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1 **Responses of herbaceous plants to urban air pollution: Effects on**
2 **growth, phenology and leaf surface characteristics**

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15 *“Solardome fumigation experiments clearly demonstrated the potential for realistic*
16 *levels of exhaust pollution to have direct adverse effects on urban vegetation”*

17

18 **Abstract**

19 Vehicle exhaust emissions are a dominant feature of urban environments and are widely
20 believed to have detrimental effects on plants. The effects of diesel exhaust emissions on
21 12 herbaceous species were studied with respect to growth, flower development, leaf
22 senescence and leaf surface wax characteristics. A diesel generator was used to produce
23 concentrations of nitrogen oxides (NO_x) representative of urban conditions, in solardome
24 chambers. Annual mean NO_x concentrations ranged from 77 nl l⁻¹ to 98 nl l⁻¹, with

1 NO:NO₂ ratios of 1.4-2.2, providing a good experimental simulation of polluted
2 roadside environments. Pollutant exposure resulted in species-specific changes in
3 growth and phenology, with a consistent trend for accelerated senescence and delayed
4 flowering. Leaf surface characteristics were also affected; contact angle measurements
5 indicated changes in surface wax structure following pollutant exposure. The study
6 demonstrated clearly the potential for realistic levels of vehicle exhaust pollution to have
7 direct adverse effects on urban vegetation.

8

9 **Key words:** vehicle emissions, nitric oxide, nitrogen dioxide, senescence, leaf contact
10 angle.

1. Introduction

The characteristics of urban air pollution have changed significantly over recent decades. Concentrations of traditionally important pollutants such as sulphur dioxide (SO₂) and black smoke have declined substantially, whilst road traffic emissions have emerged as the major cause of poor air quality (Brophy et al, 2007). The urban environment contains a complex mixture of air pollutants, the exact composition of which varies both over time and between individual towns and cities due to changes in patterns and sources of emissions. Typically, however, urban air quality is dominated by emissions from road traffic.

Diesel and petrol fuelled vehicles are responsible for the generation of a wide range of pollutants, with concentrations and relative proportions of pollutants depending on vehicle technology and operating conditions (Colvile et al., 2001). In terms of their effects on plants and their relatively high concentrations in exhaust emissions, nitric oxide (NO) and nitrogen dioxide (NO₂) are the most important phytotoxic pollutants associated with road transport. However, trace amounts of other nitrogen-containing compounds such as nitrous acid (HONO), nitrous oxide (N₂O) and ammonia (NH₃) may also be present in vehicle emissions. During combustion, other pollutants, including sulphur dioxide (SO₂) and volatile organic compounds (VOCs), are emitted, together with carbonaceous particles from incompletely burnt fuel droplets (Colvile et al., 2001).

In the urban environment NO is oxidised to NO₂ through reaction with ozone (O₃). In the open atmosphere this reaction occurs rapidly. However, close to large sources of NO, such as busy road junctions and street canyons, the supply of O₃ may be rapidly exhausted and a large proportion of the NO left unoxidised, leading to characteristically high levels of this pollutant in these areas (AQEG, 2004).

Previous research has shown that at high concentrations, many of the pollutants present in exhaust gases can be damaging to plants (Ackerly & Bazzaz, 1995; Grantz et al., 2003; Wellburn, 1990). Much of this research has, however, looked solely at the individual components of exhaust emissions and there is very little information on the impacts of the particular mix of pollutants characteristic of urban areas.

The ecological effects of roads and traffic have been recently reviewed by Spellerberg (1998) and Bignal et al. (2004). Measurable plant responses to traffic emissions have been reported in a number of studies using either transects away from roads or exposures to different traffic densities. For example, Angold (1997) and Bernhardt-Romermann et al (2006) reported changes in plant community composition with increasing proximity to roadsides in England and Germany, respectively. Gratani et al. (2000) reported positive relationships between traffic density and photosynthetic activity, stomatal conductance, total chlorophyll content and leaf senescence of *Quercus ilex* L. in Rome. In a similar study in Finland, Viskari et al. (2000b) also related changes in epistomatal wax structure to traffic density. These studies highlight the diversity of plant responses associated with environments adjacent to busy roadsides.

Although fumigation experiments with exhaust gases under controlled conditions have tended to confirm the findings from field studies (Bahl & Kahl, 1995; Kammerbauer & Dick, 2000; Viskari et al., 2000a,c; Weil & Schaub, 1999), a major criticism of all these experiments is their short duration (generally < 20 days). It is not possible to determine the implications of long-term exposure to realistic concentrations of vehicle emissions from such short-term studies.

The study reported in this paper makes use of a unique pollution exposure system that was designed to expose plants to stable and realistic concentrations of urban pollutants under near ambient conditions of light, temperature and humidity. The long-term stability of the system allowed experiments to take place over entire growing seasons and to be repeated from year to year. During the three year period from 2000-2003, a range of plant species of different morphologies and

functional types, including trees, shrubs, herbaceous vegetation and bryophytes, were exposed to vehicle exhaust emissions. Ashenden et al. (2003) summarise some of the key findings from the overall research programme, highlighting effects of pollutant exposure on the growth, physiology and phenology and of a number of plant species. The current paper provides a detailed description of pollutant conditions and their effects on 12 common herbaceous plant species. The overall aim of this study was to evaluate the effects of exposure to a realistic mixture of urban air pollutants on the growth, phenology and leaf surface characteristics of native herbaceous vegetation.

