

Stomata and plant water relations: does air pollution create problems?

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Abstract

Small changes in the gaseous composition of the atmosphere have many different impacts on terrestrial plants. Some of the most important involve changes in stomatal control of leaf conductance. Evolution has provided highly complex mechanisms by which stomata respond to a wide range of environmental factors to balance the conflicting priorities of carbon gain for photosynthesis and water conservation. These mechanisms involve direct responses of the guard cells to aspects of the aerial environment, and hormonal communication within the plant enabling conductance to be adjusted according to soil moisture status. Various aspects of these delicately balanced mechanisms can be disturbed by air pollutants. Impairment of the regulation of plant water use by SO₂ and O₃ has been known for some years, but there are still many obstacles to our understanding of the variations in response between species, or even between genotypes of the same species. A surprising outcome of some recent studies is the suggestion that CO₂ pollution may disrupt the control of water relations in some species because their stomata do not close sufficiently in CO₂-enriched air. It has often been taken for granted that the elevation of atmospheric CO₂ would lead to economies in water use by plant canopies, but the underlying assumptions are now being seriously questioned. © 1998 Elsevier Science Ltd. All rights reserved.

1. Introduction

When plants moved from aquatic to terrestrial environments during the course of evolution, they entered situations where the availability of water is variable, and where its shortage is encountered from time to time and must be endured. As the competition for light led to the evolution of taller plants, upward transport of water obtained from the soil became necessary, and this could only take place along gradients of water potential. Thus the tissues of land plants became able to tolerate progressively lower water potentials, and as the aerial environment became more thoroughly explored, some degree of moderate water stress became a regular occurrence for most individuals. The morphological and anatomical evidence from fossil plants suggests that water stress was the most significant constraint to colonization of land. Stomata appeared around 400 million years ago at a time when the CO₂ concentration in the atmosphere was probably much higher than at present. They were usually sparsely distributed, and might have

been as important for generating a transpiration stream to carry mineral nutrients as for permitting the entry of CO₂ (Edwards, 1998). Subsequent evolution has led to the development of stomata protected by guard cells, which are highly specialized sense organs that are able to respond to a wide range of environmental factors and to signals generated within the plant (Mansfield, 1986; Davies and Zhang, 1991). The ability of guard cells to adjust their turgor to give an elaborate degree of control of leaf conductance enables stomatal density to be very large compared with that in primitive plants. Indeed, modern research has revealed that stomatal density has changed over geological time according to the CO₂ concentration of the atmosphere (McElwain, 1998). As CO₂ concentration decreases, stomatal density increases. Even over recent millennia the changes in CO₂ concentration have been sufficient to induce changes in stomatal density (Beerling and Chaloner, 1992; Beerling and Chaloner, 1994). Greater density makes it possible for plants to benefit from increases in the amplitude of the changes in leaf conductance as stomata open and

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close. Thus, when the atmospheric CO₂ concentration is low the maximum attainable conductance rises, which benefits photosynthetic CO₂ fixation. This does, however, increase the risk of water stress, and the ability of the guard cells to regulate conductance is clearly of very high priority.

When we consider the influence of atmospheric pollution on plant–water relations, it is important to recognise that evolution has provided plants with mechanisms capable of regulating leaf conductance so that basic physiological needs (especially water conservation and CO₂ uptake) can be fulfilled with great efficiency. Past changes in atmospheric composition have exerted strong influence on the evolutionary changes in leaf anatomy and physiology, and it is clear that water-use efficiency is a vital component of a plant's fitness to exploit the aerial environment.

The impact of human activities on the gaseous composition of the atmosphere has produced some changes which have occurred at earlier points in the Earth's history, in particular the rise in CO₂ concentration, though the rate at which it is now happening may be unparalleled. Large increases in the levels of tropospheric ozone comparable to those now found periodically in some parts of the world are, on the other hand, unlikely to have occurred before our own times, even though biological processes may well have played some role in ozone formation, since land plants began to emit non-methane hydrocarbons into the atmosphere (Raven and Yin, in press). Emissions of nitrogenous gases (NO_x and NH_y) have risen dramatically over the last decade and their impact on the nitrogen cycle has attracted much attention (Vitousek, 1994). There is evidence that terrestrial vegetation may have coped with some major surges in availability of combined nitrogen in the past, probably as brief occurrences on a geological time scale (Raven and Yin, in press). This does not, however, provide much reassurance because we now face modifications to atmospheric chemistry in an assortment that is probably unprecedented, and which will cause global environmental changes that pose threats to many human activities.

Seen in this broad perspective, the impacts of air pollutants on vegetation assume much greater importance than has generally been recognised. The support for research has often been within a limited economic frame, such as defining reductions in productivity of a particular crop, or has been related to particular conservation issues such as forest decline. This has meant that experimental studies and modelling have often focussed on individual pollutants and single crop or tree species. Some work has been done on combinations of two pollutants (e.g. SO₂+NO₂), and there have been highly focussed studies on ecological systems of conservation value that appear vulnerable for special reasons.

