Effects of Acid Mist, Ozone and Wind on Norway Spruce

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Contents.

Abstract.

This project was set up to test the hypothesis that treatment with wind will alter pathways for pollutants into the needles of Norway spruce, and will render them more susceptible to air pollution. To test this hypothesis two year old seedlings were treated with either acid mist or ozone in open-top chambers, combined with treatments in a controlled environment wind tunnel. Acid mist was applied at pH = 2.5 and pH = 5.0 as a control. The ozone treatment was designed to fumigate the plants with air containing on average 140 nmol mol^{-1} of ozone, and was compared with a charcoal-filtered control. The wind treatments were applied in a controlled environment wind tunnel, at various times during the year, usually for 48 hours at 16 m s^{-1}, which were compared with a 'no-wind' control.

The main measurements made on the Norway spruce seedlings in this project were of gas exchange and frost hardiness. In 1990 two series of gas exchange measurements were made, shortly before and immediately after the wind treatments, during August and September. In 1991 the plants from the acid mist experiment were measured during August, while the plants from the ozone experiment were measured in November, both after the wind treatments. Frost hardiness was assessed for plants from both pollution experiments in November 1990, while in 1991 only the frost hardiness of the seedlings from the ozone experiment was determined, in early October, immediately after the last wind treatment. Measurements of visible injury, whole plant transpiration rates, chlorophyll concentrations and a destructive harvest, were also carried out, to support the findings of the main experiments.

Treatment with acid mist reduced the maximum assimilation rate in 1990. In 1991, when a better balanced fertilizer was used, no effects were found. The dark respiration, and consequently the light compensation point, was reduced by the acid mist treatment, contrary to expectation, which may indicate damage to repair mechanisms, rendering the plants more susceptible to further pollution treatments. The freezing temperature, killing 50% of the shoots (*LT50*), was reduced by 7 °C in November 1990 in acid mist treated plants. Visible injury to current-year foliage, also recorded in November 1990, was about 20% for the seedlings treated with acid mist at pH = 2.5, while no damage was found on the pH = 5.0 control plants. In 1991, when a different spraying regime was employed, visible damage was so severe that no frost hardiness measurements were carried out. The ozone fumigation produced only limited effects, which suggests that Norway spruce planted in upland Britain is unlikely to be seriously affected by ozone episodes. Assimilation rates were found to be reduced, but only when comparing the response at all photon fluxes, using a combined curves analysis. There was also a reduction in stomatal conductance in September 1990, but this effect had disappeared three weeks later. The stomata of seedlings treated with ozone responded faster to changes in the photon flux, contrary to results reported in the literature, and the cause of this response remains unclear. Like the ozone treatment, the wind treatments had only limited effects on the Norway spruce seedlings. Treatment in the wind tunnel reduced the dark respiration rate and light compensation point of seedlings from the acid mist experiment, but in the ozone experiments no effects were found. Again, this probably indicates impaired repair mechanisms. A significant result of this study was the reduction of the whole plant transpiration rate by the wind treatment in 1990. This was attributed to damage to the root systems, caused by movement of the stem, resulting in a reduced capacity of water uptake from the soil, rendering the seedlings more susceptible to drought.

This study on Norway spruce showed that synergistic interactions, which would have proved the main hypothesis of this study, were only found for a few of the variables measured, probably a consequence of the tough cuticle on this species. The ozone and wind treatments did not interact significantly to produce effects on any of the parameters measured in this study, and therefore the working hypothesis was not substantiated.

This study did suggest that the nutritional status of trees can strongly modify the effects of air pollution. The capacity of trees to absorb and neutralize pollutants is higher when all elements are available in sufficient amounts, compared to deficient growing conditions. This study also demonstrated that high wind speeds may reduce the effects of pollutants on tree foliage, but this depends on the timing of the high winds.

Chapter 1.
Introduction.

Air pollution was recognised as a phytotoxic agent during the 1950s and 1960s around point sources, such as smelters, when Gorham & Gordon (1960, 1963) recorded a drastic decline in the terrestrial and aquatic flora in the immediate vicinity of Sudbury, Ontario, Canada. This damage, however, was only observed on a local scale, and the influence of smelter pollution was found to be negligible some 25 km away from the smelters. The concept of long-range transport of acidity, and effects more than 1000 km away from sources of primary pollutants, was taken to the general public by Svante Odén towards the end of the 1960s, when he pointed to the long-range transport and deposition of acidic substances from central and western Europe to Scandinavia (Odén, 1967, as quoted in Brydges & Wilson, 1991). This was followed in 1971 by a case study to the United Nations by the Swedish Royal Ministries for Foreign Affairs and Agriculture, outlining the need for reductions in emissions on both a local and international scale.

By 1980 it was clear that some lakes and rivers were being acidified by wet and dry deposition of sulphur and nitrogen oxides, resulting in the loss of fish and other aquatic species (Harvey, 1980; Muniz & Leivestad, 1980). In addition, Abrahamsen (1980) found that increased deposition of inorganic nitrogen from the atmosphere increased tree growth, but predicted a similar negative long-term response as that following the application of unbalanced fertilizers, after some delay due to deficiencies in other elements. This predicted decrease in forest health did indeed appear during the early 1980s across large areas in central Europe and eastern North America, and the main emphasis of air pollution research shifted from aquatic systems to forests (Martin, 1986).

There is now a general consensus that forest health has declined during the last few decades across large areas of Central Europe (Skeffington & Roberts, 1985; Krause *et al.*, 1986; Rehfuess, 1987) and eastern North America (McLaughlin, 1985; Johnson, 1987; Peart *et al.*, 1992).

Several hypotheses have been proposed and tested to explain forest decline (Schulze & Freer-Smith, 1991). Extensive damage could for instance be caused by epidemics of one or more biological agents. However, there is no evidence linking specific fungi or insects with the observed widespread decline of forests in Europe and North America. Most research in this field has therefore been concerned with the effects of pollutants on the relationship between plants and pathogens or pests (e.g. Bates *et al.*, 1990; Ranta, 1990; Tiedemann *et al.*, 1990; Bolsinger *et al.*, 1992; Hiltbrunner & Flückiger, 1992), i.e. indirect effects of air pollutants.

Forest decline could be a consequence of forest practice (e.g. change from broadleaf to coniferous forest) and/or of extreme climatic effects (frost, drought, or high winds). If a change from broadleaf to coniferous forest were the main cause, in continental Europe this would have been most pronounced at lower elevations, where Norway spruce (*Picea abies* (L.) Karst.) has been introduced, but the reduction in health of Norway spruce has been associated with higher altitudes (McLaughlin, 1985). Similarly, in North America there has been much less concern that deterioration has occurred in red spruce (*Picea rubens* Sarg.) at low elevations than at high elevations (Peart *et al.*, 1992). A German study, reported by Schulze & Freer-Smith (1991), was unable to link historic records of forest damage with climatic extremes, dismissing the theory that forest decline is primarily caused by incorrect forest practice, or the consequence of extreme climatic conditions. However, extreme climatic conditions or incorrect forest practices can trigger a visible decline, after forests have been weakened by other factors, such as air pollution (McLaughlin, 1985).

There have been numerous reports linking forest decline with anthropogenic pollutants, either through direct effects on above-ground parts of plants, or through indirect effects, such as a change in habitat, in particular the soil condition, or interactions between plants and other organisms. Atmospheric pollutants can occur at concentrations, large enough to affect plants even after short episodes of exposure (acute damage), but much more common is a decline attributable to the cumulative effects of the long-term deposition of anthropogenic pollutants (McLaughlin, 1985), where atmospheric concentrations may be below the level for acute damage, but which are large enough to alter the plants' sensitivity to other stresses, such as drought and frost. To allow comparisons of studies with different exposure regimes, effect studies have been analyzed as dose-response relationships (Darrall, 1989), i.e. for gaseous pollutants such as ozone the concentration multiplied by the duration (Reich, 1987), or for wet deposited pollution the total input expressed in weight or moles per unit land area (Leith *et al.*, 1989). This approach may in due course lead to the determination of critical loads, i.e. "a quantitative estimate of an exposure to one or more pollutants below which significant harmful effects do not occur on specified sensitive elements of the environment, according to present knowledge" (Last & Watling, 1991). This is especially important with acid mist and rain, since sulphur and nitrogen are essential elements to plant growth, and problems are caused more by their amount and chemical form, than by some inherent toxic feature (Brydges & Wilson, 1991).

One common characteristic of many reported declines in tree health is their tendency to occur initially with greater frequency and severity at high elevation sites (McLaughlin, 1985; Rehfuess, 1987), where forests encounter generally more severe climatological conditions than their lower elevation counterparts. Mountainous terrain frequently experiences higher wind speeds (Grace, 1977; Rizzo & Harrington, 1988; Crossley *et al.*, 1992), and higher ozone concentrations than at low altitudes (Reiter & Kanter, 1982; Skärby & Selldén, 1984). Forests at higher altitudes are also subjected to orographically enhanced deposition (Hill *et al.*, 1988), and regular cloud immersion (Chandler *et al.*, 1988; Schmitt, 1988), and commonly consist of conifer species, having a high surface area throughout the year (Lindberg *et al.*, 1988). In addition, abrasive damage caused by high wind speeds more commonly occurs at higher altitudes, resulting in increasing average wind speeds in the canopy. The higher average wind speeds at higher elevations increase atmospheric deposition rates, compared to forests at lower elevations (Lovett & Reiners, 1986).

It is therefore most likely that forest decline can not be attributed to one individual factor, but is a consequence of complex interactions, involving both biotic and abiotic agents, including habitat variables such as wind, drought and frost, which influence tree responses to air pollutants (McLaughlin, 1985; Rehfuess, 1987; Blank *et al.*, 1990*b*; Schulze & Freer-Smith, 1991).

1.1. Physiological Responses of Trees.

The susceptibility or tolerance to air pollutants of the physiological and biochemical features of plants was reviewed by Wolfenden & Mansfield, (1991). Pollution can cause changes in plant-water relations (Eamus *et al.*, 1989*b*; Barnes *et al.*, 1990*a*), primarily by affecting the functioning of the epidermis. There are two ways in which pollution can interfere with the essential role of the epidermis: (1) some workers found damage to the cuticle or its associated waxes, resulting in an increase in permeability to water vapour (Rinallo *et al.*, 1986; Mengel *et al.*, 1989; Percy *et al.*, 1990); and (2) a modification of stomatal behaviour (Barnes *et al.*, 1990*a*).

Air pollutants can alter carbon assimilation (Eamus & Fowler, 1990; McLaughlin & Kohut, 1992), which in turn can influence root growth. Plants generally respond to growth limiting conditions or resources by changing their resource partitioning in favour of the part of the plant that draws most upon the growth limiting part of their environment (Hunt & Nicholls, 1986). This means that an increase in photosynthesis would result in a decrease in shoot to root dry weight ratio, and *vice versa*. The sensitivity of the photosynthetic capacity to air pollutants varies widely with species, and also with environmental conditions (Darrall, 1989), but generally an inhibition of the net photosynthesis is reported after exposure to pollution, possibly due to increased respiration requirements (McLaughlin *et al.*, 1991). A reduced photosynthetic capacity has frequently been found to result in a relative reduction in root growth, due to the reduced availability of assimilates to the root system (McLaughlin & McConathy, 1983).

Air pollution can interfere with the natural processes of winter hardening (Fowler *et al.*, 1989; Sheppard *et al.*, 1993*b*). Damage to Norway spruce trees in central Europe, latent at first, suddenly became evident after a series of severe frosts between 1979 and 1983, when severe air frosts occurred after a series of extended periods of mild weather (Bosch *et al.*, 1986; Rehfuess, 1987). Similarly, winter injury to natural stands of red spruce in the northeastern United States has been relatively common only during the last three decades, and anthropogenic pollutants have frequently been linked with the observed reduction in frost tolerance, although only circumstantial evidence of increases in damage coinciding with increased pollution emission is available (DeHayes, 1992).

High wind speeds, experienced at higher altitudes, can also affect plants water relations, through changes in stomatal behaviour (Dixon & Grace, 1984; MacKerron & Waister, 1985) or growth and partitioning (Russell & Grace, 1979), or by abrading the plant cuticles (Pitcairn & Grace, 1985; Hadley & Smith, 1986) and roots (Rizzo & Harrington, 1988). In turn, malfunctioning stomata, or a damaged cuticle, provide pathways for further pollution damage (van Gardingen *et al.*, 1991).

Climate probably had an important synchronising role at the inception of forest decline in the early 1970s in Europe (Rehfuess, 1987), and possibly in the eastern United States in the 1960s (Johnson, 1987). Subsequently, major increases in forest decline have been associated with dry seasons. This may be a consequence of roots being restricted to upper soil horizons which themselves are responsive to drought. Although no major droughts have occurred in Europe in recent years, forest damage has continued to increase at higher altitudes (the main location of naturally occurring Norway spruce), where cation leaching has continued (Schulze & Freer-Smith, 1991).

The potential effects of pollutants and climatic factors on forests are presented in a diagram in FIGURE 1.1. Direct effects are indicated by large arrows (⇨), and indirect effects (interactions) are represented by dotted lines. This diagram shows that ozone, acid mist, frost and wind can all have direct effects on the above-ground parts of trees. Wind and drought can directly affect the root systems, and acid mist indirectly through acidification of the soil. In addition, ozone and acid mist can alter the effects of frost and wind on tree foliage and of drought on root systems. Similarly, high wind speeds may change the response of the aerial parts of trees to pollution, while drought can reduce the effects of

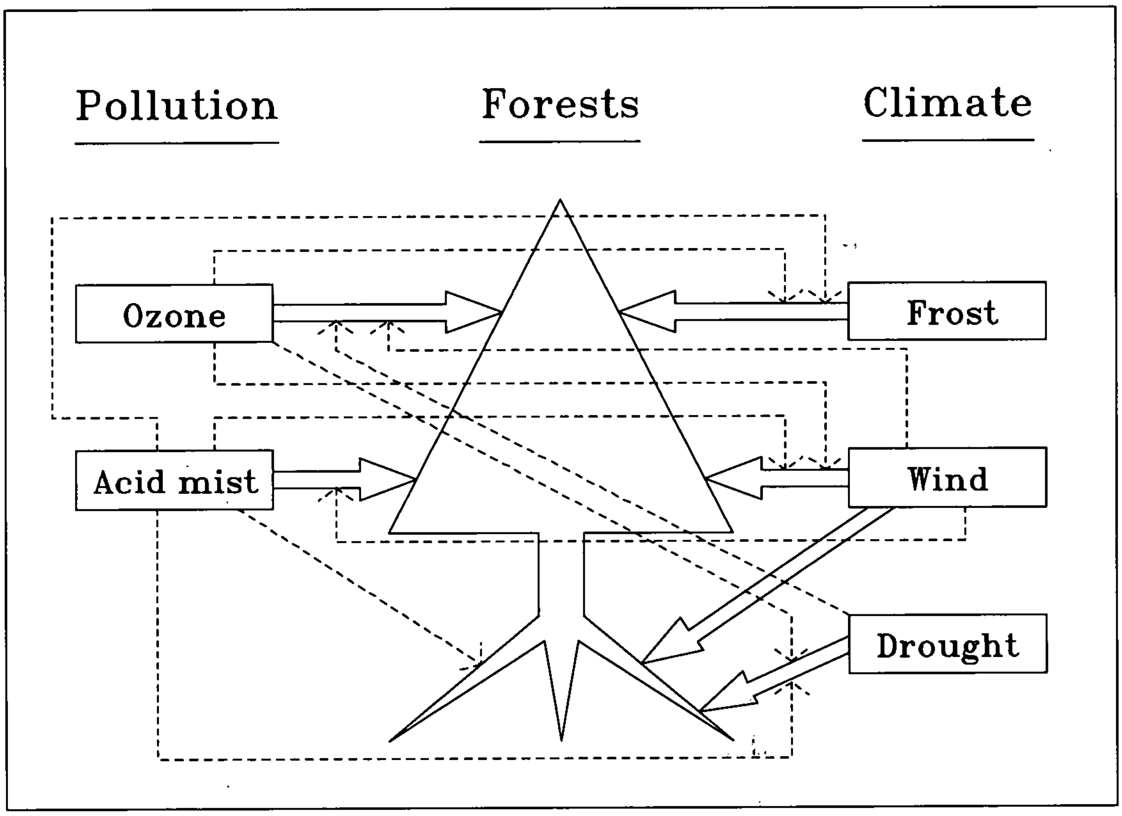


Figure 1.1. Schematic representation of the potential effects and interaction of pollution and climatic factors on forests. (See text for a further explanation.)

ozone.