2. Materials and Methods

2.1. Fumigation system

The solardome fumigation facility was situated at the Henfaes Farm site of the University of Wales, Bangor (National Grid Ref. SH 654732) in North Wales. The facility was developed from an earlier fumigation system described in detail in Rafarel & Ashenden (1991). The system consisted of four Solardome® 1 glasshouses (Solardome Industries Ltd, Southampton, Hampshire), constructed on an east-west line. The domes had a diameter of 3.1m and a floor area of 7.55m². Each dome was 2.11m high and had an internal volume of 12.0m³.

Air was circulated through the domes using fan filter units (Roof Unit Group, West Midlands, UK) fitted with activated charcoal filters. Control solardomes received charcoal-filtered air. The complex mixture of pollutants characteristic of roadside urban environments was simulated in replicate polluted domes, using the exhaust emissions from a 3.5 Kw diesel generator (Generac® ED 4000, Lombardini, Italy). An earlier feasibility study (Moonen et al., 1999) had demonstrated that emissions from a diesel generator, rather than a car engine or petrol generator, were closest to a typical urban pollutant mixture. A proportion of the output from the generator was mixed thoroughly with the filtered air stream. Control of the amount of exhaust gases supplied to treatment domes was

achieved through the use of an adjustable back-pressure valve (manually in 2000 and automatically, using a guillotine valve, in 2001-2) to achieve target concentrations.

The target NO_x concentration in solardome chambers was set at 100 nl l⁻¹, which is typical of busy urban roadside monitoring sites such as London's Cromwell Road (Bower et al., 2007). The relative concentrations of NO and NO₂ were also set to replicate the characteristically high NO:NO₂ ratio of urban roadside sites with a target ratio of 1.5 - 2.0 (AQEG, 2004). Control solardomes received charcoal-filtered air

2.2. Air quality measurements

NO_x concentrations were determined using a Model 200A chemiluminescent NO_x analyser (Advanced Pollution Instrumentation Inc., San Diego, USA). Particulate concentrations were measured using a 3-stage particle impactor (Dekati Ltd, Finland) which classified particles into three size classes: > 10 µm, 10 - 2.5 µm and 2.5 - 0.3 µm. The impaction substrates were coated with 4.0 µl of a saturated solution of Apiezon-L vacuum grease (Apiezon Products, UK) dissolved in toluene at 25°C. Particulate concentrations were determined gravimetrically

Benzene and toluene were used as indicator species for the range of VOCs produced by combustion processes; these were measured using standard thermal desorption tubes (102 mm x 6 mm stainless steel, Supelco, USA). Tubes were packed with Chromosorb-106 and a known volume of air was drawn through them, using a low-volume pump (typically 100 ml min⁻¹) for between 1 to 7 days. Exposed tubes were thermally desorbed using a Perkin-Elmer ATD-400 Cold Trap (Perkin Elmer, USA) and subsequently analysed by gas chromatography and mass spectroscopy (Hewlett Packard (now Agilent), Canada. Models 5890A series 2 and 5972 respectively). Specific ion detection (m/z 78 and 91 for benzene and toluene respectively) followed the method outlined in

Binnie et al. (2002). Calibration was carried out using Supelco JMHW VOC mix in methanol, further diluted in methanol. Reported values were blank corrected.

Measurements of HONO were carried out simultaneously in all four domes on 6 separate occasions, from 10 October 2001 to 27 June 2002. Air was sampled continuously over 2 weeks using a low volume pump (flow rate 350 ml min^{-1}) on to two tubular denuders in series, each coated with sodium carbonate. HONO was trapped on the K_2CO_3 coating of the denuder as a nitrite ion. Following extraction into water, the nitrite ion was measured spectrophotometrically using a modified Griess-Saltzman procedure (Hargreaves, 1989). All samples were blank corrected.

Levels of SO_2 in both the pollution and control domes were checked during the summer of 2000 and again in June 2001, using a UV fluorescence SO_2 analyser (Model 4108, Dasibi, Environmental Corp., California, USA). Ad hoc measurements of ozone were also carried out at different times of day and on days with a wide range of weather conditions, using a UV Photometric O_3 analyser (Dasibi, Environmental Corp., California, USA).

2.3. Plant exposure experiments

Plant exposure studies took place between 2000 and 2002, with the majority of the work focused in the summers of 2000 and 2001. Species were selected to be both characteristic of the urban environment and representative of a range of plant functional types. A summary of the species used and their dates and duration of exposure is given in Table 1.

2.4. Plant propagation and cultivation

Seeds of characteristic native herbaceous plant species were obtained from a number of research and commercial seed suppliers (NERC Unit of Comparative Plant Ecology, Sheffield, UK; Kew Seed Bank, London, UK; and Emorsgate Seeds, Norfolk, UK). Seeds were germinated in John Innes seed

compost and then single plants of each species were transplanted into 4 litre pots filled with John Innes No. 2 compost obtained from the local garden centre. Five replicate pots per species, each containing a single plant, were placed in each solardome. Plants were kept well watered using a combination of automatic watering via an aerosol misting system and hand watering of containers. Plants were not fertilized during the experiments and any differences in soil conditions between pots were assumed to be minimal. Potential between-treatment differences in soil conditions associated with exhaust emissions were not measured. However, the existence of any such differences would be considered part of the plant-soil system response to fumigation, with associated impacts on plant performance being recorded as part of the measurement protocol described below.