We now begin to recognise that interactions between plants and the chemical composition of the atmosphere are of fundamental importance in relation to many aspects of the causes and impacts of global environmental change. Only with an adequate understanding of underlying principles will it be possible to formulate strategies for coping with the highly complex issues that are now coming to the fore in debates about the impacts of environmental and climate change.

The water relations of plants have been studied for well over a century, but during the past decade much new information has come to light and some long-held concepts have had to be abandoned. Before we review the impacts of air pollutants, it is appropriate to summarize our present understanding of the normal mechanisms used by higher plants to regulate their water status.

2. Mechanisms for the regulation of plant water status

Plants that can survive the extremes of water availability—desiccation or waterlogging—require special adaptations that are usually both anatomical and physiological in nature. The focus here will not be on such plants, but on the species of more favourable habitats where, when water availability changes, survival depends mainly on physiological controls that can come into operation quickly and effectively.

2.1. Root-to-shoot communication

For over half a century it was believed that a plant's defences against drought in the soil only become operative after there has been some loss of turgor in the shoot. In the late 1980s it was found that this is incorrect, and that there is a mechanism in which root-to-shoot communication plays a vital part. The discoveries were made after it was found that reactions were occurring in leaves in the very early stages of soil drying, well before there was any drop in the water content of the shoot. Partial stomatal closure, and reduced rates of expansion of new leaves, were observed with the result that the expenditure of water was reduced. Thus the remaining water in the soil was being used with greater economy some time before the tissues of the shoot were themselves suffering from reduced water content. Further research revealed that there is an ability to 'sense' drying of the soil around just part of the root system, and to communicate the information to the shoot. The form of communication does not appear to require any reduction in the flux of water to the shoot.

There has been some controversy about the nature of the signal passing from root to shoot. It is now firmly established that the hormone abscisic acid (ABA) is at least a principal component. The most important

physiological evidence came from experiments in which the root systems of plants were split between two separate containers and allowed to become well established. If one container was deprived of water while the other was well watered the shoot remained adequately supplied with water but, regardless of this, stomatal conductance was greatly reduced. The part of the root system in drying soil contained much more ABA than the moist half, and the ABA content of the xylem sap was increased and was well correlated with the decline in stomatal conductance. Other experiments were performed on plants in containers with a few root tips protruding from the bottom. If these were allowed to dry out partially, stomatal conductance was reduced but quickly rose again when the drying roots were excised.

With such clear experimental evidence available, the role of root-to-shoot signals in regulating plant water relations is now well established. More information will be found in reviews by Davies and Zhang (1991) and McDonald and Davies (1996). The possibility that other factors may also be involved in plants under drought is, however, still being debated (Munns and Cramer, 1996). It is also possible that when stomatal closure occurs in plants in waterlogged soil, ABA is not the only agent implicated (Else et al., 1996).

2.2. Responses of stomatal guard cells

Notable advances have also been made in recent years in our understanding of mechanisms within stomatal guard cells. With regard to the control of leaf conductance during periods of soil drought, the intracellular signalling systems in guard cells are of interest, and may be of particular importance in relation to the impacts of air pollutants.

It is now firmly established that Ca^{2+} ions are employed by plant cells as intracellular second messengers which couple both external and internal stimuli to a wide range of physiological responses. The clearest experimental evidence has come from studies of stomatal guard cells, where several different stimuli have been shown to cause elevations in intracellular Ca^{2+} . One of the endogenous stimuli employing Ca^{2+} in this way is ABA, indeed it is now thought that an increase in the concentration of free Ca^{2+} in the cytoplasm is a major early component of the cellular mechanism leading to the control of guard cell turgor after ABA arrives at the outer surface of the plasma membrane (McAinsh et al., 1997). It has, however, been found that other major regulators of stomatal opening also appear to operate via elevations of cytosolic Ca^{2+} in the guard cells. CO_2 , which also induces stomatal closure, does so (Webb et al., 1996) and, curiously, so does auxin which can counteract effects of ABA and CO_2 and thus cause stomatal opening (Snaith and Mansfield, 1982; Irving et

al., 1992). This apparent contradiction raises some very challenging questions about how, when different stimuli rely on intracellular signalling based on elevations of cytosolic Ca^{2+} , the cells manage to decipher the messages and respond in different ways. It now appears that each stimulus delivers its own specific ' Ca^{2+} signature', with information encoded in the periodicity and amplitude of oscillations in intracellular Ca^{2+} (McAinsh and Hetherington, 1998). Relatively simple linkage to the mechanisms determining guard cell turgor might explain how quite different final effects are achieved (De Silva and Mansfield, 1994). Variations in response are also dependent on whether or not a cell is competent to accept a stimulus, and this has led to the concept of 'physiological address' (McAinsh et al., 1997). Although we have little understanding at present of the cellular properties underlying these changes in competence, we do know that environmental factors during the growth of a plant are of great significance (e.g. Allan et al., 1994), and consequently this is one area in which research on the impacts of environmental pollutants should focus.