1.2. Acidic Cloudwater.

The association between altitude and forest health for Norway spruce and silver fir (*Abies alba* Mill.) in the Federal Republic of Germany and other parts of continental Europe, and red spruce in parts of the eastern North America, led to interest in the chemical climate at high altitude sites (Fowler *et al.*, 1991). At most of these sites orographic cloud is common, for example during the 1985-1987 period the mountain summits of the Appalachian Mountains were shrouded in clouds for approximately 30 to 50% of the time (Saxena & Lin, 1990; Mohnen, 1992). In western Britain low cloud is present for some part of about 250 days every year above 400 m a.s.l. (Chandler *et al.*, 1988) and the same was found for the high altitude forests (above 800 m a.s.l.) of the Federal Republic of Germany and other central European countries (Schmitt, 1988). The cloudwater present at these sites generally contains much larger concentrations of the major ions than rain collected at the same sites (Fowler *et al.*, 1988*a*), and is therefore an important carrier for pollutant deposition to high elevation ecosystems (Schlesinger & Reiners, 1974; Dollard *et al.*, 1983; Waldman *et al.*, 1985).

1.2.1. Reported field conditions.

The concentrations of the major ions in rain, SO_4^{2-}, NO_3^-, and NH_4^+ and H^+ (Waldman *et al.*, 1985; Weathers *et al.*, 1988; Mohnen & Kadlecek, 1989; Saxena & Lin, 1990), generally show an increase with altitude when orographic cloud is present, due to the capture of cloud droplet as the rain falls through these hill clouds. Concentrations of the major ions in orographic clouds are generally large compared with typical concentrations in rainfall. Cloud and rain water measurements on the slopes of Great Dun Fell in northern England (847 m) showed differences in the concentrations of the major ions between a factor of 1.5 and 8 (Fowler *et al.*, 1988*b*), while other studies showed even larger differences.

At 800 m altitude in central Germany increases of up to 15 times were found by Schmitt (1988), while Lovett *et al.* (1982) reported concentrations of several important ions in cloud water in northeastern United States to range from 150 to 430 percent of the corresponding concentrations in bulk precipitation. The low-level orographic cloud is readily scavenged by rainfall from higher levels, enhancing rainfall by the seeder-feeder process. Therefore larger concentrations in precipitation reaching the ground may result (Fowler *et al.*, 1988*a*).

Concentrations of SO_4^{2-}, NO_3^-, Cl^-, H^+, NH_4^+, Na^+ and Mg^{2+} in cloud water are, however, strongly dependent on altitude (see FIGURE 1.2). The largest concentrations occur at cloud base (Mohnen, 1992), and decrease with height as the liquid water content of clouds increases with height (Fowler *et al.*, 1988*a*). Between 600 and 847 m at Great Dun Fell, droplets increased in size from 7.5 to 10.0 µm radius, while at the same time concentrations of different ions within the

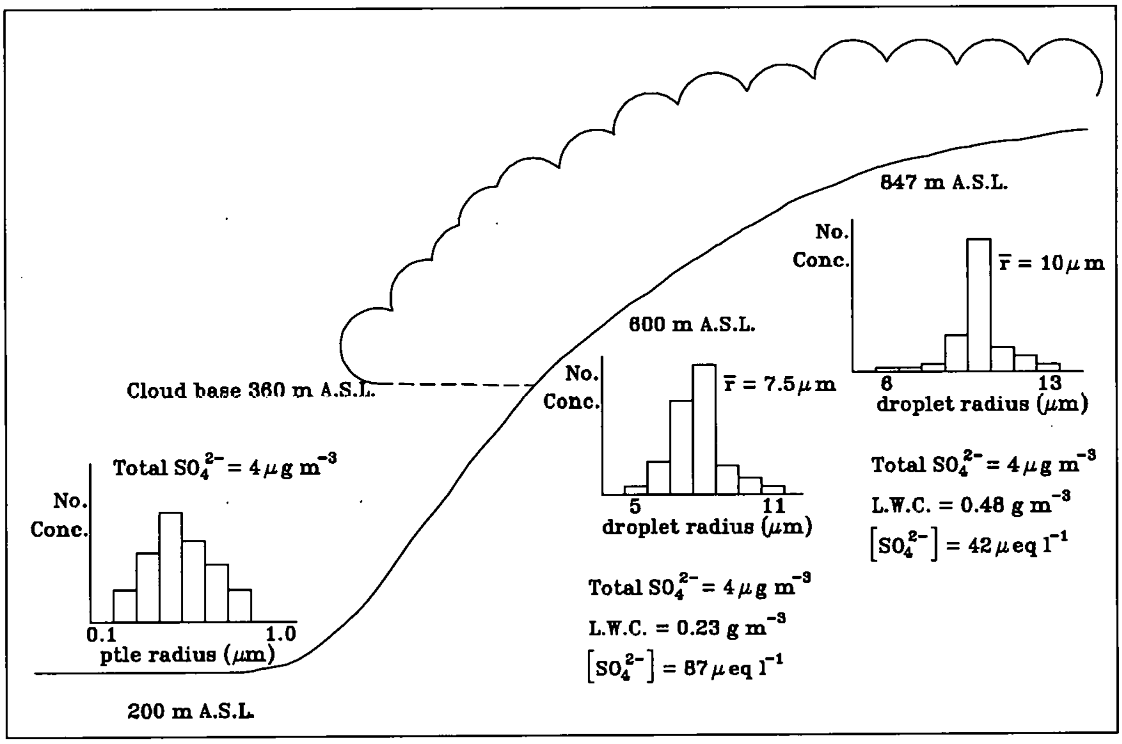


Figure 1.2. An illustration of the changes at Great Dun Fell, U.K., in particle size and ionic concentrations as aerosols are activated into cloud droplets and grow as they are advected up the hillside. L.W.C. = liquid water content of the clouds. (From: Fowler *et al.*, 1991.)

droplets decreased (Fowler *et al.*, 1991).

In many areas the wet deposition of pollutants is episodic, i.e. a large proportion of the annual deposition occurs on relatively few of the wet days during the year (Puxbaum *et al.*, 1988). This episodicity of wet deposition is only poorly correlated with the well-known episodicity of rainfall, but is more readily explained by the air masses previously passing over major sources of air pollutants (Smith & Hunt, 1978). This causes large variations in concentration of the major ions in rain and mist at the same measuring sites. Weathers *et al.* (1988) measured the pH of cloud water at four sites in the eastern United States in 1984 and 1985, and found the acidity ranging from pH = 2.4 to pH = 5.5. Saxena & Lin (1990) prepared a table of reported cloud acidities, which included seven studies of ground-based collections during the last decade, showing a range between pH = 2.1 and pH = 4.9. Schmitt (1988) analyzed cloud water collected at 800 m in the Taunus Mountains in central Germany between the autumn in 1983 and the spring of 1986, and found the pH-values for single events ranging from pH = 2.4 to pH = 7.0. In Britain measurements of ion concentrations in cloud water were reported by Gervat (1985), who found the acidity of precipitating stratocumulus clouds in April 1983 to range from pH = 3.5 to pH = 5.0, and that of low stratus from pH = 2.5 to pH = 2.9. Crossley *et al.* (1992) reported values for cloud water collected at Dunslair Heights in southern Scotland in 1988, with a maximum acidity of pH = 2.9 and an average of pH = 3.8.

The sub-micron aerosols present at low elevations are deposited relatively slowly but after growing into cloud droplets, 4 or 5 µm (radius), they impact quite efficiently on vegetation, and impaction efficiency is also increased at higher wind speeds (Lovett, 1984; Gallagher *et al.*, 1992). This results in a considerable hydrological input at higher altitudes. Lovett *et al.* (1982) estimated the annual gross deposition of liquid cloud water to a subalpine ecosystem in the northern Appalachian Mountains, as 84 cm, compared to an estimated annual precipitation of 180 cm. In upland Britain cloud deposition has been estimated to contribute up to 30% of the wet deposited sulphur and nitrogen (Fowler *et al.*, 1991). However, the total amounts of deposited cloudwater are likely to be less important than the concentrations of major ions for studies of effects on foliage (Fowler *et al.*, 1991), especially as evaporation of the liquid water following cloud water deposition may further increase concentrations and the phytotoxicity of deposited H^+, SO_4^{2-}, NO_3^- and NH_4^+ (Unsworth, 1984; Milne *et al.*, 1988).

1.2.2. Potential effects of acidic cloud water on plants.

It has been known for a considerable time that solute concentrations are larger in cloud droplets than in rain drops (Mrose, 1966), and considerable amounts of cloud water are intercepted by conifers growing at high altitudes (Schlesinger & Reiners, 1974). However, much of the earlier research on air pollution was concerned with gaseous pollutants, such as SO_2, NO_2 and O_3 (Roberts, 1984; Darrall, 1989; Saxe, 1991; Wolfenden & Mansfield, 1991), and most research into effects of wet deposition, acid rain and mist, has started relatively recently.

The effects of wet deposition on trees may be investigated at sites of interest or in controlled experiments in chambers or lab conditions. The first method, assessing plants growing at higher elevations for various parameters, involves correlating findings with the pollution climate as measured at the site(s). In doing so, Katzensteiner *et al.* (1992) found that needle loss, as a measure of tree vigour, was more severe at higher elevations and on west facing slopes, subject to the prevailing winds. Tree vigour was associated with higher inputs of nitrate and sulphate at the exposed sites, resulting from cloud water deposition, and lower soil pH-values. A study at two sites in the Great Smoky Mountains in western North Carolina, USA, one frequently above cloud base (1950 m) and one typically below cloud base (1715 m; McLaughlin & Tjoelker, 1992), indicated a larger decline at the higher elevation site in the radial growth of dominant trees during the last two decades (McLaughlin *et al.*, 1990), a greater relative height growth of sapling-sized trees at the lower elevation site (Andersen & McLaughlin, 1991), and a larger retention of ^{14}C in labelled foliage at the lower site (Andersen *et al.*, 1991). This was explained primarily by higher respiratory losses at the higher site, as the net photosynthesis was generally comparable between sites (McLaughlin *et al.*, 1991), while water stress experienced at the two sites was discarded as a possible explanation for the observed decline.

An alternative approach to investigate the effects of wet deposition on trees is in controlled experiments, where small trees, most frequently seedlings (due to operational problems with larger trees), are exposed to known amounts and concentrations of acid mist or rain, while other parameters potentially influencing tree growth can be kept constant (Unsworth, 1991). Such controlled experiments yield data that are easier to interpret, i.e. any effects found can usually be attributed to a particular treatment, but care must be taken in the extrapolation of the results to the field. Effects found using seedling material may not reflect the response of mature trees in the field. Measurements on mature red spruce trees from the northern Appalachians (Sheppard *et al.*, 1989) and seedling material in open-top chambers (Fowler *et al.*, 1989) showed a similar degree and seasonal development of frost hardiness, and Fincher (1992) reported similar alterations in cellular structure between mid-summer and mid-winter for mature red spruce foliage, and that of seedlings, but these may be exceptions.

Fowler *et al.* (1989) summarized the results of a large experiment in 1987 on the effects of acid mist (at six levels of acidity) on red spruce seedlings. It was shown that the acid mist significantly reduced the frost hardiness, as expressed in lethal temperatures, at acidities of pH = 3.5 and below, compared to the pH = 5.0 control. Another effect found was an increased photosynthesis on a projected needle area basis, when comparing the pH = 2.5 treatment with the control, which was related to an increased chlorophyll content of the needles. When the photosynthesis was expressed per unit chlorophyll, a reduction was found for the high acid treatment (Eamus & Fowler, 1990). No significant differences in biomass accumulation or in height growth between treatments were found, although the partitioning to the roots tended to decrease with increasing acidity of the mist (Deans *et al.*, 1990). However, significant effects were found in root morphology, larger amounts of coarse roots, needed for firm anchorage of trees, were produced in the pH = 5.0 control treatment, and in the production mycorrhizal fruiting bodies of *Thelephora terrestris* Ehrenb., an early successional fungus generally associated with seedlings and young stands, which was significantly reduced in the high acidity treatments (Deans *et al.*, 1990). Leith *et al.* (1989) reported significant effects of the acid mist treatments on foliar injury, with plants treated at pH = 2.5 and pH = 2.7 being significantly more damaged after ten weeks than those sprayed with pH = 3.0 acid mist, which in turn were significantly more damaged than the plants from the remaining three treatments. Damage continued to increase, and six weeks later the plants sprayed with mist at pH = 3.5 were also significantly more damaged than those from the pH = 4.0 and pH = 5.0 treatments, which remained indistinguishable throughout the experiment (Leith *et al.*, 1989). In these experiments effects were related to the acidity of the mist applied, but due to the experimental layout it was not possible to differentiate between effects caused by the concentrations of the different ions present in the mist, i.e. H^+, NH_4^+, SO_4^{2-} and NO_3^-.

Other, similar experiments have been reported, mostly with similar effects. Neufeld *et al.* (1985) found variable effects on photosynthetic rates and visible damage for four broad-leaf species, but foliar injury only after application of acid rain at pH = 2.0. At pH = 3.0 and above no effects were observed. The extent of the damage was related to the wettability of the leaves of the different species, those with low water-holding capacities showing less damage (Haines *et al.*, 1985). McLaughlin & Tjoelker (1992) conducted greenhouse studies to determine the specific effects of simulated acid deposition on growth and physiology of red spruce seedlings. They found that significantly reduced whole-plant and root growth was associated with reduced photosynthesis to dark respiration ratios at higher rain and mist acidities. Acidic rain treatments caused decreases in the number of infected short roots and in the percent mycorrhizal infection in northern red oak (*Quercus rubra* L.; Reich *et al.*, 1985) and Scots pine (*Pinus sylvestris* L.; Dighton & Skeffington, 1987), but Meier *et al.* (1989) found no significant changes in the frequency of ectomycorrhizae on red spruce.

Some workers have examined other parameters. Percy (1983) applied rain at four acidities to three *Pinaceae* species, and demonstrated a statistically significant reduction at pH ≤ 4.6 in the number of primary needles and axillary meristems, as well as a decrease in total seedling height, hypocotyl, and cotyledon elongation in two or more of the four species studied. Percy (1986) studied 11 commercially important temperate tree species, and found substantial differences in their respective sensitivities, when assessed for germinative capacity, seedling survival, macroscopic foliar injury, and growth and morphological parameters. Jacobson *et al.* (1987) also studied a number of variables, such as dry mass of stems, numbers of female flowers produced and dry mass of flowers and immature fruit, and found that foliar symptoms were the most sensitive of all the variables measured.

Numerous other experiments have been carried out during the last seven to ten years, with different backgrounds and different aims. Several workers have tried to identify which of the ions, or which combination of ions found in polluted clouds was most damaging. Generally, mists containing sulphate appear to be more damaging than those containing nitrate. Mist containing sulphate led to higher foliar necrosis and decreased growth in red spruce compared with mist containing nitrate (L'Hirondelle *et al.*, 1992). The alteration of crystalline wax structures of cabbage was similar for leaves treated with nitric acid solutions, but less severe, than for leaves treated with sulphuric acid solutions (Adams *et al.*, 1990). Large effects on the frost hardening process of red spruce were found for mists treatments containing sulphate and ammonium ions, while misting with a nitrate solution had no effect (Cape *et al.*, 1991; Jacobson *et al.*, 1992).