2.5. Plant measurements

During experiments, non-destructive measurements of plant growth, leaf senescence and flowering were made by carrying out regular counts of the total numbers of live, dead and chlorotic leaves and, where applicable, flower buds, heads and seed heads, for each plant. However, not all parameters were recorded in every year. The static contact angle of distilled water drops was measured on individual detached leaves in 2000 and 2001, to determine the leaf surface wettability and provide an indication of the condition of epicuticular waxes (Cape, 1983). A 4 μ l droplet of distilled water was placed on the leaf surface, using a syringe, and a picture taken of the droplet within 30 seconds, using a camera fitted with a macro lens. The resulting picture was scanned into a computer, magnified and the angle between the water droplet and the leaf was measured using Adobe Photoshop 5.0 software.

2.6. Statistical analysis

Statistical analysis was carried out using the R statistical package, Version 1.6.0 (CRAN, 2003). All the data shown are based on the solardomes as the treatment replicate, with two replicates for both the control and pollution treatments. Individual measurements from plants were treated as

pseudoreplicates since they were replicates of samples and not independent treatment replicates. For flowering, senescence and contact angle measurements, a nested ANOVA was carried out. The design was, therefore, 2 treatments x 2 replicate solardomes x 4-6 pseudo-replicate plants per species.

The time-series data from the regular leaf count and plant height measurements taken in 2001 were analysed using derived variables analysis (Crawley, 2002). This method removes pseudoreplication over time by reducing the repeated measures into a set of summary statistics (slopes, intersects and means). Regressions were carried out between day of fumigation and leaf count for each dome and slopes and intercepts calculated. Where necessary, response variables were transformed to obtain linear regressions. These derived variables were then investigated using two-way ANOVA. Following Sokal & Rohlf (1998), arcsine transformations were applied to all proportion data before analysis. Count data were transformed using square root or log (1+value), to maintain zeros in the dataset whilst stabilising the variance (Crawley, 2002). Back-transformed means are presented in the results.

3. Results

3.1. Pollutant concentrations

From Figure 1 it can be seen that the fitting of the computer-operated control valve in early 2001 reduced fluctuations in average weekly NO_x concentrations.

Table 2 shows the approximate concentrations of different pollutants in the pollution treatment during the main periods of the plant exposure experiments. In 2000, NO_x concentrations were approximately 20% lower than the target 100 nl l⁻¹. Target concentrations were, however, achieved in 2001 and 2002. During 2000 and 2001 the NO:NO₂ ratio was close to the lower end of the target range of 1.5 – 2.0; in 2002, the ratio rose to 2.2.

Concentrations of particulate matter appear to have been fairly constant between 2001 and 2002. The system change that took place in March 2002 caused changes in the level of a number of pollutants, leading to an increase in VOC and HONO from 2001 to 2002. In addition, although there was no overall change in NO_x levels, there was an increase in the NO:NO₂ ratio. This would have led to a fall in average NO₂ concentrations from 40 nl l⁻¹ in 2001 to 30 nl l⁻¹ in 2002. Changes to the system also led to a doubling of HONO concentrations in the polluted solardomes between the 2001 and 2002 main experimental periods. Measurements of HONO in the control treatment show good agreement between years.

In both 2001 and 2002, there were very few coarse particles in the polluted domes, with more than 99% of the mass of particles measured having a diameter <2.5 µm. Alterations to the system in early 2002 caused a large increase in the levels of toluene in the treatment domes and a smaller increase in benzene concentrations. This led to a change in the benzene : toluene ratio, from 5.1 to 1.4. There was also a moderate increase in the levels of VOCs measured in the control domes. SO₂ concentrations in the pollution and control domes were below 2 nl l⁻¹, the limit of detection of the instrument. Ad hoc measurements of O₃ showed levels in all four solardomes to be very similar, with concentrations ranging from 5 to 15 nl l⁻¹, averaging approximately 10 nl l⁻¹.

3.2. Plant height measurements (2001)

Figure 2 shows the average plant height in the pollution and control domes for *C. album* and *S. oleraceus*. Five weeks in to the experiment in 2001, plants of both species were smaller in the pollution treatment than in the control (*C. album* $F_{1,2} = 169.9$, $p = 0.006$; *S. oleraceus* $F_{1,2} = 23.38$, $p = 0.040$). Over the course of the experiment this significant difference was lost.

3.3. Leaf count measurements (2001)

During 2001, regular measurements of leaf production were taken throughout the growing season. Figure 3 illustrates the effects of pollutant exposure on the pattern of leaf development for *C. nigra*, *R. acetosa*, *S. squalidus*, *P. annua*, *C. album* and *S. oleraceus*.

Results from the derived variable analysis showed no significant treatment effect on leaf count for *S. squalidus*, *P. annua*, *C. album* or *S. oleraceus*. However, there was a significant treatment-related reduction in the slope of the regression between leaf count and length of fumigation ($F_{1,2} = 340$, $p = <0.01$) for *C. nigra*, indicating that pollutant exposure slowed down the production of plant leaves. Although not statistically significant, there was a greater number of leaves of *R. acetosa* in polluted domes, compared to controls ($F_{1,2} = 8.51$, $p = 0.10$). ANOVA carried out on measurements from individual time periods for *C. nigra*, showed that the treatment effect on leaf number was not significant until 11 ($F_{1,2} = 23.35$, $p = 0.04$) and 15 ($F_{1,2} = 479.7$, $p = 0.002$) weeks of fumigation.

