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# Effects of wind and abrasion on cuticular integrity in *Fagus sylvatica* L. and consequences for transfer of pollutants through leaf surfaces

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## ABSTRACT

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The transfer of pollutants from the atmosphere to the internal tissues of a leaf may involve several routes and physical processes. Wind and abrasive damage, which are components of weathering, cause changes in leaf surface characteristics and cuticular integrity which influence gaseous diffusion and the uptake of solutes.

Wind-induced components of weathering of individual leaves of *Fagus sylvatica* were simulated by the use of a miniature wind tunnel and surface abrasion. The consequence of exposure to wind was widespread disruption of trichomes, and evidence of smoothing of cuticular wax was obtained using low-temperature scanning electron microscopy (SEM). An abrasive treatment also damaged trichomes, created cuticular lesions visible by SEM and significantly increased total surface conductance, to water vapour, on both adaxial (astomatous) and abaxial (stomatous) surfaces. The structural integrity of the cuticle and the spatial distribution of cuticular lesions was investigated by using ruthenium red as a convenient dye tracer.

Leaves were found to be most susceptible to abrasive damage on their abaxial surfaces, where the spatial distribution of lesions was significantly clustered. The occurrence of these lesions may be related to a natural structural and conductive heterogeneity across the leaf surface. Sites most vulnerable to damage were trichomes and protruding veins.

Exposure of *F. sylvatica* leaves to natural weathering processes in an exposed upland site increased uptake of (<sup>35</sup>S)sulphate from droplets placed on the adaxial cuticle by 30-fold on average compared with leaves grown in a sheltered location at the same altitude. The spatial heterogeneity of sulphate uptake measured at discrete points over a single leaf was also greater in leaves from the exposed site.

Consequently, weathering may increase the heterogeneity of gas exchange and the uptake of solutes, by increasing leakiness at localised sites across the leaf surface.

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## INTRODUCTION

The vegetation of the British uplands experiences considerable stress from pollutants in cloud water, rain and in gases, as well as climatic stresses imposed by low temperature and high wind speeds (Grace and Unsworth, 1988). The nature of pollution stress is evident from analysis of rainwater and cloud water at hill sites, and includes high concentrations of acidity, heavy metals, sulphate and nitrate ions, as described by Unsworth and Crossley (1987), Fowler et al. (1988) and Lee et al. (1988). The manner in which this material enters the plant and the interactions between pollution stress and climatic stress are not well understood and represent significant gaps in our understanding of the mechanisms of pollutant damage to plants.

Leaf surfaces are capable of taking up pollutant ions from solution and pollutant gases from the atmosphere. The chemical and physical properties of the cuticle (see review by Riederer, 1989) determine the ways in which the exchange occurs between the plant and atmosphere. Water loss from the leaf is determined by the surface conductance ( $g_{\text{sur}}$ ), which is equal to the sum of cuticular and stomatal conductance. When leaves are abraded by wind action or blown particles (soil or ice), surface conductance increases over the period of exposure as damaged microsites increase in number (Grace, 1974; Armbrust et al., 1974; MacKerron, 1976; Wilson, 1984; Pitcairn and Grace, 1984). Leaves may then also become more susceptible to the uptake of pollutants.

Recent studies have indicated that stomatal conductance to water vapour, and consequently photosynthetic activity, is not uniform across a leaf surface (Kappen et al., 1987; Terashima et al., 1988; Daley et al., 1989). Differences in gaseous exchange may exist owing to variation in stomatal aperture caused by heterogeneity of stomatal response to their environment. This study describes a measuring system to detect pathways through which pollutants may enter leaves. The effects of high wind speed and abrasion on leaf surface structure and conductance are examined and the spatial distribution of cuticular damage and uptake of ( $^{35}\text{S}$ )sulphate are measured.

## MATERIALS AND METHODS

Seeds of *Fagus sylvatica* were sown into a potting mixture of sand, gravel and compost, and raised under glass at the Institute of Terrestrial Ecology, Edinburgh, for one growing season. Plants were transferred to an open-top glass shelter on the King's Buildings campus of the University of Edinburgh at the beginning of the second season. After the second flush of leaves, 20 plants were placed in a growth cabinet (Model 2340G3, Fisons, Loughborough, UK) set to a day/night thermal regime of 19°C–12°C. The photoperiod was 16 h and the day/night relative humidity was 80%/60%.