Similar effects on growth have been shown. When cations are readily available nitrogen deposition will stimulate growth without symptoms of damage, but when supplies of cations are limited, additional nitrogen will exacerbate nutrient shortages, causing visible damage, especially if ammonium (instead of nitrate) is utilised (Schulze & Freer-Smith, 1991). L'Hirondelle *et al.* (1992) found that seedlings of low nutrient status were more sensitive than well-fertilized ones, but did not mention in what chemical form the nitrogen fertilization was applied.

1.3. Ozone.

Ozone concentrations in the lower troposphere depend on transport from the free troposphere, i.e. above the planetary boundary layer, and *in situ* photochemical production. This local ozone production mainly results from reactions between nitrogen oxides and volatile hydrocarbons (Ashmore *et al.*, 1985; Saxe, 1991), in the presence of solar radiation (Guicherit & van Dop, 1977; Reiter & Kanter, 1982; PORG, 1987).

In the presence of solar radiation (280 nm < λ < 430 nm) nitrogen dioxide (NO_2) is dissociated into nitric oxide (NO) and an oxygen free radical (O):

$$NO_2+radiation=NO+O \tag{1.1}$$

This oxygen free radical readily reacts with oxygen (O_2) to form ozone (O_3):

$$O+O_2+M=O_3+M \tag{1.2}$$

In the absence of any other reagents, nitric oxide and ozone react again to form nitrogen dioxide and oxygen:

$$O_3+NO=O_2+NO_2 \tag{1.3}$$

However, if nitric oxide is scavenged by other oxidants, such as volatile hydrocarbons, the ozone concentration will increase. This results in a diurnal fluctuation in ozone concentrations in the planetary boundary layer, with peaks occurring during the mid-afternoon (Singh *et al.*, 1978; Martin & Barber, 1981; Ashmore *et al.*, 1985), as ozone is only produced during the day, while at night the ozone is readily depleted due to the presence of nitric oxide or due to dry deposition (PORG, 1987).

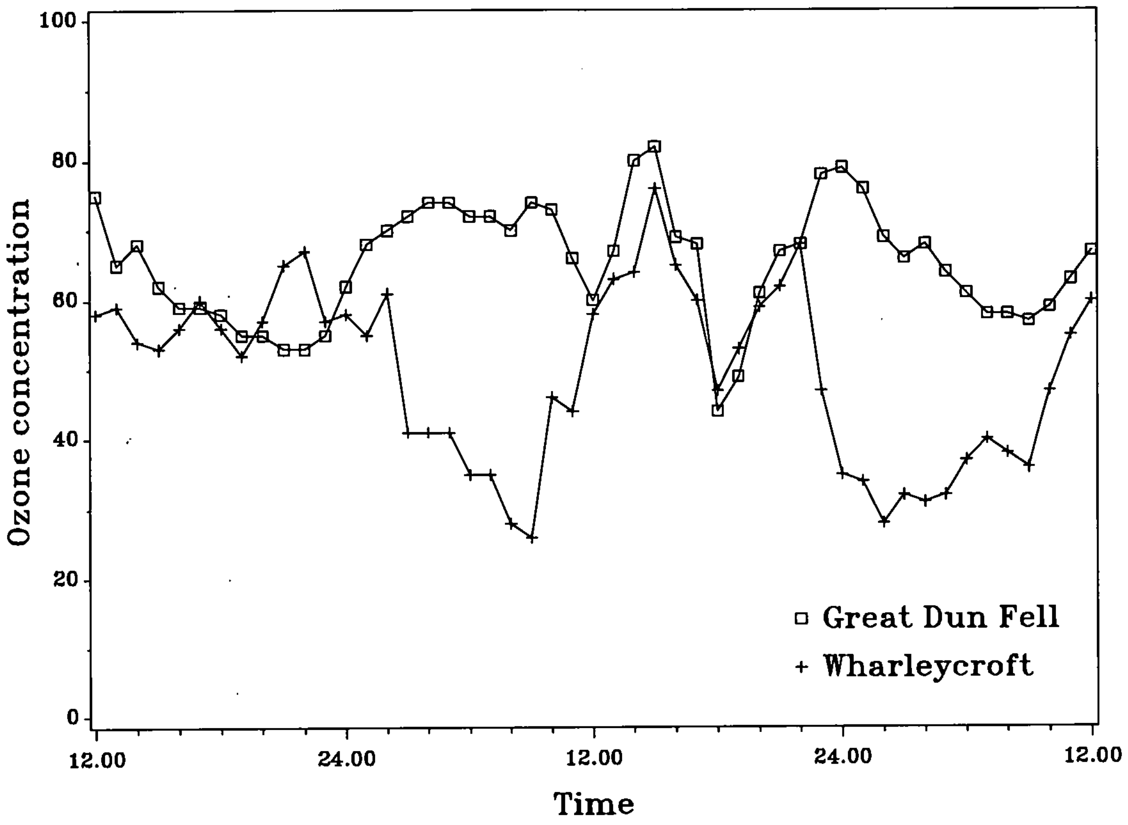


Figure 1.3. Time series of hourly mean ozone concentrations (in nmol mol^{-1}) at Great Dun Fell (847 m a.s.l.) and Wharleycroft (206 m a.s.l.) for 48 hours from noon on 12 May, 1988. (From: Gay, 1991.)

High elevation sites are often above the mixing layer temperature inversion at night (Mohnen, 1992), away from sources of nitric oxide, and ozone concentrations at such sites are therefore more determined by influxes from the free troposphere (Singh *et al.*, 1978; Reiter & Kanter, 1982), and are more constant throughout the day (Broder & Gygax, 1985; Lindberg *et al.*, 1988; Adams & Eagar, 1992). FIGURE 1.3 shows the hourly average ozone concentrations for a 48 hours period, at two monitoring stations, one at Great Dun Fell at 847 m a.s.l., the other at Wharleycroft some 8 km distant, at 206 m a.s.l. (Gay, 1991). This graph shows the diurnal fluctuation found at lower altitudes, together with the more constant level at higher altitudes. Similar to that at higher altitudes, the amplitude in ozone concentrations at lower altitudes, in winter is low (Martin & Barber, 1981; Reiter & Kanter, 1982).

Ozone at concentrations in the range 50 to 500 nmol mol^{-1}, has been known to have negative effects on many different species of vegetation (Miller and Parmeter, 1965; Skärby & Selldén, 1984; Ashmore *et al.*, 1985; Reich & Amundson, 1985). Concentrations have exceeded natural background concentrations on most days during the growing season, in central and northwestern Europe, in the United States and Canada, and especially in southern California (Reich, 1987), although it is difficult to establish what "natural background concentrations" are (Singh *et al.*, 1978). Saxe (1991) estimated that the ozone concentrations, due to anthropogenic influences, have doubled from about 10 to 15 nmol mol^{-1} at the end of the last century to the present 20 to 30 nmol mol^{-1}.

Due to practical constraints, most research has focused on short-term effects, simulating the ozone exposure at low-elevation sites, although it is generally accepted that exposure to ozone is greater at high-elevation sites, where maximum concentrations are not vastly different, but the diurnal fluctuations, typical of low-elevation sites, are small (Adams & Eagar, 1992).

1.3.1. Reported field conditions.

At remote sites, away from sources of nitrogen oxides, maximum concentration of ozone are generally found during early spring (Derwent *et al.*, 1978; Singh *et al.*, 1978; Martin & Barber, 1981), suggesting a stratospheric source rather than photochemical production (Singh *et al.*, 1978). Exchange between the stratosphere and the troposphere is most effective during late winter and spring, out of phase with the major cycle in photochemical tropospheric ozone production (Logan, 1985). Reiter and Kanter (1982), on the other hand, reported that ozone from the stratosphere rarely reaches the lower troposphere, and that the influence of the stratospheric ozone pool is found only at higher altitudes, well above the planetary boundary layer. However, most of the sites used by Singh *et al.* (1978) are located at high elevations, while the events reported by Derwent *et al.* (1978) and Martin & Barber (1981) were linked with influxes from the stratosphere.

Maximum levels are on average found during the afternoon, but can occur at other times, and concentrations of up to 80 nmol mol^{-1} occur occasionally.

Higher ozone levels, due to anthropogenic production, occur downwind of primary pollutant sources (Apling *et al.*, 1977), where nitrogen oxides and volatile hydrocarbons react in the presence of solar radiation to form ozone (Guicherit & van Dop, 1977; Ashmore *et al.*, 1985). Meteorological parameters favourable for photochemical ozone production include atmospheric stability, high insolation and temperatures, and low wind speeds (Guicherit & van Dop, 1977). Such conditions are generally associated with an anticyclone over northern Europe (Derwent *et al.*, 1978; PORG, 1987). This provides clear skies, high temperatures and modest wind speeds moving air masses which have crossed the major source areas of precursor gases in central Europe (Apling *et al.*, 1977).

From systematic measurements carried out in the Netherlands during the late 1960s and early 1970s, it has been established that hourly recorded maximum ozone concentrations may exceed 200 nmol mol^{-1} (Wisse & Velds, 1970; Guicherit *et al.*, 1972). During the dry summer of 1976 this anthropogenic production resulted in maximum one-hour mean concentrations exceeding 150 nmol mol^{-1} in the Rhine valley (Skärby & Selldén, 1984), while in Britain urban levels exceeding 200 nmol mol^{-1} and rural levels exceeding 250 nmol mol^{-1} were measured (Apling *et al.*, 1977). However, concentrations in 1976 were exceptional, and maximum hourly levels between 120 and 160 nmol mol^{-1} are more common (Harrison & Holman, 1979; Martin & Barber, 1981).

Similar maximum hourly concentrations of ozone of up to about 150 nmol mol^{-1} have been reported for eastern North America. Vukovich *et al.* (1977) provided monitoring data for 1973 to 1975, and showed maximum area-averaged concentrations across most of the northeastern United States of about 130 to 140 nmol mol^{-1} in both 1973 and 1974. During 1987, the highest hourly mean ambient ozone concentration measured in Duke Forest, Durham, North Carolina, was 135 nmol mol^{-1}, with only a few days with maximum concentrations over 100 nmol mol^{-1} (Richardson *et al.*, 1992). At the same site in 1988 the maximum hourly concentration was 125 nmol mol^{-1}, but ozone levels exceeded 100 nmol mol^{-1} more often than in 1987 (Sasek *et al.*, 1991). Wolff *et al.* (1977) did not give maximum concentrations, but showed ozone concentrations to be higher than 80 nmol mol^{-1} across a very extensive area of the northeastern United States, and therefore the maximum concentrations will have been considerably higher than that at some locations. Meagher *et al.* (1987) reported on long-term monitoring at five sites in the southeastern United States, where maximum recorded levels were in the range of 120 to 140 nmol mol^{-1}, with one exception at 166 nmol mol^{-1} at one site during 1980.

Ozone concentrations in southern California, where a large industrial area frequently experiences meteorological conditions conducive to photochemical ozone production, are known to reach much higher levels (Tilton & Meeks, 1989). Measurements in the Los Angeles air basin during photochemical smog episodes have shown surface oxidant concentrations of over 500 nmol mol^{-1} (Blumenthal *et al.*, 1978). In this study oxidants appeared to be used as a synonym for ozone. Similar surface levels of over 500 nmol mol^{-1} of ozone were calculated by Peterson & Demerjian (1976), using a three-dimensional photochemical diffusion model.

Interestingly, it is not just Europe and North America that suffer from high ozone concentrations. Measurements in Baghdad, a growing industrial city in a developing country, with a similar latitude and topography to the Los Angeles basin, showed monthly averages of around 100 nmol mol^{-1}, with a maximum recorded value of 285 nmol mol^{-1} (Kanbour *et al.*, 1987).

1.3.2. Potential effects of ozone on plants.

Most of the earlier air pollution research has concerned gaseous pollutants, including photochemical oxidants. Some studies have looked at oxidants other than ozone, such as peroxyacetyl nitrate (PAN; Tilton & Meeks, 1989; Okano *et al.*, 1990), hydrogen peroxide (Chandler *et al.*, 1988; Dollard *et al.*, 1988) and formaldehyde (Altshuller & McPherson, 1963), but virtually all research on photochemical oxidants has concentrated on ozone, as this is the most important photo-oxidant in relation to plant injury (Saxe, 1991)

The effects of ozone have been studied on a variety of processes at all levels of plant function (Reich, 1987; Kickert & Krupa, 1990), including photosynthesis (Wallin *et al.*, 1992*b*), dark respiration (Wallin *et al.*, 1990), stomatal conductance (Reich *et al.*, 1985), growth and carbon allocation (McLaughlin & McConathy, 1983), visible leaf injury (Smith *et al.*, 1990), chlorophyll content (Brown *et al.*, 1987), leaf aging (Keller, 1988), and frost hardiness (Barnes & Davison, 1988).

Low concentrations of ozone well within the ambient range have been found to cause disturbances to photosynthesis (Wolfenden & Mansfield, 1991), including reductions in the activity of ribulose bisphosphate carboxylase/oxygenase (Rubisco; Lehnherr *et al.*, 1988; Dann & Pell, 1989) and in light saturation and apparent quantum yield (Reich 1983; Wallin *et al.*, 1992*b*). The sensitivity of photosynthesis to air pollutants varies widely between species and also with environmental conditions (Darrall, 1989). In general, an equivalent dose within a single growing season affects agricultural crops more than hardwood species, which in turn are more sensitive than conifers (Reich, 1987). Reductions in photosynthesis across a range of species depend linearly on the exposure time and ozone concentrations, in plants that are not water-stressed, i.e. the ozone dose (Amiro *et al.*, 1984; Reich & Amundson, 1985). This is shown in FIGURE 1.4, which is taken from the review by Reich (1987), and present the pooled data of reductions in photosynthesis against the ozone dose (FIGURE 1.4a), and ozone absorbed by the foliage, relative to its lifespan (FIGURE 1.4b). This shows that even when species as diverse as conifers (mostly pines) and agricultural crops are pooled, there is a good correlation between the reduction in photosynthesis and the ozone absorbed by the foliage. Whether this relationship should be