3.4. Senescence responses

Plant senescence was assessed by measuring the proportion of dead leaves, as a percentage of the total number of leaves per plant/pot, over time. As both *L. corniculatus* and *C. album* drop dead leaves, this assessment was not possible for these species; the percentage of dead stems was,

however, used as a proxy for *L. corniculatus*. Table 3 shows the percentage of dead leaves in the treatment and control solardomes. For all species, there were more dead leaves on plants exposed to vehicle emissions, compared to controls. For *L. autumnalis*, *R. acetosa*, *P. annua* and *P. pratense*, this difference was statistically significant.

3.5. Flowering responses

A number of species, particularly annuals, flowered during the experiment and it was therefore possible to make an assessment of the effects of pollution treatment on reproductive phenology. Although measurements of first flowering dates were not made during these experiments, recorded patterns of flower development indicated that, for some species, plants exposed to vehicle emissions may have had delayed flowering compared to control plants. This is illustrated by flower counts made for *S. oleraceus* at four points during the summer of 2001 (Table 3).

Whilst the difference was not quite statistically significant, 100% of plants in the control domes were in flower after 5 weeks' fumigation, compared to only 33% in the polluted domes ($F_{1,2} = 16.0$, $p = 0.057$). Measurements of the percentage of different flowering stages also support the hypothesis that pollutant exposure delayed flowering. After 7 weeks' fumigation, a greater proportion of the flowers in the pollution treatment were buds ($P < 0.059$), whilst a greater proportion of those in the control were dead and about to set seed. Two weeks later, a significantly greater proportion of flowers on plants in polluted domes were as buds and open flowers compared to control plants ($P = 0.04$), and a significantly smaller proportion had set seed ($P = 0.009$). At the end of the experiment, after 12 weeks' fumigation, the differences had narrowed and were no longer significant.

There is also evidence of delayed flowering in other species. In 2001, five of the 24 *C. nigra* plants in the experiment had developed flower buds by the September measurement period. Of these, only one was in the pollution treatment. In 2002, after 9 weeks fumigation, *P. annua* plants in the pollution domes had significantly less flower buds than control plants ($F_{1,2} = 65.03$, $p = 0.015$).

3.6. Leaf surface wax responses

Contact angle measurements were only carried out in 2000 and 2001 (Figure 4). For species with a contact angle of less than 90°, there was a general trend for either no treatment effect or for an increase in contact angle in response to the pollution treatment. This difference was only significant for *R. acetosa* in 2000 ($F_{1,2} = 498.3$, $p = 0.002$); in 2001 this difference was not significant. The trend for species with a contact angle greater than 90° is less clear. Both increases and decreases were found in response to pollutant exposure. *Lotus corniculatus* and *S. oleraceus* showed a decrease in contact angle relative to the control, although this was only statistically significant for *S. oleraceus* ($F_{1,2} = 51.64$, $p = 0.019$).

4. Discussion

4.1. Plant growth response to exhaust emissions

The low level of solardome replication in this study, and associated low statistical power, is one of the reasons why, despite the often large percentage differences between treatments, relatively few statistically significant results were found. Consideration is, therefore, given to non-significant results in order not to overlook potentially important trends, particularly where the percentage difference between treatments is high or the results support a general trend. Furthermore, whilst pollutant uptake may have been altered during periods of plant watering, this was not measured and can be considered to reflect the variability in uptake associated with different atmospheric humidities and rainfall intensities in the natural environment.

Growth measurements suggest a small, species-specific stimulation or inhibition of growth in response to pollutant exposure. For some species, notably *C. nigra*, *R. acetosa* and *P. annua*, effects

of pollutant exposure did not become apparent until approximately nine weeks after fumigation, and appeared to be cumulative over time.

Interestingly, height data suggest that young plants are more sensitive to exhaust emissions than older ones, with the strong negative growth effects shown by the annual species *C. album* and *S. oleraceus* when they were seedlings, being lost as the plants matured. These results suggest that, for some species, the biggest effects of the pollution treatment are associated with the early stages of the life cycle. This theory is supported by work by Whitmore and Mansfield (1983) who found that, for *P. pratense* and *Dactylis glomerata*, the effects of overwinter fumigation with $66 \text{ nl l}^{-1} \text{ NO}_2$ were greater for plants fumigated from seedling emergence than for plants that began fumigation as established seedlings. The fact that the strong initial growth suppression of *C. album* and *S. oleraceus* seen in this study disappeared over time implies that, for much of the experiment, plants in the polluted domes must have had a higher relative growth rate than those in the controls.

4.2. *Effects of pollutant exposure on plant phenology*

Premature leaf senescence was one of the most consistent responses across species. Several other studies have also reported increased leaf senescence in response to air pollution. For example, Saxe (1994) demonstrated premature senescence for several species of potted plants following exposure to high levels of NO_x . Lane & Bell (1984) also reported a significant increase in the dead leaf mass of *L. perenne* and *Phleum pratense* exposed to low levels of either NO_2 or NO , while Viskari et al. (2000a) reported signs of accelerated cell senescence of *Picea abies* exposed to exhaust gases with $50\text{-}200 \text{ nl l}^{-1} \text{ NO}_x$.