High-speed wind treatments (about 30 m s<sup>-1</sup>) were applied to the adaxial

surface of single leaves by use of a miniature wind tunnel. Two pumps (Models MU19/65 and F65DE, Charles Austin, Byfleet, UK), placed in parallel, were used to draw air from outside the building into the wind tunnel. The air was filtered by several layers of muslin cloth at the inlet. The air flow rate was controlled by a flowmeter (Gapmeter type PGM series 15 mm, Platon International, Basingstoke, UK) and air speed was calculated from the flow rate and the diameter of the tube at the air inlet to the wind tunnel. The wind tunnel consisted of a perspex box (dimensions: 7 cm  $\times$  5 cm  $\times$  16 cm). An air inlet was positioned at one end and the opposite end was open. An entry port was located along one side so that a leaf could be positioned in front of the air inlet. The wind tunnel was positioned so that a leaf could be treated without damage to the rest of the plant. Each treated leaf was exposed to an air speed of approximately 30 m s<sup>-1</sup> for 7–8 h day<sup>-1</sup> for 4 days.

Images of undamaged and wind-damaged leaves were obtained by low temperature scanning electron microscopy (LTSEM). Rectangular areas (about 10 mm  $\times$  15 mm) were cut from each leaf and mounted on aluminium stubs using a cryo-adhesive (Tissue-Tek II OCT Compound, Lab-Tek Products, Miles Laboratories, Naperville, IL). The mounted specimens were then rapidly frozen by plunging into sub-cooled liquid nitrogen (about 63 K) before transfer to a cryogenic preparation system (SP2000, Bio-Rad Microscience, Hemel Hempstead, UK formerly, Emscope Laboratories, Ashford, UK). The specimens were transferred under vacuum to the SEM (S250 MK1, Leica Cambridge, UK formerly, Cambridge Instruments, UK) cold stage which had been pre-cooled to 115 K. When necessary, ice contamination on the leaf surface was removed by briefly raising the temperature of the SEM stage to 200 K to partially freeze-dry (Jeffree and Read, 1991). After precooling to below 150 K the specimens were transferred to the cold stage of the cryogenic preparation system and sputter-coated with gold in an argon atmosphere (20 mA, 0.1 Torr, 2 min). The specimens were examined at 115 K in the SEM. Photographic images were recorded on black and white 120 film (TMX 120, Kodak Ltd., Hemel Hempstead, UK).

An abrasive treatment was carried out by detaching a pristine leaf and wiping its surface(s) 40 times with a pure bristle paint brush (mean bristle diameter: 92  $\mu$ m, total width: 35 mm). To prevent water loss from the base of the petiole the cut surface was sealed with silicone rubber (Silflo impression material, Flexico Developments Ltd., Potters Bar, UK). The leaf was supported on a thick sponge mat to protect the lower facing surface whilst the opposite surface was brushed in a longitudinal direction. Leaves were treated on the adaxial, abaxial or both surfaces.

The spatial distribution of cuticular lesions was assessed using a method modified from that described by Heslop-Harrison and Heslop-Harrison (1982). After abrasive treatment, leaves were floated on ruthenium red solution (0.25 mg ml<sup>-1</sup> water) for 30 min to stain the epidermal cell wall be-

neath discrete cuticular lesions. Chlorophyll was then extracted in 100% methanol for 1 h. This treatment enabled dye penetration to be more easily observed. After rinsing with distilled water, each leaf was mounted on a microscope slide with soft paraffin wax (with ceresin, congealing point 60°C, BDH Chemicals, Poole, UK).

Observations on the uptake of ruthenium red dye were made using a light microscope (Ortholux II, Leica Cambridge, UK formerly, Leitz, Wetzlar, Germany) fitted with a camera (Orthomat-W, Leica Cambridge, UK formerly, Leitz, Wetzlar, Germany). Additional lighting was provided by a fibre-optic source (Lux 150, Fort Ltd., Tonbridge, UK). Images were recorded on 35 mm colour transparency film (Ektachrome, Kodak Ltd., Hemel Hempstead, UK).