2.3. New physiological insight and future research

In the context of the impacts of ozone on plants, Heath and Castillo (1987) wrote of the need for "excellent concepts in physiology and biochemistry to form our predictions to be tested in the field". This statement challenges us in various ways, but it also presents us with an enigma: how do we identify excellence? In the fields we have been discussing, the concepts defined as excellent now are very different from the ones accepted in 1987. Entirely new insights have emerged and several long-held beliefs have disappeared. But some current notions will in turn be displaced as knowledge progresses in the future. Because the issues associated with pollution and climate change are forecast to become progressively more formidable, we must at the very least ensure that our experimental studies take fully into account the most recent understanding of the basic processes in plants. We must also be prepared to re-examine and where necessary reinterpret data obtained in the past, and this will be my main objective in this brief review.

3. Air pollutants and water conservation

3.1. Sulphur dioxide, nitrogen oxides and ozone

Plants exposed to toxic levels of air pollution in the field often display symptoms of water stress. Godzik and Piskornik (1966) found that detached leaves of *Aesculus hippocastanum* (horse chestnut), taken from trees growing near an industrial source of SO_2 , wilted

much more rapidly than those from trees in an unpolluted area. They carefully distinguished between effects on leaves which showed visible lesions and those which did not, and concluded that physical injury, or indeed any visible change in appearance, was not a prerequisite for the marked differences in the ability to conserve water. Subsequent studies on several different plant species confirmed that SO_2 can disturb stomatal functioning. Majernik and Mansfield (1970, 1971) found that the normal diurnal cycle of opening during the day and closing at night was not affected, but the apertures achieved during the day were higher in plants exposed to SO_2 . Black and Black (1979) confirmed these findings—conductance increased by 20–25% when *Vicia faba* was exposed to SO_2 concentrations as low as 17 ppb—and showed that the enhanced opening was associated with damage to the epidermal cells adjacent to the stomata. This suggested that a mechanical effect might provide a simple explanation for the greater stomatal opening, i.e. the epidermal cells would offer reduced resistance to the deformation of the guard cells which is necessary to open the stomatal pore (Mansfield and Freer-Smith, 1984). This suggestion found support from observations by Neighbour et al. (1988) on two species of birch (*Betula pendula* and *Betula pubescens*) exposed to mixtures of SO_2 and NO_2 in concentrations ranging from 20 to 60 ppb. Excised leaves showed an increased rate of water loss depending on the concentrations of the two pollutants to which they had been exposed for the previous 2 months (Fig. 1). Examination of frozen hydrated specimens under the electron microscope showed very clearly that some of the

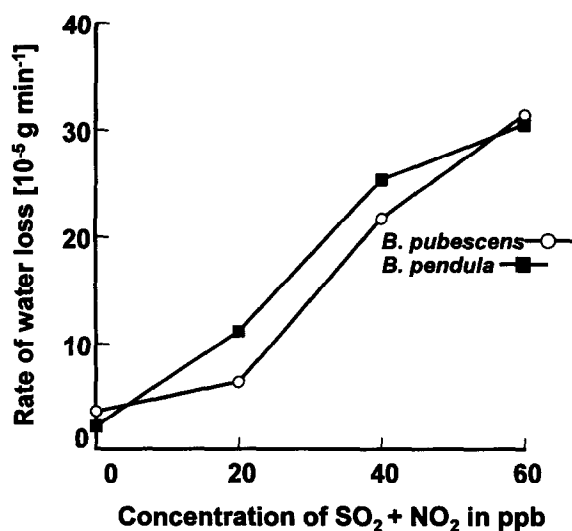


Fig. 1. Rates of water loss from excised leaves of two species of birch which had been exposed to SO_2 and NO_2 pollution for at least 21 days. Plotted from tabulated data of Neighbour et al. (1988), where statistical information will be found.

stomata on the polluted leaves were surrounded by severely damaged epidermal cells. It was also found in *B. pubescens* that the transpiration rates of the intact plants were greatly increased after exposure to the two pollutants (Fig. 2), showing that the observations on excised leaves are indicative of important changes in the daily cycle of transpiration. The rate of transpiration at night in the plants exposed to the two highest concentrations of $\text{SO}_2 + \text{NO}_2$ was roughly equivalent to that during the day in the controls, and this was probably the most surprising observation in this study. I have long been intrigued by the data published by Loftfield (1921), in what can truly be called one of the classics of the plant physiological literature of the first half of this century. Much of his work was undertaken at the laboratories of the American Smelting and Refining Company near Salt Lake City, and he explained the reasoning behind the research as follows

“.....prevention of smelter injury at that time was based upon the ‘sea captain’ plan of smelter operation, by which the manager kept informed of unfavourable conditions when plants were especially susceptible to SO_2 injury, and reduced operations within the smelter accordingly.”

Loftfield’s task was described thus “exact information was needed as to when stomata opened and closed during a 24-hour period”. This was an early and remarkable example of environmental responsibility being shown by industry, and it was unfortunate that some of Loftfield’s findings about stomatal behaviour must have proved so very unhelpful to the company, for example “It is as unusual to find movement identical on two successive days as for weather to be the same”. Nevertheless, his data provided invaluable records of daily patterns of stomatal movement which are unsurpassed in the literature, and he was undoubtedly a very careful observer whose conclusions must be taken seriously. The importance of the daily course of stomatal conductance in controlling rates of pollutant deposition is now well established (Fowler, 1992). The timing of pollution episodes, particularly of O_3 , varies from one location to another and may be very important in determining the impacts on vegetation (Lefohn and Manning, 1995).