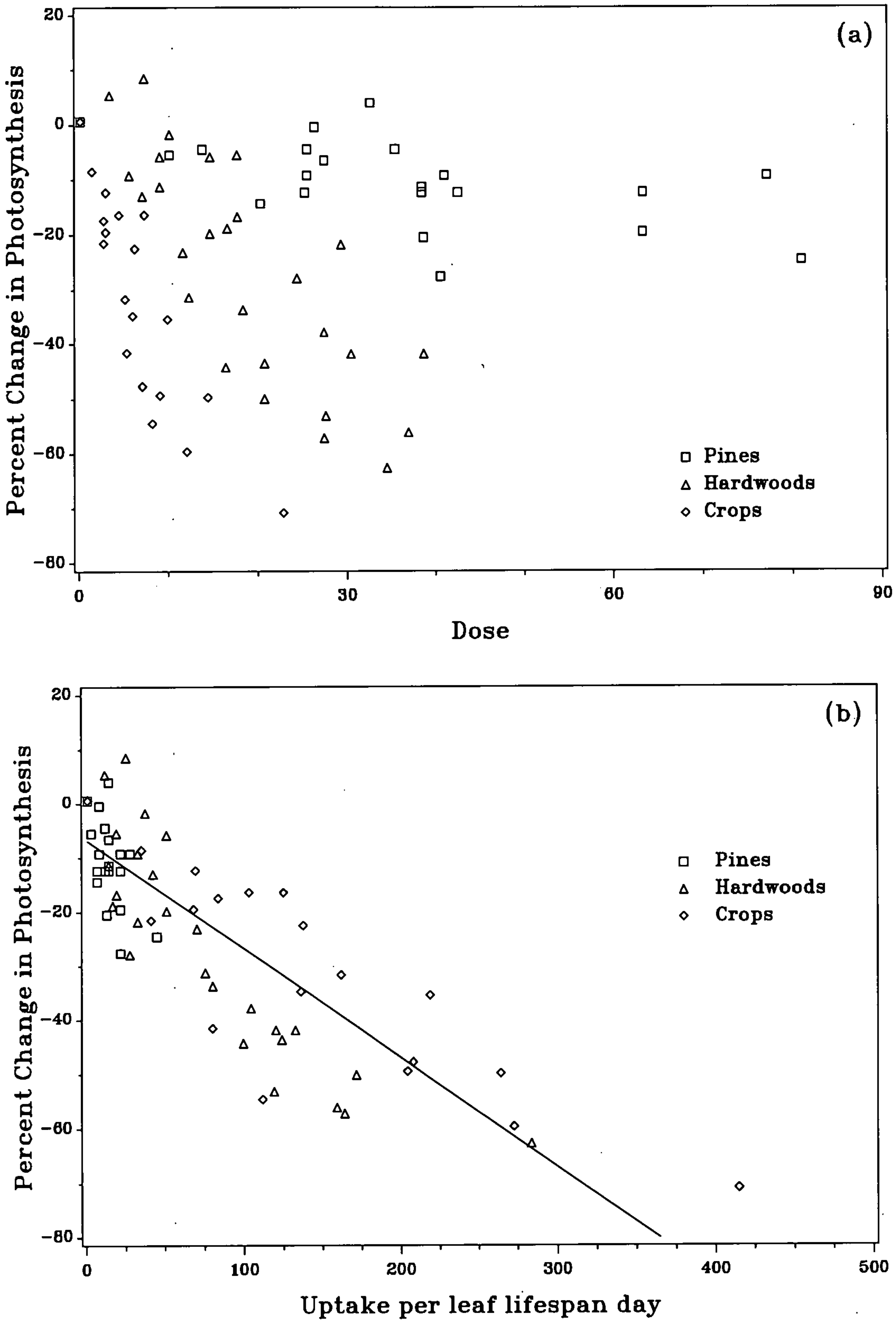


Figure 1.4. Percent reduction in the net photosynthesis of conifers, hardwoods and agricultural crops in relation to (a) the total ozone dose (in ppm-h), and (b) the ozone uptake divided by days in the leaf lifespan (in μg cm^{-2} day^{-1}). (From: Reich, 1987.)

represented by a straight line remains to be established. The line fitted by Reich would indicate a reduction of about 7% when no ozone is absorbed, which would suggest that either a non-linear regression should be used, or that other parameters, other than the measured ozone concentrations had been altered by the treatments.

There have been relatively few reports of effects of ozone on dark respiration, but in general respiration seems to increase in response to fumigation with gaseous air pollutants, including ozone (Reich, 1983; Skärby *et al.*, 1987; Wallin *et al.*, 1990; Wallin *et al.*, 1992*b*), above a threshold concentration (Darrall, 1989). This increase is likely to reflect both the activity of repair processes and a direct effect on the rates of maintenance respiration (Darrall, 1989). Not all studies have measured significant responses. Wallin *et al.* (1992*a*) found no significant response in dark respiration, while decreases in dark respiration have also been reported (Yang *et al.*, 1983; Lehnherr *et al.*, 1988).

Changes in stomatal opening have been frequently reported in response to various air pollutants, including ozone. At concentrations below 200 nmol mol^{-1} a diversity of stomatal responses has been reported (see review by Darrall, 1989). Usually the stomatal conductance is unaffected by ozone, except for a decline paralleling that in photosynthesis (Reich *et al.*, 1985; Temple, 1986; Temple *et al.*, 1988; Richardson *et al.*, 1992). Several authors concluded that ozone inhibits photosynthesis directly, rather than cause stomata to close (with the increased diffusive resistance limiting CO_2 uptake), probably causing stomatal closure in response to increased internal CO_2 concentrations (Reich *et al.*, 1985; Temple, 1986; Sasek *et al.*, 1991). However, changes occur during exposure to ozone. Walmsley *et al.* (1980) found an acclimation of stomatal resistance in radish (*Raphanus sativus* L.) in response to long-term ozone exposure, so that leaves developed under the exposure regime were less affected by ozone. Similarly, beech (*Fagus sylvatica* L.) leaves which had expanded in May, during the first flush of shoot growth, had smaller stomatal conductances in polluted (unfiltered) air than in clean filtered air while the conductance of leaves of the second, or lammas, flush of growth was larger in polluted than in clean air (Taylor & Dobson, 1989).

Studies of responses of plants to ozone have also suggested that dry matter partitioning may be strongly affected (Wolfenden & Mansfield, 1991). Initially root growth appears to be most affected (McLaughlin & McConathy, 1983; Smith *et al.*, 1990), but after prolonged exposure growth of all plant parts is reduced (Temple *et al.*, 1988; Fernandez-Bayon & Ollerenshaw, 1990; Smith *et al.*, 1990), though root growth remains the most affected, causing changes in the shoot-root ratios (Darrall, 1989).

Visible symptoms of leaf damage, frequently linked with early senescence, following fumigation with ozone have been shown for many crop species (e.g. Smith *et al.*, 1990; Ojanperä *et al.*, 1992), but tree species, and especially conifers have been found to show less visible damage (Sasek & Richardson, 1989; Sutinen *et al.*, 1990). Brown *et al.* (1987) reported visible injury, in the form of severe, uniform brown necrosis and shedding of needles, to occur only after a frost in late autumn.

Treatments with elevated ozone concentrations have been shown to affect the chlorophyll content of plant foliage. Some studies found increases in chlorophyll content (Reich *et al.*, 1986), but generally reductions have been reported (Brown *et al.*, 1987; Wallin *et al.*, 1990), although the effects have been shown to depend on the physiological growth stage of the plant material investigated (Smith *et al.*, 1990). The effects of ozone were similar on chlorophyll *a* and *b* (Richardson *et al.*, 1992; Wallin *et al.*, 1992*a*). In Norway spruce, changes in chlorophyll content are only found after prolonged exposure to elevated concentrations of ozone (Wallin *et al.*, 1990; Wallin *et al.*, 1992*a*), or after fumigation at higher ozone concentrations (Brown *et al.*, 1987).

Results obtained by a number of researchers have indicated that fumigation with ozone accelerates the ageing processes in plants. This has been reported as early senescence and drop of poplar (*Populus spp.*) leaves (Reich *et al.*, 1984; Reich & Lassoie, 1985; Keller, 1988), birch (*Betula pubescens* Ehrh. and *B. verrucosa* Ehrh.) leaves (Mortensen & Skre, 1990) and ponderosa pine (*Pinus ponderosa* Laws.) needles (Coyne & Bingham, 1982), a reduction in the quantity of ribulose bisphosphate carboxylase/oxygenase (Rubisco), as an important foliar storage protein (Dann & Pell, 1989), or severe chlorosis and wilting of spring wheat (*Triticum aestivum* L.) flag leaves (Ojanperä *et al.*, 1992).

Early senescence often brought on by ozone, together with increased rates of dark respiration, may impose a limit on the resources needed for normal winter hardening (Wolfenden & Mansfield, 1991). Increases in susceptibility to frost in spruce following exposure to ozone have been observed (Barnes & Davison, 1988; Cape *et al.*, 1990). Contrasting reports showing no effect of ozone exposure on the frost hardiness (Lucas & Penuelas, 1990), were associated with considerably lower ozone concentrations. Evidence that the timing, rather than the degree of frost-hardening process may be disrupted was provided by Lucas *et al.* (1988), who observed a dose related increase in frost damage of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) during early autumn, after exposure to ozone some weeks earlier. By late autumn all shoots appeared to have hardened equally to freezing temperatures.

Keller & Häsler (1984, 1987) found that the stomata of ozone fumigated Norway spruce needles became 'sluggish' in their closing response to darkness. They interpreted this as a latent injury that increased the risk of injury by drought, which has often been observed or suspected in areas with air pollution, particularly on days with rapidly changing light conditions (clouds). Similarly, Barnes *et al.* (1990*c*) recorded a slower stomatal response in increasing water deficit in needles previously exposed to ozone. Temple (1986), however, did not find any 'sluggishness' in the stomatal light response in the field-grown cotton (*Gossypium hirsutum* L.) exposed to a full season of ozone.

Field and laboratory studies have established that plant water status can strongly influence plant responses to ozone (Temple *et al.*, 1988; Meier *et al.*, 1990). Drought-stressed plants have generally shown reduced responses to ozone, presumably due to decreased stomatal conductance and lower rates of gas exchange (Tingey & Hogsett, 1985). For field-grown cotton, a species capable of maintaining turgor under drought stress, this reduction in response to ozone was only found under severe water stress (Temple *et al.*, 1988), further supporting the hypothesis that stomatal conductance is the major controlling variable.

There are examples of photosynthesis being inhibited with no significant effects on stomata (Lehnherr *et al.*, 1988), of stomata being affected with no effect on photosynthesis (Skärby *et al.*, 1987; Greitner & Winner, 1989; Eamus & Murray, 1991), of equivalent and synchronous inhibitions (Wallin *et al.*, 1990; Wallin *et al.*, 1992*a*), and of photosynthesis of younger leaves, developed during ozone fumigation, being unaffected by ozone, unlike older leaves on the same plants, indicating some form of adaptation to high ozone concentrations (Walmsley *et al.*, 1980). Reich (1983) reported both photosynthesis and dark respiration to be negatively affected by chronic ozone exposure, but at different times of the leaves' life cycle. Therefore, photosynthesis, transpiration and dark respiration can each be inhibited separately, without effects on the others, indicating that ozone affects plants at a multiplicity of sites (Saxe, 1991).

A very reactive pollutant, such as ozone, might be expected to react with cuticular constituents (Wolfenden & Mansfield, 1991), but typical ambient concentrations of ozone do not appear to have any effect on the ability of water to penetrate the cuticle (Barnes & Davison, 1988), which would increase when ozone reacts with cuticular components (Kerstiens & Lendzian 1989*b*). Therefore, it is generally agreed that the phytotoxic action of ozone occurs after the passage of atmospheric ozone through the stomatal pore into the substomatal cavity of the leaf interior (Reich, 1987), with minimum canopy resistances between 50 and 100 s m^{-1} for crop plants (Leuning *et al.*, 1979; Wesely *et al.*, 1982). This agrees well with laboratory studies, showing that cuticular ozone uptake is at least four orders of magnitude smaller than the flux through open stomata (Kerstiens & Lendzian, 1989*a*).

Biological response of plants to ozone stress is dependent on a number of factors, including species (Cooley & Manning, 1987; Adams & Eagar, 1992) and cultivar (Barnes *et al.*, 1988; Richardson *et al.*, 1992), developmental stage (Cooley & Manning, 1987; Krupa & Kickert, 1987), and environmental conditions, such as drought (Tingey & Hogsett, 1985). In addition, both average ozone concentrations (Hogsett *et al.*, 1985; Cooley & Manning, 1987) and peak concentrations are important in eliciting plant responses (Hogsett *et al.*, 1985), while the extent of damage induced often depends on the exposure dose (Reich, 1987). Lee *et al.* (1988) concluded that while no single index was deemed 'best' in relating plant response to ozone exposure, the top-performing exposure indices were those that (1) cumulate the hourly concentrations over time, (2) emphasize concentrations of 60 nmol mol^{-1} and higher, and (3) phenologically weight the exposure such that greatest weight occurs during the plant growth stage.

1.4. Wind.

There are two ways in which wind can affect trees at higher altitudes (Reiners & Lang, 1979; Grace, 1989*b*), short periods of extremely strong winds (acute wind stress), or prolonged exposure to moderately high wind speeds (chronic wind stress).

Extremely strong winds (e.g. tropical storms, hurricanes) generally result in damage on a large scale, uprooting trees or breaking stems, generally 1 - 5 m from the ground (Putz *et al.*, 1983; Foster, 1988*a*), and studies in naturally regenerated forests in the northeastern United States suggest that catastrophic wind damage from hurricanes and avalanches has generated much of the recent structural patterns and dynamics of the upland forests (Henry & Swan, 1974; Reiners & Lang, 1979).

The second mode by which wind may cause damage to trees, chronic wind stress due to prolonged exposure to moderately high wind speeds, is more subtle, but can exert equally strong effects in the long run (Wilson, 1980; Peart *et al.*, 1992). The occurrence of tree lines is generally accepted to be caused by the local microclimate (Reiners & Lang, 1979), but various hypotheses have been reported. Usually the summer temperatures are thought to be of key importance, and a mean temperature of 10 °C for at least one month is often quoted as a minimum requirement for tree growth (Pears, 1972; Grace, 1989*b*).However, the higher wind speeds at higher altitudes will affect the temperature regime of plants, by reducing the foliage temperature which can be considerably higher than air temperature in sheltered conditions. Wind can also abrade plant cuticles (Hadley & Smith, 1983; Hadley & Smith, 1986; Grace, 1989*b*), causing desiccation in winter when the soil is frozen, and lost water can not be replenished (Grace, 1989*b*).

1.4.1. Reported field conditions.

Wind speeds generally increase with altitude (e.g. Crossley *et al.*, 1992), and the highest recorded wind speeds have been at the summits of high mountains (e.g. Bliss, 1963). This is shown in FIGURE 1.5, which gives the exposure at six stations in the western Cairngorms, Scotland (Pears, 1972). Exposure was assessed with tatter flags, and although exposure is an unsatisfactory measure, and only altitude data are available for the six stations, it is clear from FIGURE 1.5 that the average wind speeds were considerably higher at higher altitudes.

The maximum wind speed found reported in the literature was more than 100 m s^{-1} on Mt. Washington, New Hampshire, USA (1917 m), where winds in excess of 45 m s^{-1} have occurred in every month for 29 years between 1932 and 1962, and the mean wind speed was 16 m s^{-1} (Bliss, 1963). Saxena & Lin (1990) reported monthly average wind speeds on Mt. Mitchell, North Carolina, USA (2038 m), to be between 5 and 8 m s^{-1} during the summers of 1986 and 1987, while it is known that wind velocities are typically much higher in winter (Hadley & Smith, 1983;

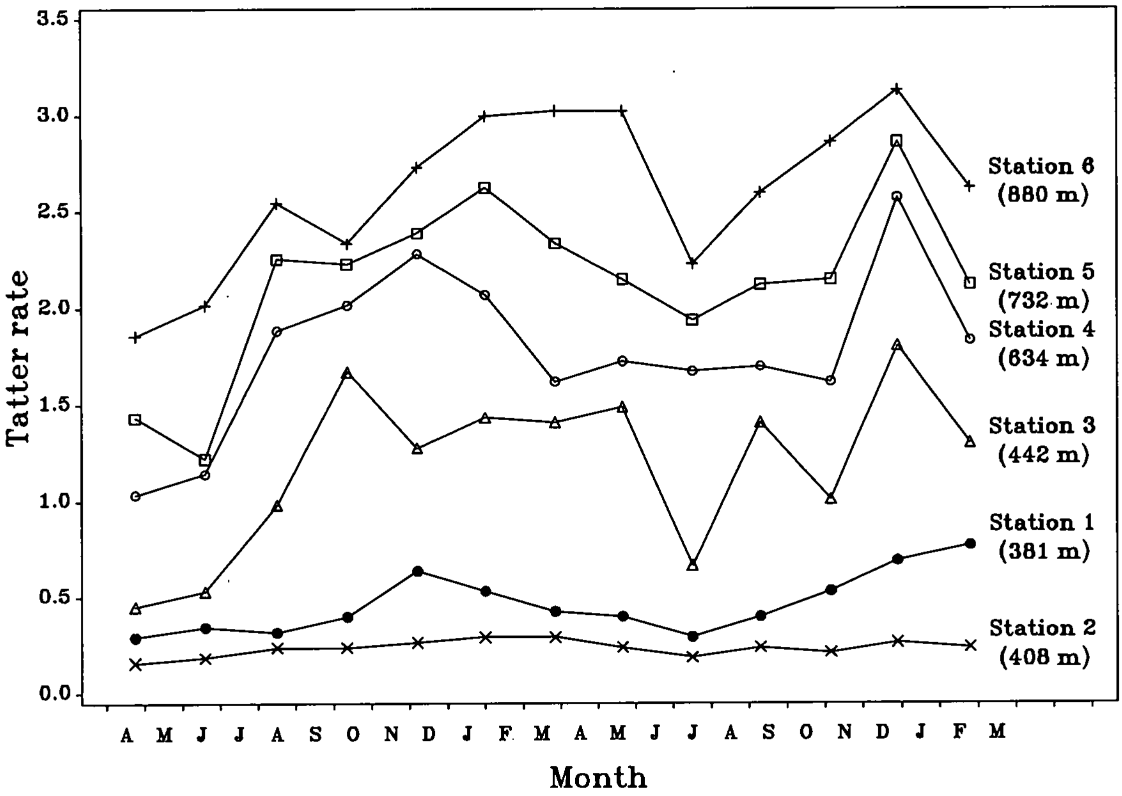


Figure 1.5. Assessment of wind exposure (in square inches of flag erosion per day) in the western Cairngorms, Scotland, for two years from March 1961. (From: Pears, 1972.)

see also FIGURE 1.5). Similarly, Crossley (1988) measured the wind speed on Castlelaw Hill (480 m) in southern Scotland during the summer of 1986, and found the average to be in excess of 10 m s^{-1}.