Abrasive damage was scored as the percentage of the area of the leaf surface which was stained with dye. The number of discrete lesions (diameter: 0.03–0.3 mm), which were seen as concentrated spots of dye were also recorded (1) per leaf, (2) per cm<sup>2</sup> of surface and (3) per mm<sup>2</sup> of dyed area. In addition the number of lesions occurring on leaf veins, classed as the major, secondary (emerging from the major vein) and the minor (tertiary) veins, and also in interveinal regions was recorded. The spatial distribution of lesions across the leaf surface was measured as the frequency of occurrence of lesions in 20 grid squares (0.4 cm<sup>2</sup>) placed at random across each of six leaf surfaces. A coefficient of dispersion was computed as the ratio of variance/mean for the frequency distribution of lesions (Sokal and Rohlf, 1987). The randomness of the spatial distribution of lesions was assessed by comparison with the Poisson distribution using the chi-squared test with  $P < 0.05$  (Sokal and Rohlf, 1987).

The effects of abrasion on total leaf surface conductance ( $g_{\text{sur}}$ ) was determined gravimetrically. The fresh weight of ten replicate leaves was measured before sealing the cut petiole with silicone rubber and applying the abrasive treatment. Then leaves were placed in a darkened incubator, set at a temperature of 18°C and re-weighed after 30 and 60 min. Total surface conductance to water loss ( $g_{\text{sur}}$ ) was estimated from the flux of water, determined by the loss of fresh weight using a microbalance (Type RE1614, Sauter, Albstadt-Ebingen, Switzerland), and the difference in vapour pressure between the leaf and air. An initially rapid decline in leaf fresh weight was taken to indicate that the stomata were open. After this initial decline, fresh weight loss was more gradual, and it was assumed that stomatal conductance had declined to a rate at which  $g_{\text{sur}}$  was predominantly owing to cuticular conductance. Leaf area was measured using a leaf area meter (Delta-t Devices, Cambridge, UK).

Effects of natural weathering on leaf uptake of (<sup>35</sup>S)sulphate were examined after placing trees at an upland hill site at Dunslair Heights, Peebleshire, 42 km south of Edinburgh (55°41'N, 3°08'W, altitude 600 m). Leaves were

harvested from plants grown for 10 weeks after bud break at either an exposed or sheltered site. Leaves were placed abaxial surface downwards on parafilm in the base of a petri dish lined with wet filter paper to produce a high-humidity atmosphere. Silicone rubber rings 1 mm long were cut from silicone rubber tubing with 4 mm internal diameter and 1 mm wall thickness (Fisons Scientific Equipment, Loughborough, Leicestershire, UK). The rings were cemented to the adaxial surfaces of the leaves with Kerr Reflect wash vinyl polysiloxane dental impression compound (Kerr UK Ltd, Peterborough, UK).

A solution containing 2.5 mM ( $^{35}\text{S}$ )sulphuric acid with a specific activity of  $2.8 \times 10^4$  MBq mol $^{-1}$  was adjusted to pH 3.0 with ammonium hydroxide. Twenty 1- $\mu\text{l}$  droplets were placed in each of the silicone rubber wells on the leaves and incubated for 5 h.

At the end of the incubation period the acid solution was flushed from the wells by washing for 10 s with a unidirectional flow of distilled water. The leaves were then blotted dry with paper tissue, the position of each well was recorded onto acetate film and numbered. The leaf tissue at the centre of each well was removed with a sharp punch 3.07 mm in diameter (disc area 7.41 mm $^2$ ) and decolourised in 1 cm $^3$  H $_2$ O $_2$  at 90°C for 24 h before scintillation counting in scintillant cocktail containing 3.3 g dm $^{-3}$  2,5-diphenyloxazole and 0.3 g dm $^{-3}$  1,4-di-2-(5-phenyloxazolyl)-benzene in 2:1 toluene/triton X-100 (Scintran, BDH Chemicals, Poole, UK).

## RESULTS

### *Undamaged leaves*

Images from LTSEM show the adaxial (astomatous) surface as gently undulating with uniformly scattered wax plates (Fig. 1a). Trichomes (leaf hairs) were densely aligned along veins but sparsely scattered across interveinal areas (Fig. 1b). The abaxial (stomatous) surface was characterised by having prominent stomata (Fig. 1c) and fewer trichomes in interveinal areas compared with the adaxial surface. Light micrographs indicated a distinct major vein with secondary veins, interspersed with a network of minor (tertiary) veins. The major and secondary veins are more prominent on the abaxial surface. There was evidence of minor damage to pristine leaves. The percentage of the leaf surface area stained by dye was greatest (0.37%) on the abaxial surface. The presence of lesions (seen as discrete points of dye staining) was rare on undamaged leaves, with an average of 1.3 and 2.5 lesions on the adaxial and abaxial surfaces, respectively (Table 1). The average leaf area was approximately 16.5 cm $^2$ .