One finding in particular is of great interest in relation to the data in Fig. 2, for Loftfield concluded that the stomata of some of the species closed very little at night. He placed the species he had observed in three groups according to their stomatal behaviour, and he used the occurrence of night opening as a major distinguishing feature. Those displaying substantial night opening included mesophytes as well as succulents, and he presented the behaviour of potato as being typical of that group. We are now familiar with night opening

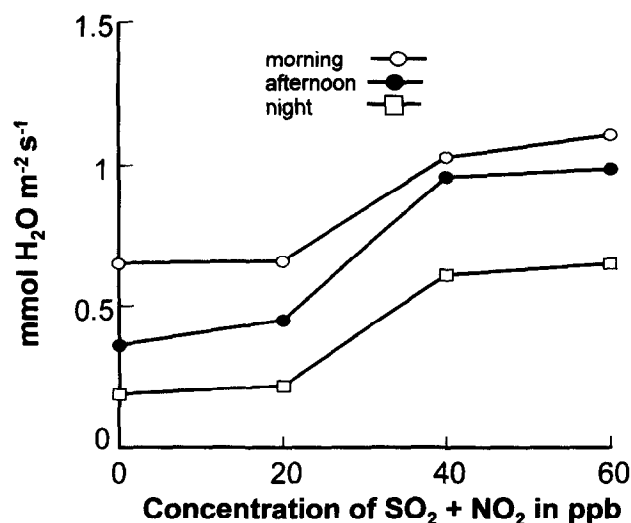


Fig. 2. Replotted data of Neighbour et al. (1988) showing effects of mixtures of SO₂ and NO₂ on transpiration of clonal *Betula pubescens* at different times of day. The plants had been exposed to the pollution for approximately 1 month prior to the measurements. Statistical information is shown in Fig. 5 of the original publication.

of stomata as an important component of Crassulacean acid metabolism and drought tolerance, but its occurrence in many mesophytes as recorded by Loftfield has never been confirmed. Unfortunately he did not report the pollution concentrations during his studies, but since the bulk of his work was done near the smelter it is at least possible that he was seeing an effect like that in Fig. 2, since many sources of SO₂ also produce NO_x (NO and NO₂).

Effects of this kind are probably associated with levels of SO₂ that are not often experienced today in countries with statutory controls on pollution emissions, but O₃ and NO_x continue to be of concern and in many places, particularly in developing countries, there are growing problems with these two pollutants. There have been very few studies of the influence of NO and NO₂ *per se* on stomatal behaviour. Ashenden (1979) found that both NO₂ and SO₂ at a concentration of 100 ppb caused a transient stimulation of transpiration in *Phaseolus vulgaris*, implying that a temporary stimulation of stomatal opening was involved. Transpiration was enhanced to similar extents by NO₂ and SO₂, both at 65 ppb, in two *Betula* species (Neighbour et al., 1988). Saxe (1986) used relatively high concentrations of NO and NO₂ (1 ppm) relevant to pollution in CO₂-enriched greenhouses, and found that NO reduced the transpiration of several ornamental plant species, while NO₂ had little effect. NO is now a very abundant pollutant in urban areas, and much more attention needs to be paid to its physiological effects on plants. Its role as a signalling compound in animals has been established in the last few years, and in the light of this information its regulatory

impacts on plant functions should be reinvestigated (MacKintosh, in press).

Disturbances of stomatal behaviour similar to those caused by SO₂ can certainly be found after exposure to O₃ pollution (Reich and Lassoie, 1984). Hassan et al. (1994) described some important work on the effects of O₃ on stomatal behaviour of *Raphanus sativus* (radish) and *Brassica rapa* (turnip). Exposure to 80 ppb ozone for 8 h daily for 1 week reduced the rate of net photosynthesis in both species, but stomatal conductance increased in radish and decreased in turnip. A dose-response study using radish showed very clearly the contrasting effects of O₃ on photosynthesis and conductance in this species (Fig. 3). The difference in the stomatal response between the two species appeared to be explained by physical damage to the epidermal cells which occurred in radish but did not appear at all in turnip. Under the scanning electron microscope many of the epidermal cells of radish had totally collapsed, and although this may not be an indication of their state *in vivo*, it does nevertheless point to a mechanical condition which would offer less resistance to stomatal opening.

The most important features of this type of injury are its occurrence at low pollutant concentrations, and the close similarity of the effects that have been found with different air pollutants, viz. SO₂, mixtures of SO₂ and NO₂, and O₃. The differences between species are remarkable, and at present we cannot explain why in some cases the epidermal cells are so susceptible to damage. It is clear, however, that injury at this point in a leaf is potentially very serious because of the loss of control of water relations. Although the guard cells themselves may remain functional, they cannot operate normally to regulate stomatal aperture because of the damage to the surrounding cells. There are also important questions about the reception of chemical signals from the roots if the essential final stage of the pathway in the epidermis is damaged.