However, it is important to note that other components of vehicle emissions may also be responsible for the observed increase in senescence in the current study. The VOC ethylene, which is also a plant hormone, is known to increase the rate of senescence (Collins & Bell, 2002). It might be expected

that increased senescence in plants exposed to exhaust gases would lead eventually to lower growth due to the reduced activity period of photosynthetically active tissue. However, this was not the case for many of the species in these experiments, suggesting the possibility that some compensatory mechanism, for example changes in photosynthetic rate, leaf surface area or root allocation, may have occurred. Gratani et al. (2000) found that although the foliage of *Quercus ilex* trees had a reduced life span following exposure to high traffic levels in Rome, this was compensated for by higher stomatal conductance, chlorophyll content and photosynthetic activity.

In this experiment, there was evidence that exposure to exhaust gases delayed flowering in a number of species. This finding supports the results of Saxe (1994) and Law & Mansfield (1982), following exposure to high concentrations of NO_x. At more realistic NO_x concentrations, Whitmore & Mansfield (1983) reported delayed development of flowering stems of *P. pratense*, although in that study there was no overall effect on the final number of flowering heads produced. Elevated ethylene concentrations have been suggested as the cause for accelerated flower development following exposure of *Petunia hybrida* at different distances from a motorway (Pleijel et al., 1994). A similar acceleration of seed production in *Lotus corniculatus* has also been shown following controlled exposure to VOCs in open top chambers (Cape et al., 2003). However, the delay, rather than acceleration, in flowering in polluted solardomes is in line with results from earlier NO_x fumigation studies and thus suggests an involvement of NO_x, rather than VOCs in the current study. A change in the timing of flowering has the potential to alter synchronicity with insect pollinators and thus the reproductive success of affected plant species, particularly annuals.

4.3. Leaf surface waxes

A number of authors have reported changes in leaf surface characteristics in response to air pollutant exposure, although the majority of this work has concentrated on the needles of coniferous trees (Turunen & Huttunen, 1990). Changes in surface waxes are a very general sign of alterations in

surface properties, including chemistry or surface roughness (Holloway, 1970), and can result from a range of natural and anthropogenic factors. Indeed, chamber environments themselves have been shown to affect leaf surface properties (Cape & Percy, 1993). Oxides of nitrogen can react directly with the cuticle, but generally only after very long exposure to a large concentrations (Lendzian & Kerstiens, 1991). Following foliar absorption, NO_x may also disrupt metabolic processes, and this could potentially affect leaf wax composition and structure indirectly, although little is known about this possible mechanism (Cape, 1994). Chui et al. (1992) showed that the addition of fertiliser to Douglas fir trees (*Pseudotsuga menziesii*) increased cuticular waxes; at the whole plant level, NO_x can act as a fertiliser, thus having the potential to affect leaf surface structure indirectly.

VOCs are very lipid soluble and may accumulate in leaf waxes, leading to changes in their physical properties and structure (Riederer, 1994). Indirect effects on plant metabolism are rare. However, a number of small organic plant molecules such as ethane and ethylene can be stimulated by plant stress, including pollution-induced stress. This may provide a potential route for the indirect effect of pollutants that do not themselves react directly with surface waxes (Cape, 1994).

The probability of particulate matter causing direct effects on leaf surface waxes depends mainly on the chemical composition of the particles. There are a limited number of studies that show impacts, usually in the form of increased water loss, of inert dusts (Eveling, 1969; Farmer, 1993). However, where studies have reported surface wax degradation following exhaust gas exposure, this has been attributed to organic hydrocarbons and NO_x (Viskari et al., 2000a) or, more specifically, the lipophilic aromatic hydrocarbons associated with vehicle emissions (Sauter & Pambor, 1989).

The cuticle has a number of important functions including the prevention of excessive water loss, regulation of solute uptake, protection of sensitive underlying tissues and acting as a barrier to pathogens (Percy et al., 1994). If the observed changes in contact angle in the current study are symptomatic of the degradation of surface waxes, this may reflect an increase in non-stomatal water loss. This could have important implications for a plant's water relations, particularly under drought

conditions, a situation which is predicted to occur with increasing frequency under UK scenarios for future climate change (IPCC, 2001).

5. Conclusion

This study has demonstrated a variety of responses of a large number of herbaceous plant species to realistic levels of a cocktail of typical urban pollutants. The results indicate that plants growing in polluted urban environments are likely to experience significant changes in the timing of key activities such as flowering and leaf senescence, as well as potentially detrimental changes in leaf surface characteristics and growth. Species differed in the magnitude of response to pollutant exposure, although differences were not consistent within taxonomic or functional groups. Urban plant communities are already strongly affected by anthropogenic influences such as disturbance and the urban heat island effect. Impacts on plant physiology, biochemistry, phenology and growth demonstrated in this and several related studies imply that traffic-derived pollution is responsible for a substantial additional stress in urban environments, with important implications for plant performance and the health and sustainability of urban ecosystems.