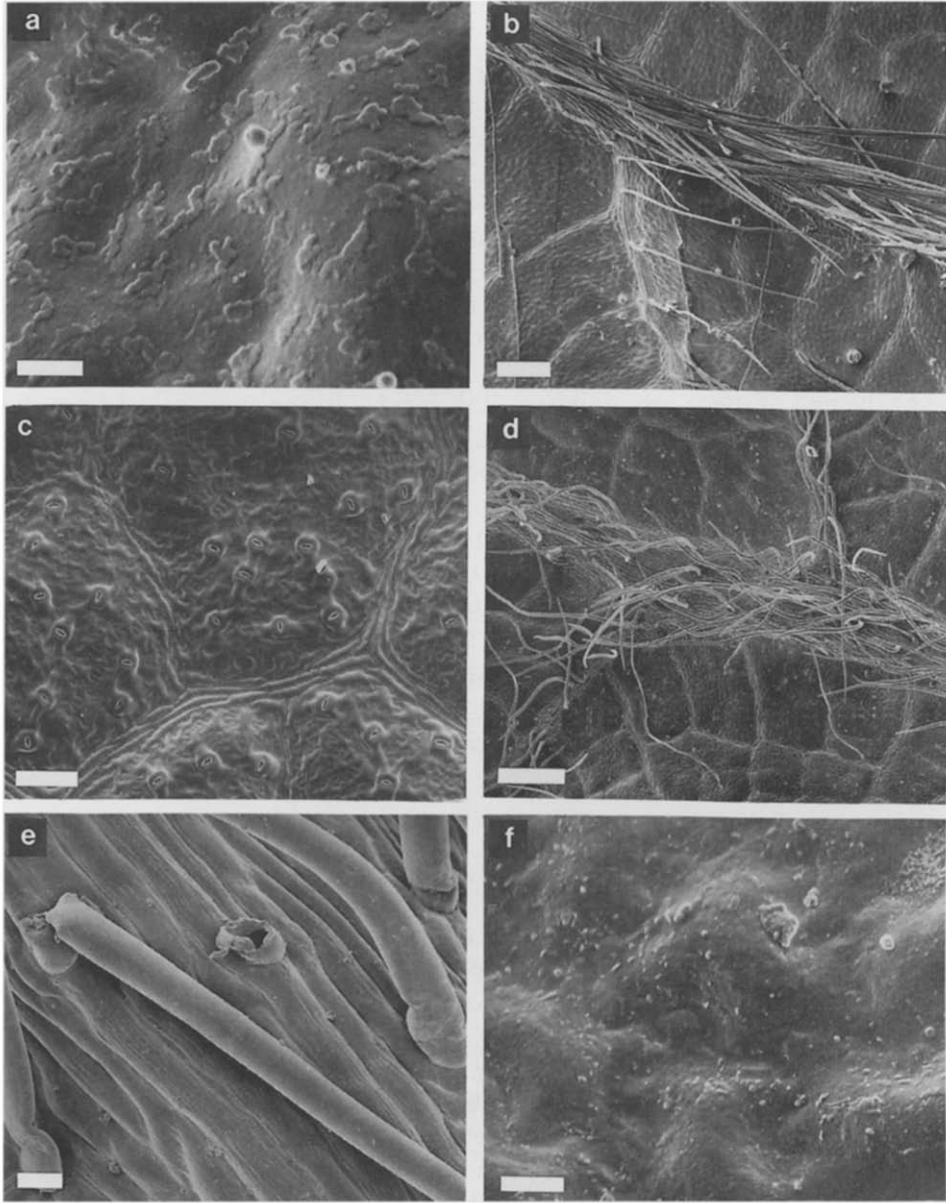


Fig. 1. Scanning electron micrographs of the leaf surface of *Fagus sylvatica*. A pristine leaf is shown with: (a) wax plates on the adaxial surface; bar, 4  $\mu\text{m}$ ; (b) trichomes densely aligned along the main vein of the adaxial surface; bar, 200  $\mu\text{m}$ ; (c) stomatal frequency across the abaxial surface; bar, 50  $\mu\text{m}$ . The adaxial surface of a wind-damaged leaf is shown with: (d) disorganisation of trichomes; bar, 400  $\mu\text{m}$ , (e) damage and removal of trichomes; bar, 20  $\mu\text{m}$ ; (f) smoothing of the wax plates, bar, 10  $\mu\text{m}$ .