Atkinson et al. (1991) looked into this matter using spring barley exposed to low concentrations of SO₂ and NO₂ (24–35 ppb of each gas). Very little difference was found in the diurnal patterns of stomatal conductance between the polluted plants and the controls. The conductance was in fact slightly reduced in the polluted air, i.e. there was no stimulation of stomatal opening like that found in *Betula* by Neighbour et al. (1988). Nevertheless, when excised leaves were supplied with a 10⁻¹ mol m⁻³ solution of ABA, the stomata that had been previously exposed to polluted air closed more slowly, and also less completely in a period of 6 h, than the controls. It was concluded that concentrations of air pollutants that did not cause any visible injury did nevertheless impair an important physiological mechanism in the guard cells.

Effects that appeared comparable to these were also found by Pearson and Mansfield (1993) in beech (*Fagus*

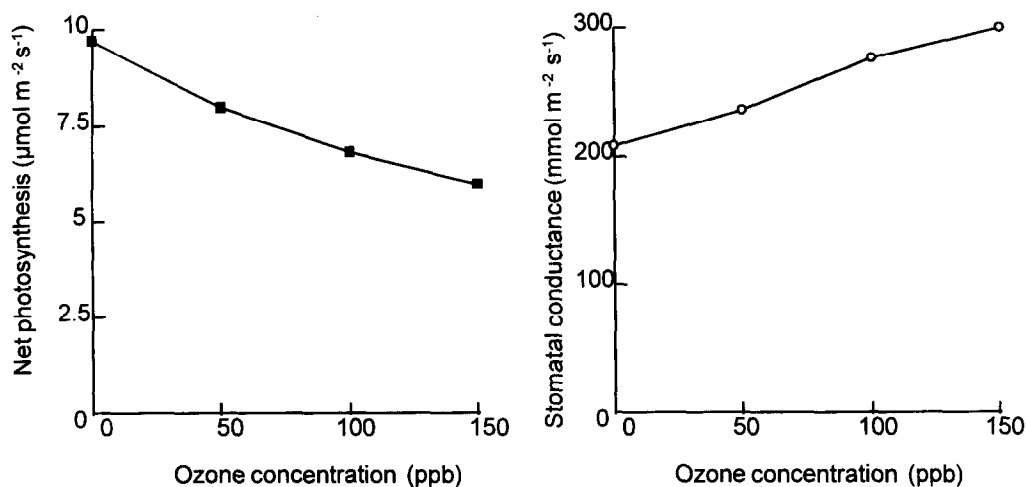


Fig. 3. Contrasting effects of increasing ozone concentrations on net photosynthesis and stomatal conductance in radish. Plotted from the tabulated data of Hassan et al. (1994). Effects of the pollution at each incremental increase in concentration were significant at $p < 0.05$.

sylvatica) exposed to realistic concentrations of O_3 . 3-year-old transplants of beech were subjected to episodes of ozone pollution in daylight chambers, higher concentrations (to a maximum of 120 ppb) being applied on days with greater incident sunlight. This provided a realistic simulation of the outside environment in parts of Europe where beech is thought to be affected by O_3 pollution. The treatments began in mid-May and measurements of stomatal conductance were made after 1 August on days when the O_3 concentration exceeded 100 ppb. Leaves of the first flush on trees that were well supplied with water displayed a decrease in stomatal conductance due to the O_3 treatment, and the magnitude of the effect rose steeply over the next 35 days. However, the situation was quite different in trees that were exposed to soil moisture deficits. The shortage of water was sufficient to cause partial stomatal closure in all the trees, but the effect was much smaller in those exposed to O_3 pollution. In consequence, the impact of O_3 was to reduce conductance on the well-watered trees, and to increase it on the water-stressed trees.

The accounts in the literature about O_3 effects on stomata have been conflicting. In general, authors have reported partial stomatal closure but some have found a stimulation of opening. The work of Hassan et al. (1994), quoted earlier, provides one explanation for the differences, and we can suggest that the water status of the plants provides another. Neither of these explanations does, however, indicate how the different responses originate at the cellular level. We do know that the oxidative products formed when O_3 enters a cell can be detoxified via metabolites such as ascorbate and glutathione, or enzymes such as superoxide dismutase (Heath and Taylor, 1997). There is also much evidence that the plasma membrane is the primary site of injury, and it is often assumed that lipids are the molecules

specifically attacked. Heath and Taylor cast doubt on this assumption and pointed to free radical mechanisms which would require other compounds such as ethylene to be present. Some association between the amount of ethylene produced by the plant and ozone injury has been established (e.g. Mehlhorn and Wellburn, 1987; Mehlhorn et al., 1990). If ozone and ethylene react to produce free radicals which are then responsible for damage to plasma membranes, it is the cells surrounding the leaf's intercellular spaces that would be the most exposed, and those around the substomatal cavity and close to the stomatal pore might be the most vulnerable. This could explain the location of the badly damaged epidermal cells reported by Hassan et al. (1994), and it is not difficult to envisage initial injury becoming quickly worse as wider stomatal opening and greater access of O_3 occurs because of the loss of epidermal turgor. Contrasting behaviour between species, such as Hassan et al. found in *Raphanus* and *Brassica*, does not necessarily require totally different mechanisms. It could, for example, be due to differences in intercellular anatomy which lead to alternative cells lining the substomatal cavity being the ones most susceptible to injury. If they were mesophyll cells and photosynthesis were reduced, the increase in intercellular CO_2 concentration would lead to partial stomatal closure by a mechanism that is now well defined (Mott, 1988). Thus we can see how small differences in internal anatomy might lead to contrary responses in terms of a vital function such as leaf conductance.