In 1988 the maximum monthly wind speed (20 minute averages) measured at a site at 602 m altitude at Dunslair Heights in southern Scotland was about 19 m s^{-1}, while at the same time the maximum recorded wind speed at the lower site at 275 m elevation was about 6 m s^{-1} (Crossley *et al.*, 1992). Weathers *et al.* (1988) reported wind speeds in the eastern United States to vary typically from less than 1 m s^{-1} in Bar Harbour in Maine, USA (at sealevel) to greater than 40 m s^{-1} Mt. Washington. Harrington (1986) estimated the mean annual wind speed at the summit of Wildcat Mountain, New Hampshire (1346 m) at 9.7 m s^{-1}, and compared this with wind speeds of over 50 m s^{-1} recorded on nearby Mt. Washington. In September 1985 a peak gust of 22.3 m s^{-1} was measured at Wildcat

Mountain, while on the same day the average wind speed on Mt. Washington was 21.9 m s^{-1} (Rizzo & Harrington, 1988), indicating the difference in wind exposure.

1.4.2. Potential effects of wind on plants.

Wind may influence biological processes in many ways (see van Gardingen & Grace (1991) for a review). Plant water relations may be modified (MacKerron & Waister, 1985), winds can cause damage to foliage (Wilson, 1980) and roots (Rizzo & Harrington, 1988), and consequently other parameters may be affected, such as photosynthesis (Caldwell, 1970), dark respiration (Russell & Grace, 1978*a*) and growth and assimilate partitioning (Russell & Grace, 1979).

Photosynthetic rates are not expected to change due to direct effects of exposure to high wind speeds (Russell & Grace, 1978*b*; Russell & Grace, 1979), but changes in stomatal conductance due to wind treatments will also affect the assimilation rates, as will changes in needle display to available radiation. Tranquillini (1969), in a comparative study over a wind speed range extending to 20 m s^{-1}, concluded that Norway spruce and larch (*Larix decidua* Mill.) were little affected by wind, but that in rowan (*Sorbus aucuparia* L.), Arolla pine (*Pinus cembra* L.) and rhododendron (*Rhododendron ferrugineum* L.) the rates of transpiration and net photosynthesis were greatly reduced at high wind speeds. Caldwell (1970) came to the same conclusion after exposing rhododendron and Arolla pine seedlings to winds at 15 m s^{-1}. However, the reduction in photosynthesis was also attributed to the substantial change in needle orientation (Caldwell, 1970). In addition, photosynthesis may be reduced following a treatment in a wind tunnel, because of a high mesophyll resistance due to a reduced water content (Grace & Thompson, 1973).

Little information is available on the effect of wind on the dark respiration. Those reports available indicate an increased dark respiration with increased wind speeds (Todd *et al.*, 1972; Russell & Grace, 1978*a*), although the underlying mechanisms were not investigated.

In contrast to the limited research on wind effects on dark respiration, the effects of high wind speeds on the stomatal conductance and transpiration rates have been investigated extensively. Wind is unlikely to affect the cuticle water loss unless there is cuticle abrasion (Hadley & Smith, 1986), but the cuticle may be damaged by abrasive particles carried by the wind, or by rubbing against other plant parts as the plant moves with the wind. Initially, damage is limited to the disruption of the epicuticular waxes (Grace, 1974; Thompson, 1974), but even this may have a major effect on the transpiration rate in some species (Pitcairn *et al.*, 1986). With more severe damage the epidermis may itself be breached, causing a very large increase in the "cuticular" conductance (Russell & Grace, 1978*a*; van Gardingen *et al.*, 1991). In contrast to the grasses used by Grace (1974) and Thompson (1974), tough-leaved conifers are less affected by severe treatments in the wind tunnel, their epicuticular wax apparently being only a small part of the total resistance to diffusion of gases (Caldwell, 1970; Grace *et al.*, 1975; Rees & Grace, 1981; van Gardingen *et al.*, 1991).

An increase in wind speeds may modify plant water relations in several ways (MacKerron & Waister, 1985). At higher wind speeds, trees are more closely coupled to the atmosphere, which results in a decreased boundary layer resistance, and a decrease in the leaf to air temperature difference (Grace, 1989*b*). High wind speeds have also been shown to cause changes in the stomatal conductance. Both increases and decreases in transpiration rates have been reported. At lower radiation levels increased wind speed almost always increased the estimated evaporation and only at the highest radiation levels were there a large number of occasions when increased wind speed led to reduced evaporation (Dixon & Grace, 1984; MacKerron & Waister, 1985), possibly due to the partial closure of stomata in response to wind speed (Grace *et al.*, 1975).

Growth and assimilate partitioning have also been shown to be affected by exposure to high wind speeds. The rate of leaf extension of tall fescue (*Festuca arundicacea* Schreb.) and perennial ryegrass (*Lolium perenne* L.) was reduced when the plants were exposed to high wind speed (Russell & Grace, 1978*b*), as was the extension growth of shoots and needles of lodgepole pine (*Pinus contorta* Dougl.; Rees & Grace, 1980). Russell & Grace (1979) demonstrated that wind may affect the distribution of assimilates between the root and shoot, or within the leaf itself.

High wind speeds can cause visible damage to plant material, initially to foliage, but ultimately virtually all plant material is susceptible to extremely high wind speeds, causing damage to forests by breaking or uprooting stems. Although the relationship between wind damage and age is quite different for conifers and broad-leaved trees, in both cases the susceptibility increases with age (Foster, 1988*a*). On a smaller scale, i.e. damage to leaves, there appears to be contradictory evidence. Russell & Grace (1979) reported damage to grass leaves to increase with wind speed and to be more pronounced on older leaves, while Wilson (1980) demonstrated that young expanding sycamore (*Acer pseudoplatanus* L.) leaves were far more susceptible than mature leaves. However, it is possible that the difference is caused by different growth characteristics. Effects of high winds on root systems have also been reported.

Several workers have used scanning electron microscopy to characterize damage caused by exposure to high wind speeds, using both artificially applied wind treatments (in wind tunnels) and plant material from windy sites, after monitoring exposure. Hadley & Smith (1986) investigated Engelmann spruce (*Picea engelmannii* (Parry) Engelm.) needles, and found that in wind-exposed needles there was the absence of an epidermal covering, including a lack of the apparent ridges of epicuticular wax observed on lee-ward needles. Stomata in wind-exposed needles also appeared to lack any accumulation of wax within the stomatal cavity (Hadley & Smith, 1986). Van Gardingen *et al.* (1991) examined the leaf surfaces of Sitka spruce and Scots pine, and found flattening and smearing of wax crystals, which were comparable to artificially abraded surfaces. On sycamore, dark and light brown lesions, caused by wind damage, were associated with collapse of epidermal and mesophyll cells as well as disruption of the epicuticular waxes (Wilson, 1984).

Wind damage occurs differentially within landscapes, and is largely controlled by topographic position and aspect (Foster, 1988*a*). Crown dieback and mortality of red spruce and balsam fir (*Abies balsamea* (L.) Mill.) in the subalpine zone of the northern Appalachian Mountains have been attributed to wind induced crown damage, through swaying of trees, and root damage, through vertical movements of roots due to exposure of the trees to wind (Harrington, 1986; Rizzo & Harrington, 1988).

1.5. Combinations of acid mist, ozone and wind.

Under natural circumstances it is improbable that the effects of a particular pollutant or stress would occur in isolation from the effects of other pollutants and stresses. A mountain forest, for example, could be exposed to large doses of ozone at some times of the year, and enveloped in (acidic) mists at other times (Wolfenden & Mansfield, 1991). It has been suggested that a combination of elevated ozone concentrations, acid mist, soil (nutrient), and climatic stress characteristics are the cause of the recent decline of Norway spruce on acidic soils at higher altitudes in southern Germany (Bosch *et al.*, 1983). Therefore, any study attempting to identify the cause(s) of the observed decline in forest health, should include the full range of environmental stresses.

Many studies on the effects of acid precipitation on photosynthesis and stomatal conductance have been carried out in combined studies of ozone effects and simulated acid precipitation, as this combination represents a more realistic air pollution environment for trees at high altitudes, where the ozone level is higher than at lower altitudes and a major share of the sulphur and nitrogen pollution arrives as occult deposition (Saxe, 1991). In fact, Blank *et al.* (1990*a*) reported a large combined experiment at the Gesellschaft für Strahlen- und Umweltforschung in Munich, Germany (GSF), in which an elevated ozone treatment was combined with acid mist at pH = 3.0, without resorting to the usual 2x2 factorial design, as did Roberts & Cannon (1989), and several other studies which excluded ambient air and cloudwater from reaching the foliage (e.g. Vann *et al.*, 1992). The effects by ozone or acid mist in these experiments could not be separated, and antagonistic interactions would mask individual effects.

Numerous reports are available on the effect of frosts or drought combined with a pollution treatment, but to date no work appears to have investigated the combined effects of pollutants and high wind speeds, to which trees growing at higher altitudes are also frequently exposed, and which can exert a considerable influence on tree growth (Harrington, 1986). Wind will replenish pollutants to which trees are exposed, both ozone and acid mist, but damage caused by high wind speeds could also provide additional pathways for pollutants to enter the plant foliage.

1.5.1. Reported field conditions.

Trees growing at higher elevations will be exposed to elevated ozone concentrations and acid mist and cloud water (Vann *et al.*, 1992), but not concurrently, as the meteorological conditions for both pollutant stresses are generally mutually exclusive.

Elevated ozone concentrations can be found at higher altitudes, due to mixing of lower tropospheric air with air from the free troposphere (Reiter & Kanter, 1982), which implies relatively high wind speeds, but concentrations do not usually exceed 80 nmol mol^{-1} (Singh *et al.*, 1978).

On the other hand, while low elevation radiation fogs are characterized by generally calm winds, cloud caps on mountains more frequently occur with higher wind speeds (Lindberg *et al.*, 1988). At the same time, increases in wind speed cause increased deposition of cloud water droplets via impaction, and although deposition via sedimentation decreased, the total deposition increased with wind speed (Lovett, 1984).

Rime ice is deposited when supercooled water droplets entrained in orographic clouds impact on canopy surfaces (Foster, 1988*b*). This process is dependent on wind speed: impaction is more efficient at higher velocities (Chamberlain & Little, 1981; Fowler *et al.*, 1991). When rime ice deposition results in physical damage to the trees, this results in higher within-canopy wind speeds, which in turn causes more mechanical foliage loss. Because current-year foliage is concentrated towards the outside of tree crowns, this will be preferentially lost, and the trees may lose a relatively large proportion of their photosynthetic capacity, as the youngest foliage is more efficient at carbon assimilation (Ludlow & Jarvis, 1971; Coyne & Bingham, 1982; Foster, 1988*b*). However, not all leaf surfaces are wetted efficiently, and the droplets may have sufficient energy to be reflected, or 'bounce' off the leaf, while losing some of its momentum (Cape, 1988). The much shallower viscous boundary layers which develop over conifer needles, relative to those over foliage of broad-leaves trees, enhance the capture of aerosols by conifers (Chamberlain & Little, 1981). Conifers commonly grow at high altitude, exposed and windy sites, attributes which further enhance particle deposition (Fowler *et al.*, 1991).

Canopy disturbances producing significant gaps in the forest will cause large increases in deposition rates locally on the down-wind sides of the gaps (Lovett & Reiners, 1986), which, if the acidity of the deposited mist is high enough to cause damage to the foliage, can result in a positive feedback (Johnson, 1987).

1.5.2. Potential effects of combinations of air pollution and wind on plants.

Although it is recognised that plants can be appreciably affected by the conditions in which they grow, including high winds (Wolfenden & Mansfield, 1991), to date no studies on the combined effects of air pollutants and high wind speeds have been reported. Ozone and acid mist applied at near ambient doses can alter epicuticular wax structure on elongating red spruce needles, through a direct pollutant interaction with wax crystallization and wax biosynthesis, respectively (Percy *et al.*, 1990), while alteration of epicuticular wax structures has also been shown in response to a treatment in a wind tunnel (van Gardingen *et al.*, 1991). Scanning electron microscope examinations of Sitka spruce foliage at high altitude (600 m and subject to cloud deposition) and low altitude (300 m and no cloud) sites revealed a faster 'weathering' of the needle epicuticular wax at the upper site together with greater accumulation of particles (Crossley, 1988). Clearly, if particles are suspended in the air, combined with high winds they could be particularly damaging to leaf surfaces, as has been shown for ice crystals (Hadley & Smith, 1986).

Treatments of acid mist and ozone have been combined frequently, and Saxe (1991) summarised that there are "sometimes additive or antagonistic interactions between ozone and acid precipitation", while Barnes *et al.* (1990*b*) concluded that when interactions were found, they were generally antagonistic. However, most frequently the results of from 2x2 factorial experiments, suggest there are no interactions between ozone and acid mist (Skeffington & Roberts, 1985; Chappelka & Chevone, 1986; Sasek *et al.*, 1991; Richardson *et al.*, 1992).

Cape *et al.* (1990) reported that acid mist and ozone both increased frost sensitivity of Norway spruce, but the effects were not additive, i.e. there was an interaction between the two pollution treatments: the temperature causing 20 % shoot death (LT20) of the seedlings treated with ozone alone, or with ozone and acid mist was only increased by a few degrees C, compared to that of the control seedlings, while the LT20 of the seedlings treated with acid mist only was increased by almost 20 °C.

In summarising the already mentioned research on combined acid mist and ozone effects on Norway spruce at the GSF in Munich, Blank *et al.* (1990*b*) concluded that the experimental evidence does not indicate that ozone/acid mist are the major factors inciting the observed Norway spruce decline on acidic sites at higher altitudes of the Inner Bavarian Forest and probably similar forest areas elsewhere. There was no consistent pattern of plant response, neither across all clones, nor within the clones in one soil, nor at the various response levels (ranging from growth rate to changes in biochemical reactions) within one clone. Measurements of plant growth showed a significant ozone/acid mist-related reduction in radial growth increments of three out of five clones (Payer *et al.*, 1990). Assessments of frost resistance showed no consistent effect of ozone/acid mist treatment, with one clone being detrimentally affected, but two other clones exhibited better frost resistance than did the controls (Senser, 1990). Visual foliar damage symptoms attributable to ozone/acid mist application have not been found (Senser *et al.*, 1990). A treatment (ozone/acid mist) induced reduction in photosynthetic capacity was found in two clones which did not show a corresponding growth reduction (Führer *et al.*, 1990). This decrease was found only in measurements of photosynthetic capacity carried out towards the end of the experiment (Blank *et al.*, 1990*b*). It appears therefore, that the results from this major study do not agree particularly well with results obtained elsewhere. It remains to be established whether these discrepancies are cause by the experimental layout, random variation due to the use of genetically diverse material, or possibly some other factor that has not been taken into account, such as antagonistic interactions between acid mist and ozone.