TABLE 1

The effects of abrasion on the uptake of ruthenium red dye and the numbers of lesions on the adaxial and abaxial leaf surfaces of *Fagus sylvatica*. Each value is the mean of ten replicate leaves. Significantly different ( $P < 0.05$ ) treatment effects in each column are denoted by a different superscript

Treatment	Percentage dye coverage	Number of lesions per leaf	Number of lesions per cm <sup>2</sup> of surface	Number of lesions per mm <sup>2</sup> of dyed area
No abrasion adaxial surface	0.17 <sup>a</sup>	1.3 <sup>a</sup>	0.08 <sup>a</sup>	0.47 <sup>a</sup>
No abrasion abaxial surface	0.37 <sup>a</sup>	2.5 <sup>b</sup>	0.15 <sup>a</sup>	0.40 <sup>a</sup>
Abrasion to adaxial surface	1.93 <sup>b</sup>	15.8 <sup>c</sup>	0.97 <sup>b</sup>	0.50 <sup>a</sup>
Abrasion to abaxial surface	2.33 <sup>b</sup>	51.3 <sup>d</sup>	3.19 <sup>c</sup>	1.37 <sup>b</sup>

### *Wind-damaged leaves*

The most prominent features of wind damage to the adaxial surface were the disorganisation, collapse, puncture and removal of trichomes (Figs. 1d and e). There was evidence of smoothing of the epicuticular wax, indicated by the absence of wax plates (Fig. 1d–f). Cracks and punctures visible by direct observation by LTSEM were observed only rarely in the cuticle covering the epidermal cells.

### *Abraded leaves*

Trichomes were damaged and disorganised after abrasive treatment. There were significant increases in dye uptake and the number of lesions per cm<sup>2</sup> and per leaf, on both adaxial and abaxial surfaces, was high compared with the unabraded leaves (Table 1 and Figs. 2a,b). The area of the leaf stained by dye uptake on the abaxial surface was greater (2.33%), though not significantly so, than that on the adaxial surface (1.93). There was also a significantly greater number of lesions on the abaxial than on the adaxial surface (3.19 cm<sup>-2</sup> and 51.3 per leaf, respectively). The number of lesions was always greatest on the veins. On the adaxial surface, 32% of the observed lesions were interveinal compared with 45% on the abaxial surface (Table 2).

After abrasion to the adaxial surface, the number of lesions per mm<sup>2</sup> of dyed leaf area (0.5 mm<sup>2</sup>) was not significantly different from that of the pristine leaves (Table 1). However, after abrasion to the abaxial surface there was a significant increase in frequency of lesions per dyed area. Also, the spatial pattern of lesions across the abaxial surface was significantly different



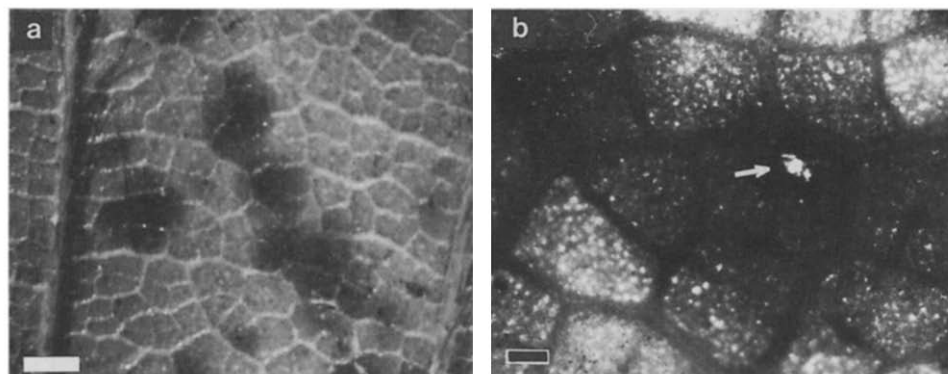


Fig. 2. Light micrographs of the abaxial surface of an abraded *Fagus sylvatica* leaf. (a) A cluster of lesions, indicated by the dark regions of ruthenium red dye, between two secondary veins; bar, 500  $\mu\text{m}$ ; (b) detail of a dyed region indicating a puncture in the cuticle (arrowed); bar, 100  $\mu\text{m}$ .

