al.* 1991), only studies in which compounds were used with $\log K_{oa}$ values comparable to those of PHE, ANT and FLUO (which have a $\log K_{oa}$ of respectively 7.4 -Tolls and McLachlan 1994-, 7.8 -Tolls and McLachlan 1994- and 8.6, calculated from De Maagd *et al.* 1998), were chosen.

From the elimination rate constants reported in these studies, the time needed to achieve 95% of the equilibrium concentration ($t_{0.95}$) was calculated with the first-order one-compartment kinetic bioconcentration model. In studies in which plants were exposed in chambers containing a ventilator, i.e. under turbulent conditions (thin boundary layers), $t_{0.95}$ values ranged from 3 days for anthracene in grass (Tolls and McLachlan 1994) to 107 days for PCB#18 in spruce needles (Reischl *et al.* 1989). Under non-turbulent lab conditions a value of 31 days for mirex (Bacci *et al.* 1990b) was found, whereas in a field study concentrations of chlorinated organic compounds (PCBs, DDT *et al.*) in spruce needles were still increasing after 5 years (Jensen *et al.* 1992). These results indicate that it is not likely that equilibrium will be reached within three weeks of exposure in an “open” greenhouse.

Conclusion

The densely hairy, partially overlapping leaves of the low growing *P. media* contain less low MW PAHs and more high MW PAHs than the almost glabrous, *P. major* and sparsely hairy *P. lanceolata* leaves, which are relatively free-standing in the air. The measured trends are consistent and indicate that leaf hairs and plant architecture can affect deposition rates of SOCs in different ways for gases and particles.

These results may be important for predictive models, in particular with regard to the concentration of SOCs in food crops. Food crops are only exposed to contaminated air for a relatively short time period and an equilibrium between air and plant will probably not be achieved. Neglecting the effects of plant architecture may result in overestimation of concentrations of gaseous SOCs in the plant. On the other hand, deposition of particle-bound SOCs may be underestimated for leaves with a dense layer of hairs. However, differences in foliar concentrations of SOCs between plant species in these experiments were not large; usually less than a factor of 5.