1.6. Objectives and Outline of this Thesis.

It has been shown that subjecting plants to a wind treatment can increase their susceptibility to air pollution. Both acid mist and ozone can only directly affect above-ground plant material after entering the foliage, and damage caused by wind, either direct physical damage, or indirect through changes in stomatal functioning, could enhance pollutant uptake.

The main objectives of this project were to investigate the effects of acid mist, ozone and wind on Norway spruce, together with the interactions between acid mist and wind, and between ozone and wind. The working hypothesis was that treatment with wind will alter the pathways into the needles of Norway spruce, and will render the trees more susceptible to acid mist and ozone. To test this hypothesis two year old seedlings were treated with either acid mist or ozone in open-top chambers, and these pollution treatments were combined with treatments in a controlled environment wind tunnel at various stages in the growing season (see Chapter 2). Several physiological parameters were measured. Photosynthesis is the primary production process in plants, and the stomatal conductance involves a compromise between the need for open stomata for assimilation purposes and closed stomata to reduce water loss, and these two processes, together with their associated parameters were measured, and reported in Chapter 3. The frost hardiness of trees has been found to be affected by anthropogenic pollutants, and the interactions between pollution and wind have been investigated (see Chapter 4). A few smaller experiments, on visible injury, whole plant transpiration rates, chlorophyll concentration and a destructive harvest, were also carried out, to support the findings of the main experiments (see Chapter 5). The results of all experiments are discussed in Chapter 6.

Chapter 2.

Plant Material and Treatments.

One common characteristic of many reported declines in tree health is their tendency to occur initially with greater frequency and severity at high elevation sites (McLaughlin, 1985; Rehfuess, 1987), where forests encounter generally more severe climatological conditions than their lower elevation counterparts. Mountainous terrain frequently experiences high wind speeds (Grace, 1977; Rizzo & Harrington, 1988; Crossley *et al.*, 1992), higher ozone concentrations than at low altitudes (Reiter & Kanter, 1982; Skärby & Selldén, 1984), orographically enhanced deposition (Hill *et al.*, 1988), and regular cloud immersion (Chandler *et al.*, 1988; Schmitt, 1988).

The effects of acidic cloud water on plant growth and frost hardiness has been studied extensively (e.g. Fowler *et al.*, 1989; Leith *et al.*, 1989), as have the effects of elevated ozone concentrations (e.g. Reich, 1987). There are, however, no reports of interactions between air pollutants and high winds, although it has been recognised that damage caused by high wind speeds can potentially result in higher deposition rates of atmospheric pollutants, thus causing a positive feedback (Lovett & Reiners, 1986; Johnson, 1987).

The treatments applied in this study were chosen to simulate the climatological conditions found at higher altitudes, and were compared with conditions more typical of a clean atmosphere at lower altitudes. Seedlings were used, in order to examine the effects of these conditions on the growth and survival of new plantations. The pollution treatments were applied in open-top chambers, a compromise between working in a fully controlled environment, and a non-disturbed environment (Unsworth, 1991). The wind treatments were applied in a controlled environment wind tunnel (van Gardingen *et al.*, 1991).

Acid mist was applied at an acidity of pH = 2.5, which represents the acidity of the most polluted cloud water found in upland Britain (Gervat, 1985; Unsworth and Crossley, 1987), and this treatment was compared with a pH = 5.0 control. The ozone applications were designed to fumigate the plants with air containing on average 140 nmol mol^{-1} of ozone. This level is close to the upper limit of ozone concentrations measured regularly in the UK (PORG, 1987). The ozone treatment was paired with a charcoal-filtered control. Both the acid mist and the ozone treatments are combined with the wind treatments, but due to space restrictions on the open-top chamber site no plants received both acid mist and ozone. The wind treatments were applied in a wind tunnel, at various times during the year, usually for 48 hours, at an average wind speed of 16 m s^{-1}, a velocity found regularly at higher altitudes. This results of the wind treatments were compared with a 'no-wind' control.

2.1. Plant Material.

Plant material was supplied by the Economic Forestry Group Nursery in Forfar. Two year old Norway spruce (*Picea abies* (L.) Karst.) seedlings were obtained as bare-rooted material, and potted up into 8" pots, containing about 3.8 litre of compost each. The seedlings had been raised from a seedlot with the Forestry Commission identity number 71(430)84013LOT3, originating from Germany.

The plants used in 1990 were collected from the nursery in April 1990, potted up within the next few days, and kept on the standing out area until they were placed in the open-top chambers. The plants used in 1991 were collected in November 1990, again potted up within the next few days, and kept on the standing out area during the winter.

The compost used in both years consisted of 50% peat, 30% loam and 20% grit. In 1990 fertilizer was added to the compost (Vitax Q4, composition 5.3-7.5-10 N:P:K plus trace elements), at a rate of 400 g per 100 litres. For the plants used in

1991 no fertilizer was added to the compost, but at the start of the growing season each plant was given 3 g of Osmocote mini (containing 18-6-11 N:P:K). A different fertilizer was used, as it was found that in previous years N:K ratios in the plants fertilized with Vitax Q4 were unusually low, and Osmocote provides the plants with a better balance of nutrients.

Table 2.1. Initial harvests in 1990 and 1991. (Height and root collar diameter in mm, shoot and root dry weights in g; ± 1 s.e.).

	1990	1991
Height	315 ± 20	305 ± 51
Root collar diameter	6.23 ± 0.60	6.66 ± 0.98
Shoot dry weight	10.0 ± 2.8	13.6 ± 5.5
Root dry weight	4.3 ± 1.1	-

During potting up in 1990, 10 plants were taken as an initial harvest. The average height, root collar diameter, and the shoot and root dry weights are presented in TABLE 2.1. In April 1991 20 plants, which had been potted up during the previous autumn, were randomly selected from the standing out area, and harvested. The average initial height, root collar diameter, and the dry weight of the shoot are also shown in TABLE 2.1. Because no final harvest was planned for this year, no attempt was made to wash the roots to obtain the root dry weight. As the plants were all raised from the same seedlot, and because the averages for the three measured variables were fairly similar in both years, albeit somewhat more variable in 1991, it can be assumed that the root-shoot ratios for 1991 were similar to those in 1990.

The seedlings were assigned at random to open-top chambers, and to the pollution and wind treatments, except for the plants receiving a wind treatment in spring 1991. This wind treatment was designed to simulate a storm at budburst, and seedlings were chosen accordingly (see Section 2.5.3.).

2.2. Open-Top Chambers.

Several methods have been used by researchers to apply air pollutants to plants (Unsworth, 1991). Closed chambers, such as greenhouses, are widely available, and their environment can be accurately controlled, but light levels tend to be reduced, and elaborate air conditioning systems are needed to control the temperature (Unsworth, 1991). Field release systems do not influence the light levels or the temperature, but only effects of pollution at ambient levels and above can be studied, and control of above-ambient concentrations needs sophisticated equipment (McLeod *et al.*, 1985). A compromise between the use of closed chambers and an open-air field fumigation system, is the open-top chamber (Heagle *et al.*, 1973; Mandl *et al.*, 1973). These consist of a transparent, cylindrical structure, without a roof, with a fan to inject air into the lower part of the chamber, which leaves the chamber through the open top (Unsworth, 1991). Due to the absence of a roof, the effect of the chamber on the light levels inside is reduced compared to those in closed chambers, while using relatively high flow rates ensures a limited effect on the temperature. Some workers have used open-top chambers intermittently, by employing removable side covers, to reduce the effects on the micro-climate (Wiltshire *et al.*, 1992; A. Crossley, pers. comm.).

The open-top chambers (O.T.C.'s) site near the Edinburgh Research Station of the Institute of Terrestrial Ecology consists of 24 chambers (Fowler *et al.*, 1989), different numbers of them being used for different experiments each year. The octagonal chambers are 3 m wide and 2.5 m high. The chambers consist of an aluminium frame glazed with 3 mm horticultural glass (60 x 60 cm panes). A frustrum partly closes the top, and a collar is mounted just below the frustrum, to reduce incursions of ambient air through the open top (Buckenham *et al.*, 1981). Air is filtered using activated charcoal, to remove gaseous pollutants (ozone, sulphur dioxide and nitrogen dioxide), and blown into the chambers at a rate of 40 m^3 min^{-1}, through a polyethylene manifold suspended at 1.8 m above the ground. In the chambers used in the acid mist experiments a polyethylene ceiling was fixed just below the collar, to exclude rain. Plants in the chambers were watered by placing the pots on capillary matting, which was irrigated using tap water as required.

The chambers inevitably affected ambient conditions. The chamber temperature was found to be elevated by up to 5 °C on warm sunny days, but typically by 1 - 2 °C compared to ambient (I. D. Leith, pers. comm.). Similar increases have been reported elsewhere (Vann *et al.*, 1992). Light levels inside the chambers were reduced by approximately 15% compared to ambient conditions (Fowler *et al.*, 1989). The floor area of the chambers is approximately 7 m^2, and allowing an edge strip of 0.3 m, a maximum of 100 plants in 8" pots could be accommodated in each chamber. TABLE 2.2 gives a summary of the properties of the open-top chambers.

2.3. Acid Mist Treatments.

The major ions in cloud water are sulphate, nitrate, ammonium and hydrogen ions (Waldman *et al.*, 1985; Weathers *et al.*, 1988; Mohnen & Kadlecek, 1989; Saxena & Lin, 1990). Only under specific conditions, in a marine climate, are other ions, such as sodium and chloride, present in substantial concentrations (Fowler *et al.*, 1988*b*; Weathers *et al.*, 1988). Therefore, the acid mist applied in this study consisted of equimolar concentrations of sulphuric acid and ammonium nitrate, at two levels of acidity, pH = 2.5 and pH = 5.0 as a control. These concentrations represent the two extremes found in cloud water (Gervat, 1985; Unsworth & Crossley, 1987; Schmitt, 1988).

2.3.1. Mist application system.

The acid mist was applied as droplets, generated by Micromax atomizers (model 84, CDA Sprayers Ltd., Bromyard, Herts). These devices consist of a disc spinning at about 5000 rpm, and were mounted in the centre of the chambers, about 1.5 m

Table 2.2. Physical characteristics of the open-top chambers used to study the effects of acid mist and ozone.

Volume	22.4 m^3
Floor area	7 m^2
Open-top Ø	3.5 m
Air flow rate	40 m^3 min^{-1}
Air filtration system	Activated charcoal
Effective filtration	100% filtration efficiency at ambient wind speeds less than 1 m s^{-1}, decreasing to 75% at wind speeds of 4 m s^{-1}
Temperature	Increase due to fan unit is 0.26°C
Temperature excess (T °C)	Within chamber increases with solar radiation St (W m^{-2}) according to: $T = 0.26 + 5.5 * 10^{-3}$ St
Distance between chambers	N-S 7 m, E-W 8 m
Mist	1.5 m above plants, 40 - 90 μm Ø droplets, at 3 mm h^{-1}

Chamber shadows do not overlap neighbouring chambers between 8.00 and 1600 GMT between 21 March and 21 September. (From: Fowler *et al.*, 1989.)

above the plants. The atomizers produced droplets with a mass mean diameter of 90 μm and a number mean diameter of 40 μm, delivered at a rate of 3 mm h^{-1} (Leith *et al.*, 1989). Before spraying, each mist solution was checked by measuring the conductivity, and the solutions were then supplied simultaneously to the atomizers in all chambers, from 25 dm^3 polypropylene bottles, through 1/4" tubing, using air compressed to a maximum pressure of 1.3 bar. The plants were not sprayed under very bright conditions, to avoid scorching and excessive evaporation of the mist solutions (Fowler *et al.*, 1989). At the end of the mist application the spray distribution in the chambers was checked, and any plants that did not receive a sufficient amount of mist, which happened occasionally depending on the wind speed and direction, were noted. However, no plants were found to systematically receive less mist than others.

2.3.2. Mist applications.

In 1990 the plants were sprayed twice a week to provide 4 mm precipitation equivalent (i.e. 4 litres per m^2 of ground area) per week. The concentrations and doses of the mist treatments are given in TABLE 2.3. The two levels of acidity were both applied in two chamber replicates. Twenty plants were available in each chamber. Spraying commenced the third week of May, and continued until the seedlings were sampled for the frost hardiness assessments in mid November.

In 1991 the plants were sprayed four times each week, but received only the equivalent of 1 mm of precipitation each time. The concentrations and the doses were the same as in 1990. Four chamber replicates for each treatment were available this year, but only twelve plants could be accommodated in each chamber. The acid mist was applied between 14 May and 11 November 1991.

The difference in number of applications is caused by the fact that in both years the O.T.C.'s were also used for other experiments. In 1990 the main experiment investigated the effect of the number of wet-dry cycles, by applying either 2 mm precipitation equivalent twice a week, or 1 mm four times a week, thereby keeping the total applied quantity constant (Sheppard *et al.*, 1993*b*), and plants used for the experiments described in this study could only be accommodated in

Table 2.3. Concentrations and deposition rates in the acid mist experiments.

A. Concentrations (mM).				
pH	H^+	NH_4^+	NO_3^-	SO_4^{2-}
5.0	0.010	0.005	0.005	0.005
2.5	3	1.5	1.5	1.5

Deposition (kg ha^{-1} $week^{-1}$).			
pH	H	N	S
5.0	0.0004	0.006	0.006
2.5	0.12	1.7	1.9

the chambers receiving the 4 mm total weekly dose in only two applications. This differed from the treatment in 1991, when all the chambers used for the acid mist experiments were sprayed with the equivalent of 4 mm each week, applied over four applications (Leith *et al.*, 1992).

2.4. Ozone Treatments.

Ozone was applied only when the climatological conditions were favourable for photochemical ozone production in the ambient atmosphere. These were arbitrarily restricted to a shortwave solar radiation exceeding 400 W m^{-2} and air temperature higher than 12 °C. This resulted in episodes of ozone exposure, much as would be expected to occur naturally, as elevated ozone concentrations are generally associated with summertime anticyclonic conditions in the United Kingdom, which provides both the meteorological conditions (Derwent *et al.*, 1978; PORG, 1987), and the necessary photochemical precursors, as air mass trajectories in general cross central Europe before reaching the country (Apling *et al.*, 1977).

The ozone treatment in the open-top chambers was aimed at a seasonal average concentration during the fumigation periods of 140 nmol mol^{-1}, which represents the maximum concentration regularly measured in the United Kingdom (PORG, 1987). This treatment was compared with a charcoal-filtered control, which, because of incursions of air through the open top of the chambers, does not reduce the ozone concentration to 0 nmol mol^{-1}. The average ozone concentration during the fumigation periods in the charcoal-filtered control, chambers was about 10 nmol mol^{-1}. However, 0 nmol mol^{-1} would be an unrealistic control, as the ambient ozone concentration always had a background level above 10 nmol mol^{-1} (Saxe, 1991).