TABLE 2

The effects of abrasion on the number of lesions on veins or interveinal regions in leaves of *Fagus sylvatica*. Each value represents the mean of ten replicate leaves. Significantly different ( $P < 0.05$ ) values in each row are denoted by a different superscript

Treatment	Number of lesions			
	Main vein	Secondary vein	Tertiary vein	Interveinal
No abrasion adaxial surface	0 <sup>a</sup>	0.3 <sup>a</sup>	0.5 <sup>a</sup>	0.5 <sup>a</sup>
No abrasion abaxial surface	0.2 <sup>a</sup>	0.3 <sup>a</sup>	1.1 <sup>a</sup>	0.9 <sup>a</sup>
Abrasion to adaxial surface	0.2 <sup>a</sup>	1.3 <sup>b</sup>	9.3 <sup>c</sup>	5.0 <sup>bc</sup>
Abrasion to abaxial surface	1.4 <sup>a</sup>	12.4 <sup>b</sup>	14.5 <sup>bc</sup>	23.0 <sup>c</sup>

from a Poisson distribution, with a coefficient of dispersion of 3.51, indicating a clustered spatial distribution.

However, on the abaxial surface there was a significant increase in frequency ( $1.48 \text{ mm}^{-2}$ ) of lesions per dyed area; this suggests there was clustering of lesions with overlapping of dyed regions. On the abaxial surface, a coefficient of dispersion of 3.51 and a significant deviation from a Poisson distribution both indicated a clustered distribution of lesions. In contrast, there was no evidence to suggest that lesions on the adaxial surface were not distributed randomly (Table 3).

TABLE 3

Statistical parameters calculated from the frequency distribution of the number of lesions in 20 randomly placed 0.4 cm<sup>2</sup> grid squares across each of six adaxial and abaxial leaf surfaces of *Fagus sylvatica*. The ratio of the variance ( $S^2$ ) to mean of the frequency distribution was used to calculate the coefficient of dispersion (CD). A coefficient of dispersion near to one indicates a frequency that is essentially a Poisson distribution, whereas a CD significantly greater than one indicates clustering. The chi-squared statistics ( $\chi^2$ ) was derived from the sum of the differences between the expected and observed frequencies

Treatment	Statistical parameters calculated from the frequency distribution of lesions			
	Mean	$S^2$	CD	$\chi^2$
Abrasion to adaxial surface	0.67	1.11	1.66	3.37ns
Abrasion to abaxial surface	1.47	5.23	3.51	62.23*

ns, no significant difference; \* $P < 0.05$ .

TABLE 4

The effects of abrasion on total surface conductance to water vapour of leaves of *Fagus sylvatica*. Each conductance is the mean of ten replicate leaves. Significantly different ( $P < 0.05$ ) values are denoted by a different superscript

Treatment	Total surface conductance (mmol m <sup>-1</sup> s <sup>-1</sup> )	SE (%)	Leaf RWC
No abrasion	6.42 <sup>a</sup>	0.52	84
Abrasion to adaxial surface	15.51 <sup>b</sup>	0.89	89
Abrasion to abaxial surface	20.93 <sup>c</sup>	1.46	87
Abrasion to both surfaces	24.38 <sup>d</sup>	0.90	85

SE, standard error; RWC, leaf relative water content.

### *Leaf surface conductance*

Abrasion of the leaf surface (s) significantly increased  $g_{\text{sur}}$  (140–280%) relative to an unabraded leaf (Table 4). Abrasion of the abaxial surface significantly increased  $g_{\text{sur}}$  by 35% relative to abrasion of the adaxial surface. A further, and significant (17%), increase in  $g_{\text{sur}}$  occurred after abrasion of both surfaces.

TABLE 5

Statistical parameters calculated from scintillation counts per minute above background in 3.07 mm diameter leaf discs exposed for 5 h to droplets containing 2.5 mM ( $^{35}\text{S}$ )sulphate as a mixture of ammonium sulphate/sulphuric acid, pH 3.0. Values are derived from cpm above background counted from samples taken from one leaf except where otherwise stated. The ratio of the variance ( $S^2$ ) to the mean was used to compute the coefficient of dispersion, CD

Treatment	Mean uptake cpm per disc	Mean uptake $\text{mol}(\text{SO}_4^{2-}) \text{ cm}^{-2}$	$S^2$ (cpm)	CD (cpm)
Sheltered	14.2	$1.13 \times 10^{-10}$	161.3	11.3
Exposed	429.7	$3.2 \times 10^{-9}$	90480.6	210.6