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References

- Ackerly, D.D., Bazzaz, F.A., 1995. Plant-growth and reproduction along CO₂ gradients - Nonlinear responses and implications for community change. *Global Change Biology* 1, 199-207.
- Angold, P.G., 1997. The impact of a road upon adjacent heathland vegetation: Effects on plant species composition. *Journal of Applied Ecology* 34, 409-417.
- AQEG, 2004. Nitrogen dioxide in the United Kingdom. Report prepared by the Air Quality Expert Group on behalf of the Department of Environment, Food and Rural Affairs, Scottish Assembly, Welsh Assembly Government and Department of the Environment in Northern Ireland.
- Ashenden, T.W., Ashmore, M.R., Bell, J.N.B., Bignal, K.L., Binnie, J., Cape, J.N., Caporn, S.J.N., Carroll, J., Davison, A., Hadfield, P., Honour, S., Lawton, K., Moore, S., Power, S., Shields, C., 2003. Impacts of vehicle emissions on vegetation. Ninth International Conference on Urban Transport and the Environment in the 21st Century, Conference Proceedings. Crete, Greece, March 2003.
- Ashmore, M.R., Bell, J.N.B., Mimmack, A., 1988. Crop growth along a gradient of ambient air pollution. *Environmental Pollution* 53, 99-121.
- Bahl, A., Kahl, G., 1995. Air pollutant stress changes the steady-state transcript levels of 3 photosynthesis genes. *Environmental Pollution* 88, 57-65.
- Bernhardt-Romermann, M., Kirchner, M., Kudernatch, T., Jakobi, G., Fischer, A., 2006. Changed vegetation composition in coniferous forest near motorways in southern Germany: The effects of traffic-born pollution. *Environmental Pollution* 143, 572-581.
- Bignal, K., Ashmore, M., Power, S., 2004. The ecological effects of diffuse pollution from road traffic. *English Nature Research Report* 580.
- Binnie, J., Cape, J.N., Mackie, N., Leith, I.D., 2002. Exchange of organic solvents between the atmosphere and grass - the use of open top chambers. *Science of the Total Environment* 285, 53-67
- Brophy, N., Dore, C., Hann, M.R., Jackson, J., King, K., Murrells, T.P., Passant, N., Thistlewaite, G., Wagner, A., 2007. Air Quality Pollutant Inventories for England, Scotland, Wales and

- Northern Ireland: 1990 – 2005. Report AEAT/ENV/R/2480. AEA Energy & Environment, Harwell.
- CAFE, 2004. Second Position Paper on Particulate Matter. CAFE Working Group on Particulate Matter, December 2004. Available at: http://ec.europa.eu/environment/air/cafe/working_groups/wg_particulate_matter.htm
- Cape, J.N., 1983. Contact angles of water droplets on needles of Scots pine (*Pinus sylvestris*) growing in polluted atmospheres. *New Phytologist* 93, 293-299.
- Cape, J.N., 1994. Evaluation of pollutant critical levels from leaf surface characteristics, in: Percy, K.E., Cape, J.N., Jagels, R., Simpson, C.J. (Eds.), *Air pollutants and the leaf cuticle*. Springer-Verlag, Berlin, pp. 123-138.
- Cape, J.N., Percy, K.E., 1993. Environmental influences on the development of spruce needle cuticles. *New Phytologist* 125, 787-799.
- Cape, J.N., Leith, I.D., Binnie, J., Content, J., Donkin, M., Skewes, M., Price, D.N., Brown, A.R., Sharpe A.D., 2003. Effects of VOCs on herbaceous plants in an open-top chamber experiment *Environmental Pollution*, 124, 341-353
- Chui, S.T., Anton, L.H., Ewers, F.W., Hammerschmidt, R., Pregitzer, K.S., 1992. Effects of fertilisation on epicuticular wax morphology of needle leaves of Douglas fir, *Pseudotsuga menziesii* (Pinaceae). *American Journal of Botany* 79, 149-154.
- Collins, C.D., Bell, J.N.B., 2002. Effects of volatile organic compounds, in: Bell, J.N.B., Treshow, M. (Eds.), *Air pollution and plant life*. John Wiley & Sons, Ltd., Chichester, pp. 173-186.
- Colvile, R.N., Hutchinson, E.J., Mindell, J.S., Warren, R.F., 2001. The transport sector as a source of air pollution. *Atmospheric Environment* 35, 1537-1565.
- Crawley, M.J., 2002. *Statistical Computing: An Introduction to Data Analysis using S-Plus*. John Wiley & Sons, Ltd., Chichester.
- Bower, J., Loader, A., Broughton, G., Stedman, J., Kent, A., Cook, A., Targa, J., Glynn, A., Willis, P., 2007. *Air Pollution in the UK: 2006*. Report AEAT/ENV/R/2544. AEA Energy & Environment, Harwell.
- Eveling, D.W., 1969. Effects of spraying plants with suspensions of inert dusts. *Annals of Applied Biology* 64, 139-151.
- Farmer, A.M., 1993. The effects of dust on vegetation - A review. *Environmental Pollution* 79, 6-75.