2.4.1. Ozone fumigation system.

The ozone fumigation system, as presented in FIGURE 2.1, was developed in

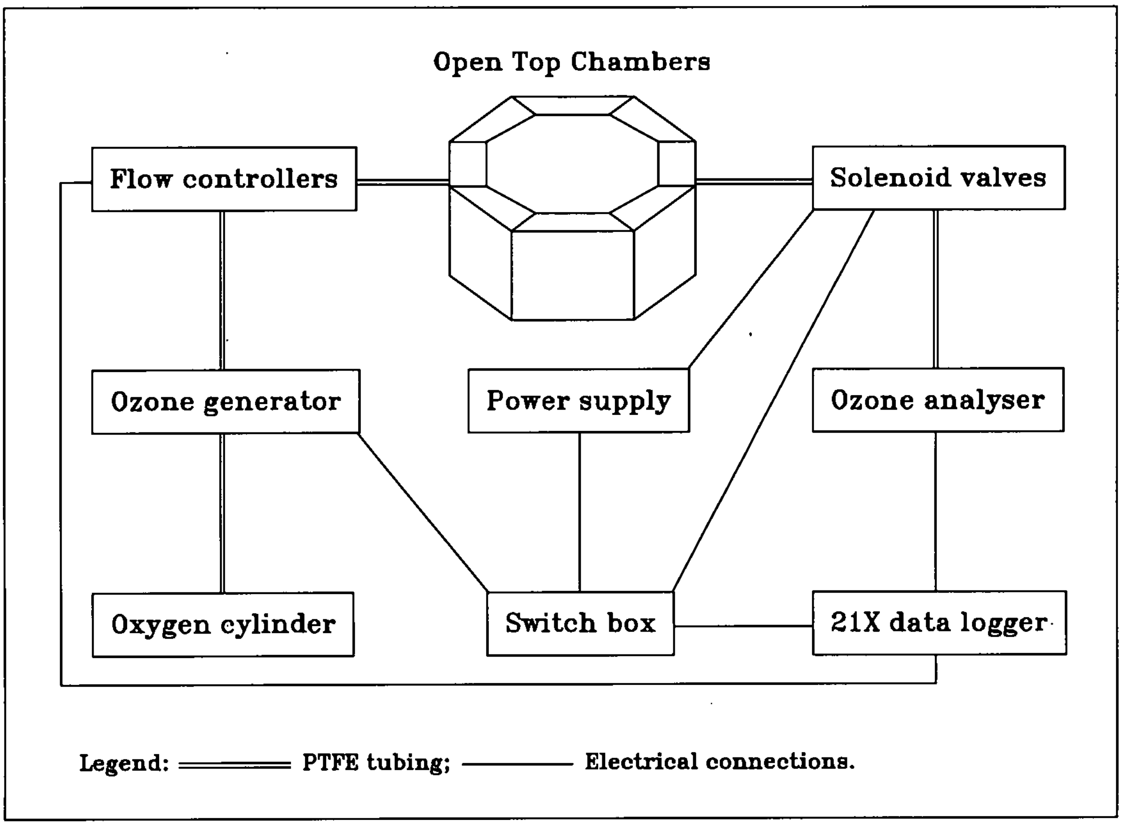


Figure 2.1. Schematic diagram of the ozone fumigation system used at the open top chamber site in 1990 and 1991.

1990. During 1988 and 1989 the ozone concentrations in the chambers were checked only once or twice throughout the day. The system was designed to sample all four ozone chambers, two at 140 nmol mol^{-1} and two charcoal-filtered controls, and ambient ozone, every half hour. The heart of the control system was a datalogger (21X, Campbell Scientific Ltd., Shepshed, Leics.), which monitored the various instruments, controlled the opening and closing of the solenoid valves, thereby selecting the source of inlet air to the ozone analyzer, and was also used to provide a voltage signal to the flow controllers to regulate the flow of the ozone into the chambers.

Ozone was produced using an electrical discharge ozone generator (Model LN 103, Brown, Boveri & Cie., Baden, Switzerland), using compressed zero-grade oxygen (BOC Ltd.) as a source gas to avoid the production of di-nitrogen pentoxide (N_2O_5; Brown & Roberts, 1988). The arc voltage was adjusted manually.

The oxygen-ozone mixture was then led through two flow controllers (Model FC280, Tylan General (UK) Ltd., Westlea, Swindon, Wilts) into the air stream into the two open-top chambers designated to receive 140 nmol mol^{-1} of ozone, using 1/4" PTFE tubing and stainless steel fittings. The oxygen pumped into the chambers did not alter the composition of the air inside the chambers, other than the ozone concentration, as the flow rate of about 1 dm^3 min^{-1} for the oxygen-ozone mixture was negligible compared to the flow of about 40 m^3 min^{-1} from the main fan units into the open-top chambers.

Air was drawn continuously from the chambers using a large diaphragm pump, through PTFE filters (Ø 47 mm, pore size 0.2 µm) and 1/2" PTFE tubing. These sample lines were sequentially sub-sampled, and the ozone concentration was measured with a UV photometric ozone analyzer (Model 427, Analysis Automation Ltd., Eynsham, Oxford). Five PTFE solenoid valves were used, four normally closed connected to the chambers, and one normally open drawing ambient air from a 1.5 m mast on top of the monitoring cabin. The four normally closed valves were connected in parallel, and together they were connected in series with the normally open valve. This allowed control of five valves with only four digital control ports on the 21X. If one of the control ports was set high, this would activate a relay in the switch box, and power would be supplied to one of the normally closed valves and the normally open valve, thereby sampling one of the chambers. If none of the control ports was set high, the normally open valve would be open, and ambient air would be sampled. Four LED's on the relay box were used to indicate which chamber was being sampled.

The two analogue output channels on the 21X were used to provide signals to the flow controllers, controlling the amount of ozone being bled into the chambers. These output voltages were set manually, and adjusted regularly, depending on the measurements during the previous half hour cycles. Ideally this should have been done automatically, but adjusting the flow into one of the chambers, would also affect the amount of ozone going into the other chamber, because the ozone generator would continue to produce the same quantity of ozone, and altering the flow rate into one of the chambers, would also alter the flow through the generator, and thus change the ozone concentration. There are two possible ways to solve this: 1. Use a stepper motor to adjust the arc voltage in the generator, but this would mean working with high voltages, and was never considered. 2. Install a third flow controller, keeping the overall flow through the generator constant by bleeding the excess ozone through a charcoal filter into the atmosphere. This would be potentially hazardous, since the ozone was generated at a concentration of about 0.6% (6 000 000 nmol mol^{-1}), and the 21X provided only two analogue output signals.

The 21X monitored five input channels, the two flow controllers, ambient temperature, global solar radiation and the ozone analyzer. The temperature was measured using a shielded platinum resistance thermometer (Vector Instruments, Rhyl, Clwyd, Wales), mounted on a mast, 1.5 m above the monitoring cabin. The global solar radiation was measured with a solarimeter (Model CM 5, Kipp & Zonen, Delft, The Netherlands), which was placed next to the temperature sensor. The flow rates, temperature and solar radiation were measured continuously, while the output from the ozone analyzer was recorded according to the sampling cycle. The four chambers and ambient air were measured every half hour, providing 6 minutes monitoring from each source. The first two of these six minutes were used to purge the tubing and the analyzer, and during the next four minutes readings were taken every 12 seconds, which was slightly longer than the internal cycle of the analyzer. Averages were stored at the end of every half hour, but could be checked manually at any time.

A further feature of the 21X program was an alarm shut-down system, which reduced the overall fumigation time, but avoided the plants being exposed to acute concentrations of ozone, as it has been suggested that short-term exposure to high concentrations can have relatively large effects (Reich, 1987). When the average concentration in either of the chambers was found to exceed 160 nmol

mol^{-1}, the generator and the power supply to the solenoid valves were switched off, the flow controllers were closed, and the four control ports for the solenoid valves were all set high, lighting up all four LED's, indicating a system shut-down. After this the 21X would not execute the program until reset by the operator. A listing of the 21X program is given in Appendix 1.

An example of the operation of the ozone fumigation system is given in FIGURE 2.2, which presents the 30-minutes average ozone concentrations in the four chambers, together with the ambient concentration for 29 August 1991. The system was switched on at 7.57 GMT, and run until 16.35 GMT (indicated by arrows, ↓). FIGURE 2.2 shows the concentrations in the four chambers were virtually the same when no ozone was applied, and considerably less than the ambient concentration, due to the charcoal filters. It also shows the normal diurnal cycle in the ambient ozone concentration (Gay, 1991), which tends to peak during the early to mid-afternoon (see section 1.2.1.).

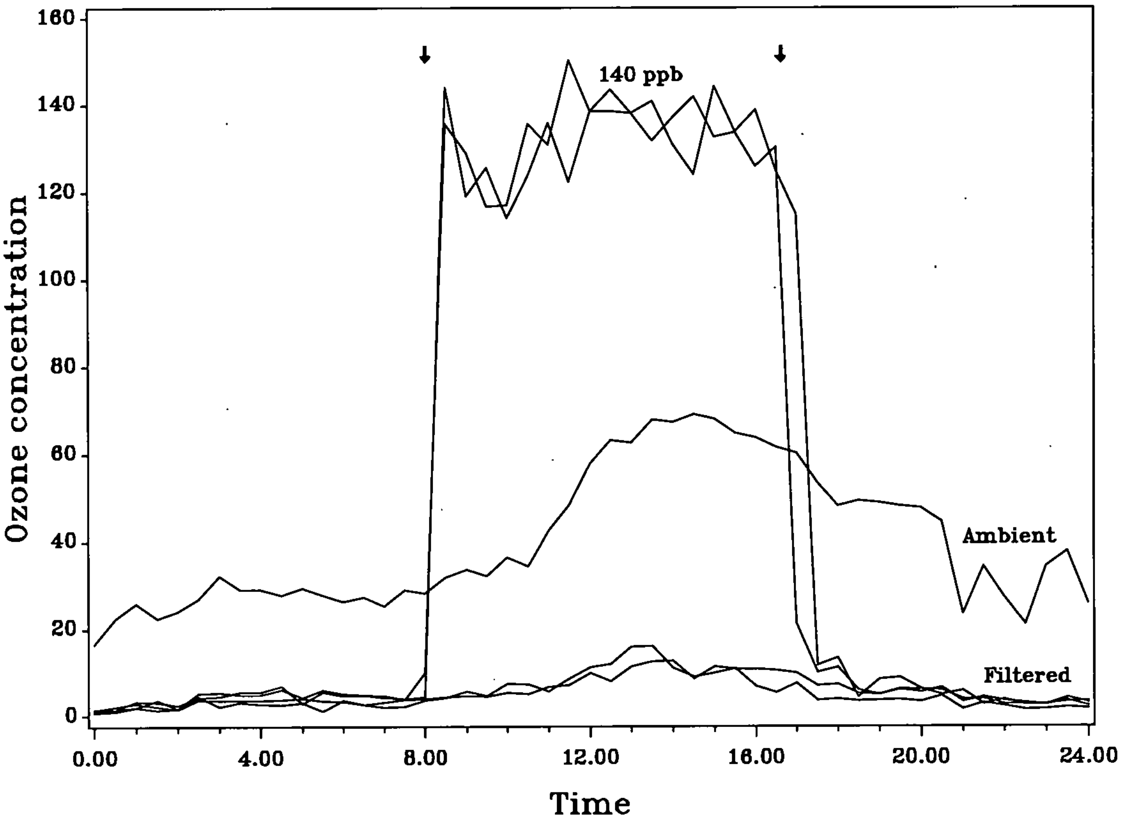


Figure 2.2. The average ozone concentrations (nmol mol^{-1}) inside the four open-top chambers, together with the ambient concentrations, on 29 August 1991.

The calibration of the ozone analyzer was checked regularly, by providing it with an air mixture of a known ozone concentration, together with the analyzers used for the UK monitoring network (PORG, 1987). These network analyzers are fully calibrated every six months, against a secondary standard UV photometer, which in turn is calibrated against the primary UK standard UV photometer, kept at the AEA Technology Harwell Laboratory, Oxfordshire. The mass flow controllers were calibrated using a 2 dm^3 soap-film flow meter, although this was not essential, as the flow rates were adjusted to obtain the correct concentration of ozone in the open-top chambers, and the actual flow rates were not important. The global solar radiation sensor was calibrated against a factory-calibrated sensor, used on the monitoring cabin 500 m from the O.T.C. site. The platinum resistance thermometer was calibrated by placing it in an ice bath, thus obtaining the exact ratio of the resistance of the sensor and leads, over the resistance of a precision resistor (with a low response to changes in temperature) across the terminal block of the datalogger, a value which is needed in the datalogger program.

2.4.2. First year, 1990.

In 1990 the ozone applications began on 13 June, and continued until 17 August. There were two periods in May when the ambient concentrations were particularly high, but the system was not operational by then, so that the plants, which were still kept on the standing out area at this time, were all exposed to ambient concentrations of ozone during these episodes, including the control plants. The highest hourly average concentrations at a monitoring station 500 m from the site were 75 nmol mol^{-1}, and the ozone concentration remained above the average background concentration for about 40 hours each. This is shown in FIGURE 2.3a, which gives the daily average (24 hours; lines) and maximum hourly average (dots) ambient concentrations from May to September 1990.

The actual days and times that ozone was applied in 1990 are shown in FIGURE

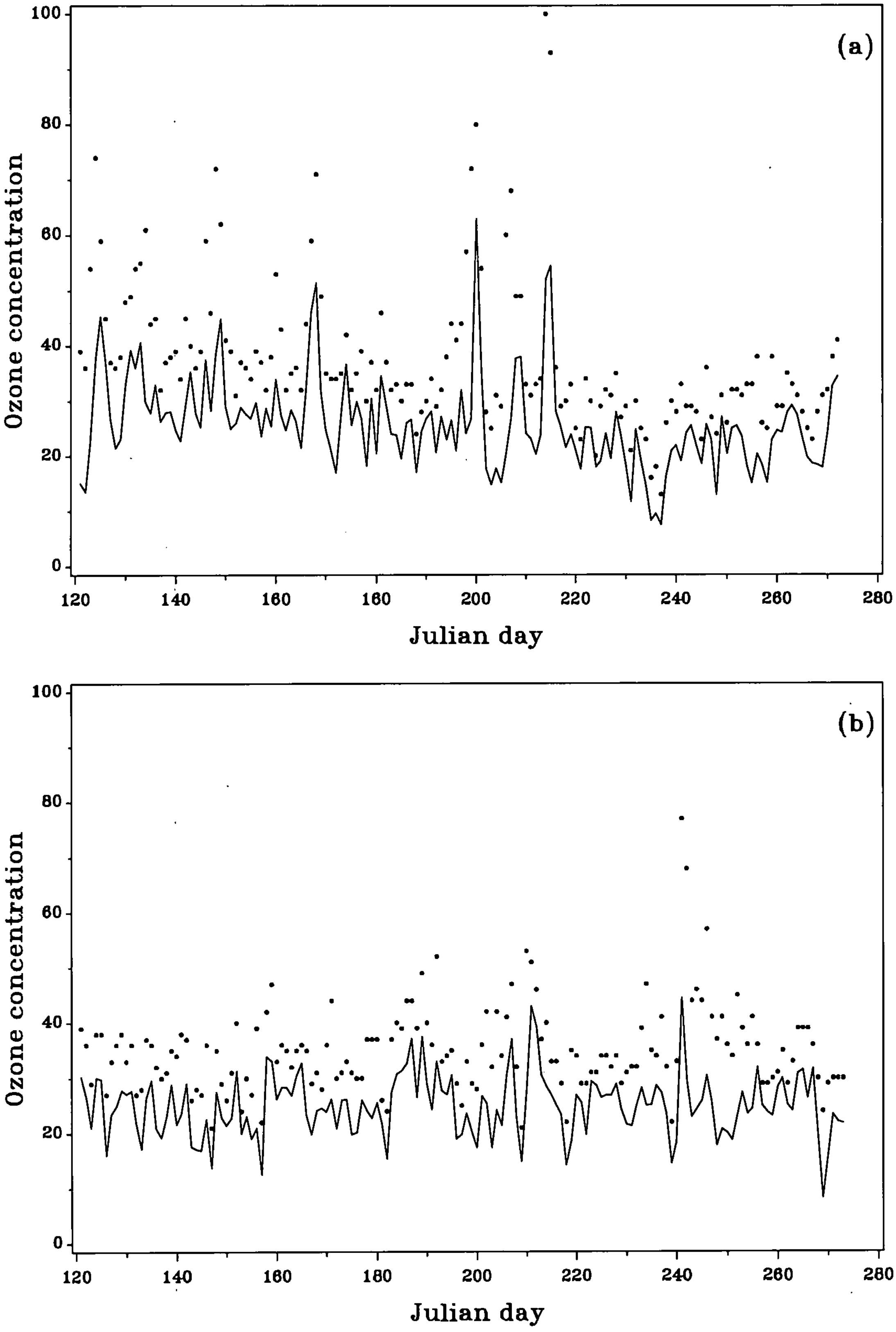


Figure 2.3. Daily average (lines) and hourly maximum (dots) ambient ozone concentrations (nmol mol^{-1}) from May to September in 1990 (a) and 1991 (b). Data collected under contract to the UK Department of the Environment, and provided by R. L. Storeton-West.