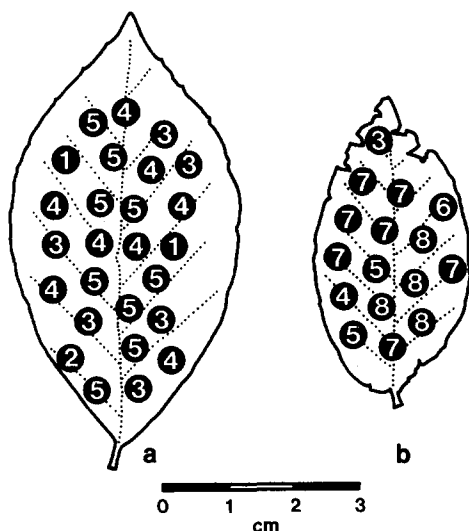


Fig. 3. Maps of the distribution of ( $^{35}\text{S}$ )sulphate uptake via the adaxial surface of *Fagus sylvatica* leaves grown in sheltered (a) and exposed (b) sites at Dunsclair Heights, Peebleshire, UK. The sample positions are indicated by black circles. Numbers within the circles represent half-order of magnitude ranges of ( $^{35}\text{S}$ )sulphate uptake (see key) from a 2.5 mM pH 3.0 solution of sulphuric acid/ammonium sulphate into 3.07 mm diameter leaf discs.

## Key to Fig. 3

Code No.	Uptake range $\text{mol}(\text{SO}_4^{2-}) \text{ cm}^{-2}$
1	$0 < 5 \times 10^{-12}$
2	$5 \times 10^{-12} < 10^{-11}$
3	$10^{-11} < 5 \times 10^{-11}$
4	$5 \times 10^{-11} < 10^{-10}$
5	$10^{-10} < 5 \times 10^{-10}$
6	$5 \times 10^{-10} < 10^{-9}$
7	$10^{-9} < 5 \times 10^{-9}$
8	$5 \times 10^{-9} < 10^{-8}$

### *Uptake of (<sup>35</sup>S)sulphate*

Uptake of (<sup>35</sup>S)sulphate via the adaxial cuticle into leaves of *Fagus sylvatica* grown in the sheltered site at Dunslair Heights was low, averaging  $1.13 \times 10^{-10}$  mol cm<sup>-2</sup> for the 26 samples. The uptake of (<sup>35</sup>S)sulphate by individual discs ranged from 0 (undetectable above background counts) to about 47 cpm, equivalent to about  $3.7 \times 10^{-10}$  mol cm<sup>-2</sup>. By contrast uptake of (<sup>35</sup>S)sulphate by the leaf from the exposed site averaged about 30-fold greater, and showed greater between-sample variance (Table 5), the counts ranging from about 6 cpm to 982 cpm above background. The coefficient of dispersion (CD) of the data for the exposed leaf (210.6) was much greater than that from the sheltered site (11.3), indicating increased spatial heterogeneity in the uptake by exposed leaves, and also indicating that the data were non-randomly distributed.

The spatial distribution of the uptake of (<sup>35</sup>S)sulphate across the leaves is mapped in Fig. 3, in which uptake by each leaf disc is indicated by a number representing half-order of magnitude of ranges, as specified in the figure legend. At this spatial resolution of mapping no large-scale pattern of variation was detected with respect to major structural features of the leaf such as edges (which were not intensively sampled in this experiment) or the main vein. However, it is evident that uptake of (<sup>35</sup>S)sulphate by individual discs varies by up to two orders of magnitude between adjacent samples separated by as little as 6 mm.

### DISCUSSION

Abrasion to leaf surfaces of *Fagus sylvatica* increased the frequency of cuticular lesions, identified as sites of dye penetration into leaves, and increased total surface conductance to water vapour ( $g_{\text{sur}}$ ). Leaves exposed to wind also showed significantly increased uptake of sulphate ions from dilute acidic solutions, and the uptake was more spatially heterogeneous over the leaf surface than in undamaged leaves. Consequently it is suggested that localised damage sites may provide entry points for gaseous and solute forms of pollutants.