It may be a valid criticism that too much research has been directed towards simple descriptions of responses, with little or no attempt to explain discrepancies between species, or within species on different occasions, at a mechanistic level. The explanation offered earlier is somewhat speculative, but supportive evidence is fairly

strong. First, there are findings of Hassan et al. (1994) that photosynthesis was inhibited by O₃ both in *Brassica* and in *Raphanus*, even though stomatal opening was induced in one and not in the other. This clearly indicates that at least some of the stomatal and photosynthetic responses must be considered separately. Farage et al. (1991) applied single acute doses of O₃ to wheat and observed depressions of photosynthesis and of stomatal conductance, but a careful analysis of the degree of stomatal limitation, estimated from the relationship between the intercellular CO₂ concentration and CO₂ uptake, showed that stomatal closure was mainly secondary, the primary effect of O₃ being on carboxylation efficiency. The importance of direct effects in the mesophyll has often been recognised (e.g. Reich, 1987; Darrall, 1989), and the elimination of stomatal closure as the principal factor in determining the O₃-induced drop in photosynthetic rate has large implications for the physiological state of the plant. A decline in stomatal conductance as a result of direct action of a chemical on the guard cells, but with no effect on the mesophyll, would improve water-use-efficiency though there might be some small loss of productivity because of stomatal limitation of photosynthesis. This is the principle underlying the notion of 'chemical anti-transpirants' which were once actively sought as an artificial means of increasing the drought tolerance of crops (Martin et al., 1983). If, on the other hand, stomatal closure takes place only after photosynthesis in the mesophyll has been inhibited, and occurs because of an increase in the CO₂ concentration in the intercellular spaces, the consumption of water by the plant will be reduced but at the expense of both a drop in plant productivity and a fall in water-use-efficiency.

There has been a considerable degree of confusion about these concepts in the literature, amidst evidence that drought may both increase and decrease the ozone tolerance of plants. Several studies on different crops in the USA have suggested that water stress can reduce ozone injury. Observations on tobacco in the 1960s showed that injury due to O₃ was greater on plants growing in moist soil (Walker and Vickery, 1961; Dean and Davis, 1967). Experiments by Tingey and Hogsett (1985) on *P. vulgaris* established that this was primarily the result of reduced pollutant uptake when the stomata are partially closed. They induced stomatal closure by applying exogenous ABA or by allowing the plants to develop water stress, and found that both treatments reduced O₃ injury to about the same degree. They also used fusicoccin to induce reopening of stomata on water-stressed plants and found that O₃ sensitivity was restored. This important study clearly indicated that water stress can protect plants from O₃ injury mainly through its influence on stomatal conductance, rather than through biochemical mechanisms.

Research on soybeans, on the other hand, has indicated that soil moisture stress may interact with O₃ pollution to cause major reductions in yields. Heggestad et al. (1985) considered ambient O₃ and soil moisture stress treatments in the field which independently caused just 5% and 4% reductions in yield, respectively. When these treatments were experienced together, however, the yield reduction amounted to 25%. Heggestad and Lee (1990) found a substantial drop in root length in soybeans grown in unfiltered compared with charcoal-filtered air. In this case the plants were grown in a greenhouse in tubes 1.5 m in length, with no water table equivalent to that in the field, and the effects of O₃ and drought treatments were in this case less than additive. A possible explanation is that during drought in the field, greater root growth is required to maintain contact with the water table, and that this process is inhibited, or takes a larger proportion of the plant's resources, when overall biomass is reduced in the presence of O₃. These data emphasise strongly that the impacts of air pollution on drought tolerance will only be adequately understood when the behaviour of the plant as a whole is monitored, and when the plant's experience of drought is comparable to that in the field.