- Grantz, D.A., Garner, J.H.B., Johnson, D.W., 2003. Ecological effects of particulate matter. *Environment International* 29, 213-239.
- Gratani, L., Crescente, M.F., Petruzzi, C., 2000. Relationship between leaf life-span and photosynthetic activity of *Quercus ilex* in polluted urban areas (Rome). *Environmental Pollution* 110, 19-28.
- Hadley, P., 2003. Engineer, Centre for Ecology and Hydrology, Bangor.
- Hargreaves, K.J., 1989. The development and application of diffusion tubes for air pollution measurements. PhD Thesis. University of Nottingham, UK.
- Honour, S.L., 2003. The effects of urban air pollution on native herbaceous plant species. PhD thesis, Imperial College, London, UK.
- Holloway, P.J., 1970. Surface factors affecting the wetting of leaves. *Pesticide Science* 1, 156-163.
- Intergovernmental Panel on Climate Change (IPCC), 2001. *Climate Change 2001: Impacts, Adaptations and Vulnerability*. Cambridge University Press, Cambridge.
- Kammerbauer, J., Dick, T., 2000. Monitoring of urban traffic emissions using some physiological indicators in *Ricinus communis* L. plants. *Archives of Environmental Contamination and Toxicology* 39, 161-166.
- Lane, P.I., Bell, J.N.B., 1984. The effects of simulated urban air pollution on grass yield 2: Performance of *Lolium perenne*, *Phleum pratense* and *Dactylis glomerata* fumigated with SO₂, NO₂ and/or NO. *Environmental Pollution* 35, 97-124.
- Law, R.M., Mansfield, T.A., 1982. Oxides of nitrogen and the greenhouse atmosphere, in: Unsworth, M.H., Ormrod, D.P. (Eds.), *Effects of gaseous air pollution in agriculture and horticulture*. Butterworths, London.
- Lenzian, K.J., Kerstiens, G., 1991. Sorption and transport of gases and vapours in plant cuticles. *Review of Environmental Contamination and Toxicology* 121, 65-128.
- Moonen, P.C., Binnie, J., Cape, J.N., Ashenden, T.W., 1999. Impacts of vehicle emissions on vegetation. Institute of Terrestrial Ecology.
- Percy, K.E., McQuattie, C.J., Rebbeck, J.A., 1994. Effects of air pollutants on epicuticular wax chemical composition, in: Percy, K.E., Cape, J.N., Jagels, R., Simpson, C.J. (Eds.), *Air pollutants and the leaf cuticle*. Springer-Verlag, Berlin, pp. 67-80.

- Pleijel, H., Ahlfors, A., Skarby, L., Pihl, G., Sellden, G., Sjodin, A., 1994. Effects of air pollutant emissions from a rural motorway on *Petunia* and *Trifolium*. *Science of the Total Environment* 147, 117-123.
- Rafarel, C.R., Ashenden, T.W., 1991. A facility for the large-scale exposure of plants to gaseous atmospheric pollutants. *New Phytologist* 117, 345-349.
- Riederer, M., 1994. Air pollutants and the cuticle: Implications for plant physiology, in: Percy, K.E., Cape, J.N., Jagels, R., Simpson, C.J. (Eds.), *Air pollutants and the leaf cuticle*. Springer-Verlag, Berlin.
- Sauter, J.J., Pambor, L., 1989. The dramatic corrosive effect of roadside exposure and of aromatic hydrocarbons on the epistomatal wax crystalloids in spruce and fir - and its significance for the 'Waldsterben'. *European Journal of Forest Pathology* 17, 444-448.
- Saxe, H., 1994. Relative sensitivity of greenhouse pot plants to long-term exposures of NO-containing and NO₂-containing air. *Environmental Pollution* 85, 283-290.
- Sokal, .RR., Rohlf, F.J., 1998. *Biometry*. W H Freeman & Company, New York.
- Spellerberg, I.F., 1998. Ecological effects of roads and traffic: a literature review. *Global Ecology and Biogeography* 7, 317-333.
- Spencer, H.J., Scott, N.E., Port, G.R., Davison, A.W., 1988. Effects of roadside conditions on plants and insects 1: Atmospheric conditions. *Journal of Applied Ecology* 25, 699-707.
- Turunen, M., Huttunen, S., 1990. A review of the response of epicuticular wax of conifer needles to air pollution. *Journal of Environmental Quality* 19, 35-45.
- UKAQA, 2006. United Kingdom Air Quality Archive. Available at: <http://www.airquality.co.uk>
- Viskari, E.L., Holopainen, T., Karenlampi, L., 2000a. Responses of spruce seedlings (*Picea abies*) to exhaust gas under laboratory conditions II: Ultrastructural changes and stomatal behaviour. *Environmental Pollution* 107, 99-107.
- Viskari, E.L., Kossi, S., Holopainen, J.K., 2000b. Norway spruce and spruce shoot aphid as indicators of traffic pollution. *Environmental Pollution* 107, 305-314.
- Viskari, E.L., Surakka, J., Pasanen, P., Mirme, A., Kossi, S., Ruuskanen, J., Holopainen, J.K., 2000c. Responses of spruce seedlings (*Picea abies*) to exhaust gas under laboratory conditions I. Plant-insect interactions. *Environmental Pollution* 107, 89-98.

- Weil, M., Schaub, H., 1999. Influence of exhaust gas and ozone on extracellular peroxidase activity of *Helianthus annuus* L. leaves. *Journal of Plant Physiology* 154, 523-528.
- Wellburn, A.R., 1990. Why are atmospheric oxides of nitrogen usually phytotoxic and not alternative fertilisers? *New Phytologist* 115, 395-429.
- Whitmore, M.E., Mansfield, T.A., 1983. Effects of long-term exposures to SO₂ and NO₂ on *Poa pratense* and other grasses. *Environmental Pollution* 31, 217-235.

Table 1. Summary of species exposed in the solardome fumigation facility each year, including start date and length of fumigation.