In artificially abraded leaves the abaxial (stomatous) surface sustained a significantly greater number of lesions, both on and between veins, than the adaxial (astomatous) surface. This is consistent with the findings of Wilson (1984), who demonstrated that the abaxial surface of hypostomatous leaves of *Acer pseudoplatanus* was more susceptible to microscopic damage owing to abrasion. In leaves of *Fagus sylvatica*, disruption of the trichomes was the main feature of both wind and abrasive treatment of the adaxial surface. The grouping of trichomes along leaf veins may increase the vulnerability of these regions to mechanical damage, and this vulnerability may be further en-

hanced on the abaxial surface by the fact that the veins protrude above the surface of the interveinal lamina.

During abrasion treatments to the adaxial surface, minimal damage to the stomata on the abaxial surface was assumed. Thus an increase in  $g_{\text{sur}}$  after abrasion to the adaxial surface, suggests that cuticular conductance had increased. Similar observations have been recorded after abrasion to *Festuca arundinacea* (Pitcairn et al., 1986), simulated wind damage to *Picea sitchensis* and *Pinus sylvestris* (Van Gardingen et al., 1990) and after natural weathering of *Pinus sylvestris* at upland hill sites (Grace, 1990).

There is evidence that the leaf cuticle is physically and chemically heterogeneous (Norris and Bukovac, 1968; Hoch, 1979, Price, 1982) and that the properties of cuticular waxes differ between the adaxial and abaxial leaf surfaces (Basiouny and Biggs, 1976; King and Radosevich, 1979; Bukovac et al., 1979; Price, 1982). In *Fagus sylvatica*, the more pronounced clustering of lesions on the abaxial leaf surface may be a consequence of heterogeneity both of morphology (and thus vulnerability to damage) and cuticle properties across the surface. A larger increase in  $g_{\text{sur}}$  after abrasion to the abaxial leaf surface compared with the adaxial, may indicate greater vulnerability of the abaxial cuticle and protruding veins, and thus a larger increase in the cuticular component of  $g_{\text{sur}}$ . Alternatively, abrasion to the abaxial surface may increase the stomatal component of  $g_{\text{sur}}$  by direct mechanical damage to stomata as described by Grace (1990), or by the indirect effects on their function of damage to the cuticle covering neighbouring epidermal cells. The extent and type of damage sustained by stomata depends on the mode of abrasive action. Passive stomatal opening may occur due to loss in turgor of epidermal cells (Meidner and Mansfield, 1968; Glinka, 1971) if they are punctured by abrasion (Heath, 1938). Abrasion to epidermal cells may also cause localised increases in  $g_{\text{sur}}$  via smoothing of epicuticular waxes, and consequently water loss, sufficient to depress turgor in the stomatal guard cells, and cause them to open (Pitcairn et al., 1986).

Schönherr and Bukovac (1972) suggest that the geometry of stomatal pores and the hydrophobic properties of the stomatal surface preclude the entry of most aqueous solutions into the leaf via the stomata. However, the uptake of foliar applied substances may occur via routes associated with the guard and accessory cells of stomata (Hunt and Baker, 1982). It has recently been demonstrated that stomatal aperture can be heterogeneous across a leaf and consequently the distribution of photosynthetic activity may also be patchy rather than uniform (Omasa et al., 1985; Farquhar et al., 1987; Kappen et al., 1987; Terashima et al., 1988; Daley et al., 1989). Clustered distribution of lesions on the abaxial surface may relate to a patchy response of stomata to abrasion. It is possible that heterogeneity, in vulnerability and response to abrasive-induced damage, exists across a stomatous surface. However, at

present there is insufficient evidence to indicate that the spatial distribution of lesions is directly related to the spatial distribution of stomatal activity.

A lesion causing damage to epidermal cells may affect single or groups of stomata owing to localised changes in epidermal cell turgor. Thus, an abrasive-induced increase in  $g_{\text{sur}}$  on a stomatous leaf surface, may indicate an indirect effect on stomatal conductance, via changes in the cuticular component of  $g_{\text{sur}}$ , or a direct effect on stomatal conductance via damage to the stomatal complex (stomatal dysfunction).

These observations demonstrate that wind- and abrasive-induced damage may create or increase heterogeneity across the leaf surface. Consequently, the exchange of pollutants (gases and solutes) between the atmosphere and the leaf increase after weathering, and may be related to heterogeneity of leaf surface characteristics. Therefore, further insight into the understanding of the pathways by which gases and solutes are transferred between the atmosphere and the leaf may be gained by measuring the spatial variation in cuticular and stomatal conductance at high resolution and the distribution of microsites across the leaf surface.

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