In summary, there is strong evidence that O₃ pollution can disturb water relations in some plant species by disrupting the control of stomatal conductance. This can take the form of abnormal opening of stomata when the guard cells gain mechanical advantage after loss of turgor by the surrounding epidermal cells. There are sufficient reports in the literature of O₃-induced increases in stomatal conductance, or of sluggish stomatal responses, to suggest that these effects are not uncommon (e.g. Skärby et al., 1987; Eamus et al., 1990; Leonardi and Langebartels, 1990). But even when this does not occur there may be a sluggish response to water stress during drought in the soil. When stomata are already partially closed in response to drought, and the access of O₃ to the mesophyll tissue is restricted, some protection may be given against injury. This possible advantage may, however, be overridden by effects on the plant as a whole, particularly O₃-induced changes in allocation between shoot and root. Grantz and Yang (1996) found for cotton that when O₃ reduces the allocation of biomass to the root system, there is impairment of the hydraulic capacity of the roots relative to the area of leaves consuming water by transpiration. The decline in root hydraulic conductance per unit leaf area was as high as 41%, and a reduction in stomatal conductance became a necessity to protect leaf water status. They proposed that a drop in the hydraulic efficiency of roots may have been a factor in injury to the whole plant by O₃ by indirectly causing a lowering of leaf gas exchange and carbon gain. In the absence of observations on the plant as a whole, it might have appeared that stomata were being inhibited directly by

O₃ when in reality the action was occurring indirectly via the roots. In practice, it is likely that ozone affects guard cells both directly (McAinsh et al., 1996) and indirectly. Maier-Maercker (1989) compared the amount of lignification of guard and subsidiary cell walls in *Picea abies* grown in clean and O₃-polluted air, and found that O₃ appeared to have caused reduced lignification of the external walls of the stomatal apparatus. Maier-Maercker and Koch (1991) attributed the irregular stomatal behaviour and delayed closure in *P. abies* to this structural change in the epidermis. Fink (1991), also working with *P. abies*, found that after exposure to O₃ there was a huge redistribution of calcium within the needles. Calcium oxalate is normally deposited within the cell walls of this species, but after exposure to O₃ the deposits were relocated within epidermal and subsidiary cells. While this has not been shown to interfere with the role of calcium in intracellular signalling, it is probable that some disturbances in this important function will occur.

It is interesting to pose the question "What would be the most advantageous response of the stomata to elevated levels of O₃ in the atmosphere?" (cf. Mansfield and Majernik, 1970). Guard cells react to many components of the environment to meet the physiological requirements of the plant, and if they were to lose turgor during episodes of O₃ pollution, leading to a drop in leaf conductance, there would be some protection for the underlying tissues. It would be important that the guard cells were not damaged (they are susceptible to irreversible changes as a result of oxidative stress [McAinsh et al., 1996]) and that they could recover turgor and return to their normal regulatory role when the level of O₃ fell again. There could also be a temporary gain in water-use-efficiency, on the principle of anti-transpirant action as mentioned before, though at the expense of some loss in CO₂ intake for photosynthesis. Kobayashi et al. (1993) considered the interactive effects of O₃ and water stress using a growth model of soybean, and one of their conclusions was that reduced water use in the presence of the pollutant could contribute to the alleviation of water stress. There is, however, little evidence that the effect of O₃ on the guard cells is sufficiently specific and short-lived for this to be of true practical value. Greitner and Winner (1988) exposed radish and soybean to 120 ppb O₃ and used gas exchange techniques and $\delta^{13}\text{C}$ values to evaluate impacts on assimilation and water use, and concluded that O₃ could act simultaneously to suppress growth and increase water-use-efficiency. The data for radish did, however, point to possible problems in the longer term. O₃ decreased stomatal conductance by about 35% in both 19- and 25-day-old leaves, while photosynthesis was unaffected in 19-day-old leaves but was reduced by 37% in 25-day-old leaves. Later work from the same laboratory showed that 120 ppb O₃ eventually

caused visible injury in radish, and obviously any early improvement in water-use-efficiency is of little value if severe toxicity appears later (Atkinson et al., 1988).

There are many papers in the literature on this topic, but there is little, if any, evidence that water-use-efficiency is improved in the long term during exposure to O₃ pollution. Where careful studies of gas exchange have been made, the marginal cost in terms of water consumption per unit of carbon gained usually increases, as found for *P. abies* and *Pinus armandi* (Wallin and Skärby, 1992; Shan et al., 1996). In the case of trees, however, genetic differences can lead to huge variations in physiological responses to stress factors such as ozone, and even in the case of a single species generalisations are dangerous (Payer et al., 1990; McLaughlin et al., 1994; Karlsson et al., 1997). This creates enormous problems for mechanistic modelling, but it does offer encouragement that tolerant genotypes might be found for use in polluted environments.

3.2. CO₂ pollution and disturbances in stomatal control

It is widely assumed that CO₂ enrichment of the atmosphere will generally be beneficial to plant growth, both through increased carbon assimilation and through gains in water economy. Stomatal opening is generally inhibited by increases in CO₂ concentration (Mansfield et al., 1990; Morison, in press). When the possibility of using chemicals to regulate stomatal opening to improve drought tolerance was being discussed many years ago, it was suggested that CO₂ is the ideal anti-transpirant because it both supports photosynthesis and reduces transpiration (Mansfield, 1976). Experimental evidence has often shown this to be true, for many plants in air enriched with CO₂ (e.g. to double the present ambient concentration) show both increased rates of biomass accumulation and greater water economy. When the data available for different plant species were considered together, there was a surprising degree of consistency in the degree of stomatal closure caused by a given increase in CO₂ concentration (Morison, 1987). Most of the early research was done on herbaceous plants, but many different species were used embracing both C₃ and C₄ metabolism, and thus the data base that provided a foundation for general conclusions was by no means a narrow one. Nevertheless, few of the experiments had been performed on trees, and now that we have data for a range of tree species some of the earlier assumptions are being strongly challenged. In many trees small or zero reductions in stomatal conductance have been found in response to CO₂ enrichment, and there are even a few examples of small increases (Norby and O'Neill, 1991; Bunce, 1992; Barton et al., 1993; Dufrière et al., 1993; Gunderson et al., 1993; Ellsworth et al., 1995; Liu and Teskey, 1995; Beerling et al., 1996). Eamus (1996)