Species	2000	2001	2002
<i>Plantago major</i>	Early July (18 wks)		
<i>Centaurea nigra</i>	Early July (18 wks)	Early June (61 wks)	
<i>Rumex acetosa</i>	Early July (27 wks)	Early June (53 wks)	
<i>Leontodon autumnalis</i>	Early July (13 wks)		
<i>Lotus corniculatus</i>	Early July (27 wks)		
<i>Trifolium repens</i>	Early July (45 wks)		
<i>Lolium perenne</i>	Early July (45 wks)		
<i>Sonchus oleraceus</i>		Early June (12 wks)	
<i>Chenopodium album</i>		Early June (12 wks)	
<i>Senecio squalidus</i>		Early June (12 wks)	
<i>Poa annua</i>		Early June (12 wks)	Mid June (14 wks)
<i>Phleum pratense</i>			Mid June (21 wks)

Table 2. Summary of average pollutant concentrations during the main plant exposure experiments, 2000-2002

	2004	2000		2001		2002	
	Annual mean conc.	10/07 – 02/10		04/06 – 17/09		10/06 – 16/09	
	Marylebone Rd, London ¹	Polluted	Control	Polluted	Control	Polluted	Control
NO _x (nl l ⁻¹)	161	77 (41)	nm	97 (20)	nm	98 (8)	6 (3)
NO:NO ₂ ratio	2.9	1.6	nm	1.4	nm	2.2	1
PM ₁₀ (µg m ⁻³)	35	nm	nm	39 (9)	nm	35 (8)	5 (3)
PM _{2.5} (µg m ⁻³)	19	nm	nm	37 (9)	nm	34 (8)	3 (1)
Benzene (ng l ⁻¹)	2.8	nm	nm	4.6 (1.7)	0.4 ³	7.3 (1.4)	3.6 (2.4)
Toluene (ng l ⁻¹)	11.0	nm	nm	0.9 (0.3)	0 ³	5.1 (1)	2.7 (1.6)
HONO (µg m ⁻³)	0.67-6.03 ²	nm	nm	5.4 (1.7)	0.1 (0.1)	10.8 (1.5)	0.1 (0.1)

Data shown are means +/-SD, nm not measured; ¹ Source: UKAQA (2006); ² 15 minute mean concentrations, October 2004, Clemitshaw et al., unpublished data; ³ one measurement only.

Table 3. Percentage of dead leaves in treatment and control domes \pm 1SE.

Year	Species	Time after fumigation commenced	% Dead Leaves		F value	P
			Polluted	Control		
2000	<i>L. autumnalis</i>	8 weeks	12.1 \pm 1	4.9 \pm 1	39.1	0.025*
	<i>P. major</i>	11 weeks	21.2 \pm 3	10.5 \pm 3	3.31	0.130
	<i>C. nigra</i>	11 weeks	7.7 \pm 1	5.8 \pm 2	0.65	0.504
	<i>R. acetosa</i>	11 weeks	25.0 \pm 3	18.4 \pm 3	2.44	0.259
	<i>L. corniculatus</i> ^a	27 weeks	30.8 \pm 4	13.5 \pm 1	17.0	0.054 ·
2001	<i>C. nigra</i>	15 weeks	14.3 \pm 2	19.5 \pm 1	7.01	0.117
	<i>R. acetosa</i>	15 weeks	35.7 \pm 1	15.3 \pm 3	41.4	0.023*
	<i>S. squalidus</i>	15 weeks	9.1 \pm 1	6.3 \pm 1	3.58	0.199
2002	<i>P. annua</i> ^b	14 weeks	77.0 \pm 2	47.6 \pm 1	100.0	0.010*
	<i>P. pratense</i> ^b	21 weeks	41.4 \pm 3	18.1 \pm 0	74.2	0.013*

^a results refer to % dead stem biomass ^b results refer to % dead of total leaf biomass;
· p<0.1 * p<0.05

Table 4. Relative proportions and number of flower buds, open and dead flowers and seed heads for *S. oleraceus* at four measurement intervals during 2001.

Date of measurements	No. weeks' fumigation		Percentage flowering part \pm 1SE			
			Buds	Open	Dead	Seed heads
12/07/01	5 weeks	Polluted	100 \pm 0			
		Control	100 \pm 0			
		F value	0.00			
		P	1.00			
27/07/01	7 weeks	Polluted	97.2 \pm 0.9	1.0 \pm 0.3	1.4 \pm 0.18	0.4 \pm 0
		Control	88.9 \pm 1.5	3.1 \pm 2.9	7.8 \pm 1.6	0.2 \pm 0.2
		F value	22.41	0.50	12.56	1.00
		P	0.042*	0.551	0.071*	0.42
09/08/01	9 weeks	Polluted	70.5 \pm 2.2	5.2 \pm 0.5	21.1 \pm 2.7	3.1 \pm 0.0
		Control	55.5 \pm 2.7	1.5 \pm 0.1	29.0 \pm 4.8	14.0 \pm 1.9
		F value	18.41	49.58	2.03	31.35
		P	0.050*	0.020*	0.290	0.030*
27/08/01	12 weeks	Polluted	58.4 \pm 1.8	2.7 \pm 0.6	13.6 \pm 1.6	25.3 \pm 0.8
		Control	53.1 \pm 0.5	2.3 \pm 0.1	11.5 \pm 3.3	33.1 \pm 3.8
		F value	8.17	0.51	0.33	4.07
		P	0.10	0.55	0.62	0.18

* p<0.1 * p<0.05

Fig. 1. Average weekly NO_x concentration in polluted solardomes, July 2000 – September 2002.

Fig. 2. Average plant height in pollution and control treatments for a) *C. album* and b) *S. oleraceus*, after 5, 7 and 9 weeks fumigation in 2001. Error bars are ±1SE

Fig. 3. Average leaf count over time for a) *S. squalidus*, b) *C. nigra*, c) *R. acetosa*, d) *S. oleraceus*, e) *C. album* and f) *P. annua*. Error bars are ±1SE.

Fig. 4. Average contact angle measurement 2000 and 2001. Error bars ±1SE