pointed out that because atmospheric CO₂ enrichment usually leads to an increase in leaf area, this needs to be accompanied by partial stomatal closure if water consumption by the tree is not to increase. Beerling et al. (1996) and Heath and Kerstiens (1997) found that in the cases both of beech (*F. sylvatica* L.) and birch (*B. pubescens* Ehrh.), the stomatal control of transpiration in elevated CO₂ appeared unable to compensate for the increase in total leaf area. In contrast the stomata of pedunculate oak (*Quercus robur* L.) did close appreciably, the fall in conductance perhaps being sufficient to reduce water consumption per tree in spite of the increase in biomass. Heath and Kerstiens found that the disappearance of the stomatal CO₂ response was associated with hot, sunny days, and further work showed that some loss of stomatal sensitivity to vapour pressure deficit under these conditions might be responsible (Heath, 1997). Heath et al. (1997) measured shoot hydraulic conductance of beech and oak after 2 years of growth in ambient air or in 600 ppm CO₂, and found a reduction in oak but not in beech. This clearly reflected the difference between the two species in transpirational-water flux in elevated CO₂.

These new research findings are beginning to change quite dramatically our opinions about the relationship between terrestrial plants and atmospheric CO₂. The issues extend far beyond questions about the ability of individual species to survive in a CO₂-enriched atmosphere. The continuance of favourable water relations is essential if forest ecosystems are to take advantage of atmospheric CO₂-enrichment, and thus contribute to the terrestrial sink for anthropogenic CO₂. The role of forests in the global carbon cycle is important in this context because of the capacity of their standing biomass and the long life span of the dominant components. If correctly conserved and managed, they have the potential to sequester and store large quantities of carbon from the atmosphere (Grace et al., 1995). There are also many implications for the modelling of future climate. Suppression of transpiration could result in reduced relative humidity, cloud cover and rainfall, and increasing temperatures (Lockwood, 1995). Sellers et al. (1996) used a coupled biosphere-atmosphere model and concluded that the expected reduction in evapotranspiration accompanying a doubling of the present ambient CO₂ concentration would substantially amplify the temperature increase resulting from the atmospheric 'greenhouse' effect.

The vegetational components of models of global environmental change are of considerable significance, and yet the assumptions currently being made about the impacts of elevated CO₂ on stomatal behaviour are little more than conjecture. The discoveries that there are major differences between species, and in particular that many trees display little or no stomatal closure in elevated CO₂, need not cause insurmountable difficulties

for modelling if the underlying causes of differences can be resolved.

4. Conclusions

Terrestrial plants have evolved highly specialized mechanisms to enable them to withstand variations in the rhizospheric and aerial environments. The ability to regulate leaf conductance is of prime importance, and once leaves are fully developed the control exerted by the stomata plays a vital part in maintaining water balance. The stomatal guard cells are remarkably versatile sense organs which detect changes in a wide range of factors of the aerial environment, and also respond to signals from the roots determined by soil moisture status.

Various aspects of stomatal function can be modified by air pollutants, and in some cases the disturbance can be sufficient to cause the plant to lose control of its water relations. There are great variations in response to a given pollutant between species, and even between genotypes of a single species, and some of the differences can probably be attributed to the precise location of initial injury to cells around the substomatal cavities. The greatest threat to the control of stomatal conductance occurs when cells of the epidermis near the guard cells are damaged, but the guard cells themselves are not. This lowers the mechanical resistance when the turgor of the guard cells increases, abnormal stomatal opening being the result, and in some cases only partial closure at night may occur. This phenomenon has been found with both SO₂ and O₃ pollution, and with mixtures of SO₂ and NO₂.

It has been generally assumed that CO₂ pollution should improve the water economy of most land plants, but inadequate attention has been paid to the relationship between CO₂-induced stimulation of leaf area and the ability of stomata to close partially, in a compensatory manner, and regulate total water consumption. There are now many examples of species, especially trees, in which the stomata show little or no closing response to elevated CO₂. This has important implications both for the survival of individual plants, and for modelling those aspects of climate change that depend on the energy relationships between plants and the atmosphere.

Serious injury to plants from air pollution has in the past often been considered to be a local or regional problem. We are now having to consider the possibilities of damage on a broader scale as pollutants such as O₃ become more widespread, alongside the omnipresent CO₂. This can no longer be regarded as an area of marginal interest within plant science. Complex mechanisms of the kind discussed here will only be unravelled by the involvement of those in the mainstream of the subject.

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