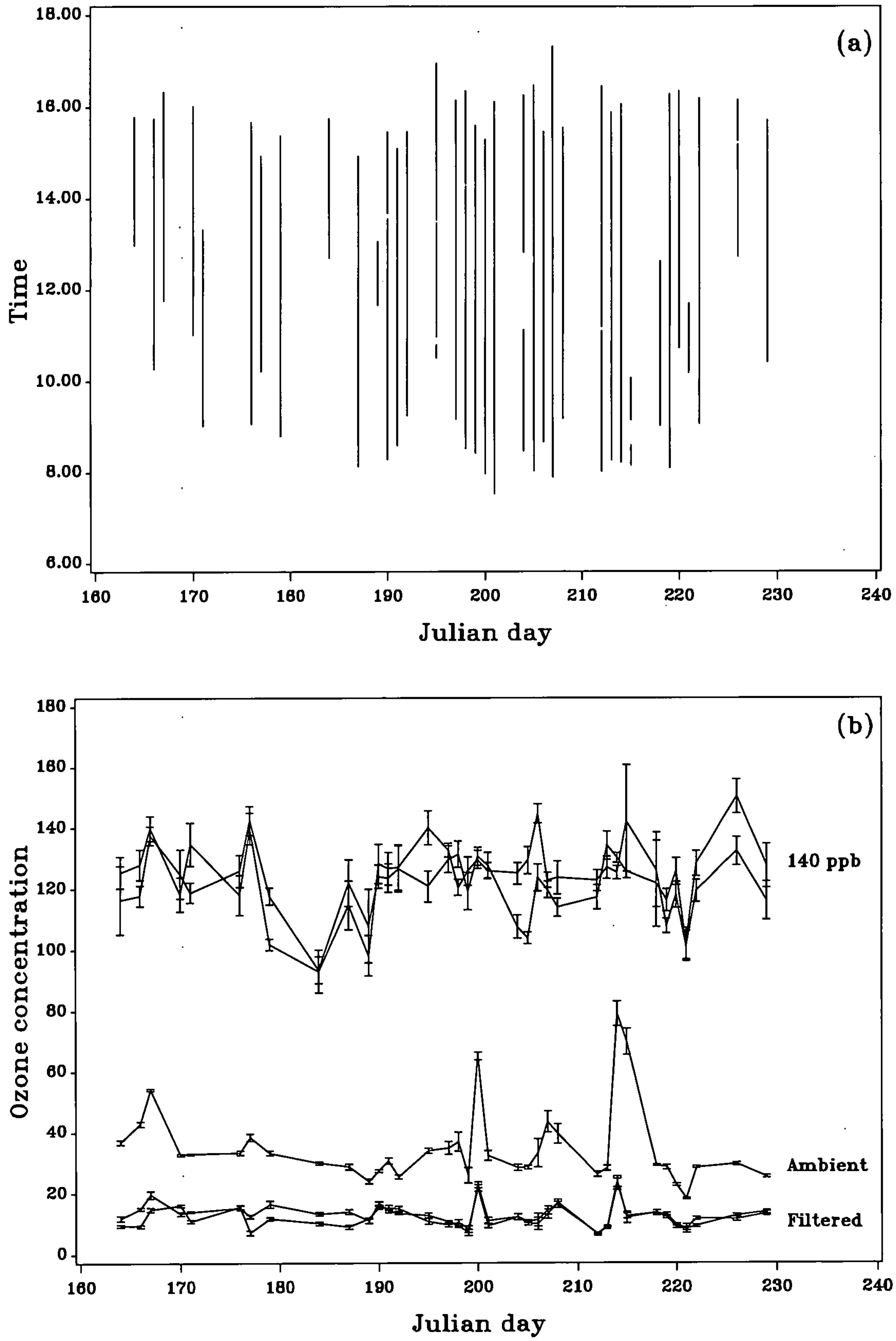


Figure 2.4. Ozone applications in 1990. (a) Vertical lines indicate the times ozone was applied on each day. (b) Daily average concentrations (± 1 s.e.; nmol mol^{-1}).

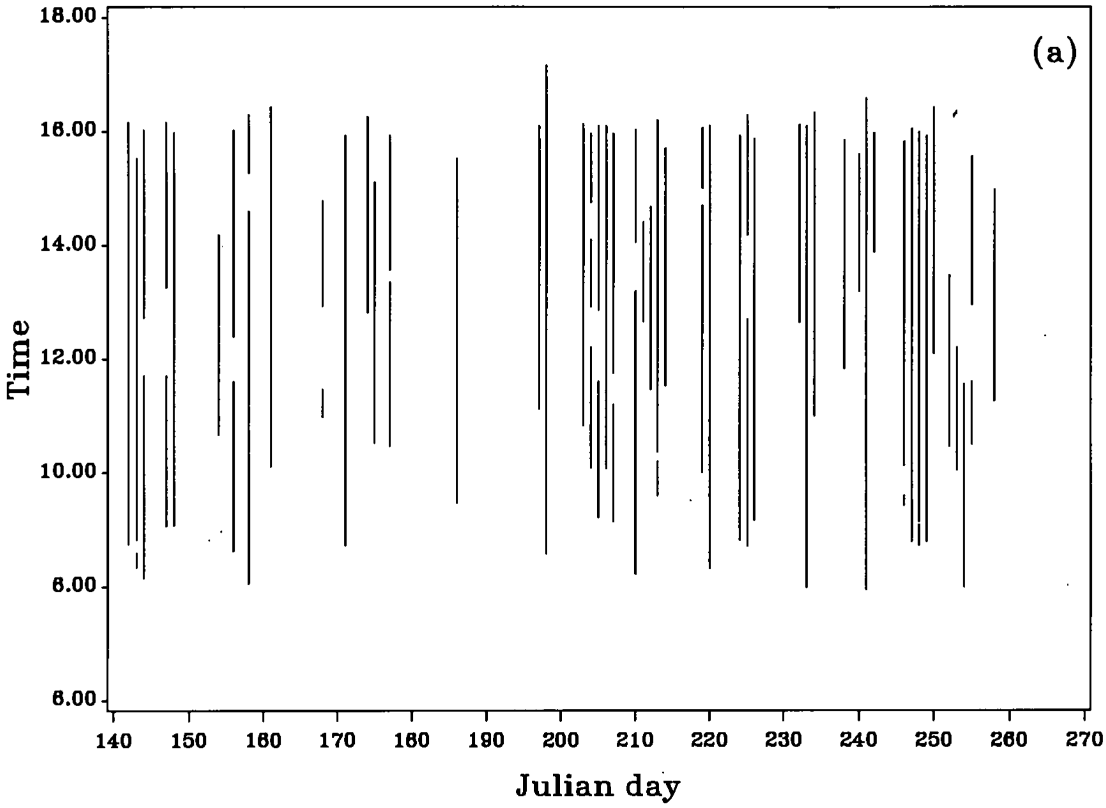
2.4a. The vertical lines indicate the times the system was run on each day. In total the plants received a treatment for just over 200 hours.

FIGURE 2.4b gives the average concentrations and their standard deviations, for the four chambers and the ambient air concentration, during the periods that ozone was applied. The average concentration during these periods for the chambers that received ozone was 123 ± 17 nmol mol^{-1}, in the control chambers this was approximately 12 ± 5 nmol mol^{-1}. The ambient ozone concentration during the fumigation periods was about 35 ± 16 nmol mol^{-1}. The objective of giving the ozone treatment at 140 nmol mol^{-1} was not quite achieved, but even under these controlled conditions the concentration in the chambers fluctuated considerably, as indicated by the relatively large standard error of 17 nmol mol^{-1}. This was partly caused by an ingression of ambient air through the open-top of the chambers (Buckenham *et al.*, 1981). This graph also shows that the filter units generally reduced the concentration in the control chambers to about a third of the ambient concentration, and the same was true for all four chambers in the ozone experiment, during the periods that no ozone was applied.

2.4.3. Second year, 1991.

FIGURE 2.5 provides the same information as FIGURE 2.4. The days and times over which the fumigation system was run in 1991 are presented in FIGURE 2.5a, and the resulting average concentrations for the four chambers, together with the ambient concentration of ozone are shown in FIGURE 2.5b. The plants received a total of 265 hours of ozone fumigation, starting on 22 May and lasting until 15 September. In 1991 the fumigation was set up early, but the weather conditions were mostly unsuitable for ambient ozone production. By the end of July ozone had been applied for only about 100 hours, against 150 hours in 1990, when the system was set up a month later.

The mean ozone concentrations during the periods that ozone was applied,



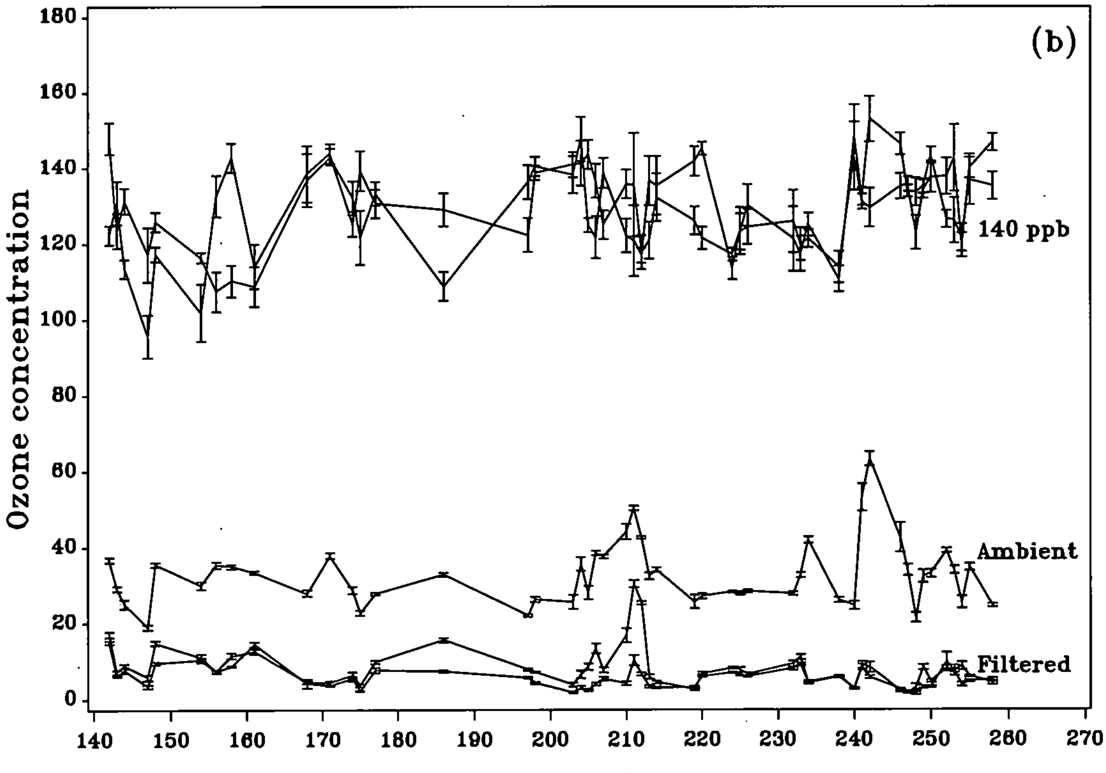


Figure 2.5. Ozone applications in 1991. (a) Vertical line indicate the times ozone applied on each day. (b) Daily average concentrations (± 1 s.e.; nmol mol^{-1}).

averaged over the season, in the two chambers receiving the high ozone treatment was 129 ± 17 nmol mol^{-1}. The concentration in the filtered control chambers was 7 ± 4 nmol mol^{-1}, and the mean ambient concentration was 33 ± 9 nmol mol^{-1}. The daily average concentrations (24 hours) are shown in FIGURE 2.3b, which also provides the maximum concentrations at the monitoring station 500 m away from the O.T.C. site. In 1990 the average concentration was 26 ± 12 nmol mol^{-1}, and the maximum recorded hourly average was 100 nmol mol^{-1}. In 1991 these values were 25 ± 9 and 77 nmol mol^{-1} respectively.

2.5. Wind Treatments.

The wind speed used in this study was dictated by the maximum velocity of 16 m s^{-1} obtainable in the wind tunnel when using Norway spruce seedlings at 30 to 40 cm height. However, this represents realistic wind speeds at higher elevations (Caldwell, 1970; Crossley, 1988; Rizzo & Harrington, 1988). The wind treated plants were compared with other plants that had not received a treatment in the wind tunnel, and had only been subjected to the low velocities experienced in the open-top chambers.

2.5.1. Wind tunnel.

The wind treatments were applied in a computer controlled environmental wind tunnel (van Gardingen *et al.*, 1991), with a working section of 1.6 m long, 0.9 m wide and 0.7 m high. Wind speeds up to 16 m s^{-1} could be generated in this wind tunnel. Two cylinders, 125 mm diameter, mounted cross-wire just upstream of the working section, generated turbulence within the working section (Dixon & Grace, 1984).

Light was provided to the working section using 400-W metal-halide fluorescent lamps (model Kolorarc MBIF/NT/H, Thorn Lighting Ltd., London) and 60-W tungsten bulbs. Three light levels were available, by using either or both of two set of lamps, the first consisting of three metal-halide lamps and six tungsten bulbs, and the second set containing six metal-halide lamps. The photosynthetically active photon flux was 160 μmol m^{-2} s^{-1} (van Gardingen *et al.*, 1991) when only the first set of lamps was on, and 355 μmol m^{-2} s^{-1} when both sets were switched on. When only the second set of six metal halide lamps was used the photon flux was about 200 μmol m^{-2} s^{-1}.

A maximum of ten plants were placed in the wind tunnel at any one time, in alternating rows of two, to minimise any sheltering effects. The distance between the plants was 34 cm. Depending on the size of the trees, there was little or no physical contact, to avoid abrasion by trees rubbing each other. Newly planted seedlings in forest plantations are generally spaced such that the canopy does not close for the first few years, and seedlings are therefore not expected to rub against each other under the influence of high wind speeds.

The wind speed was measured with a pitot static tube (Grace, 1989*a*), positioned immediately downstream of the working section, and connected to a micromanometer (Combustion Instruments, Staines, Middlesex). The wind speeds were calculated from:

$$\Delta P = 0.5 \rho u^2 \tag{2.1}$$

where ΔP is pitot reading (Pa), ρ is the air density (kg m^{-3}), and u is the wind speed (m s^{-1}). The air density, ρ, was calculated from:

$$\rho = \rho_{STP} \frac{P}{P_{STP}} \frac{T}{T_{STP}} \tag{2.2}$$

where ρ_{STP} is the density of air at standard temperature and pressure (1.292 kg m^{-3}), P is the atmospheric pressure (kPa), P_{STP} is the standard pressure (101.3 kPa), T is the temperature (K), and T_{STP} is the standard temperature (273.15 K).

The wind treatments were applied at 16.5 ± 0.5 m s^{-1}, averaged across all experiments, which is equivalent to just over 50 m s^{-1} (115 mph) at the normal meteorological measuring height of 10 m (calculated assuming a logarithmic profile (Monteith & Unsworth, 1990, page 113) above a plant canopy of 50 cm high).

The temperature and humidity in the wind tunnel were set to follow a sinusoidal curve, fluctuating by ± 2 °C and ± 5% RH respectively around a value, dictated

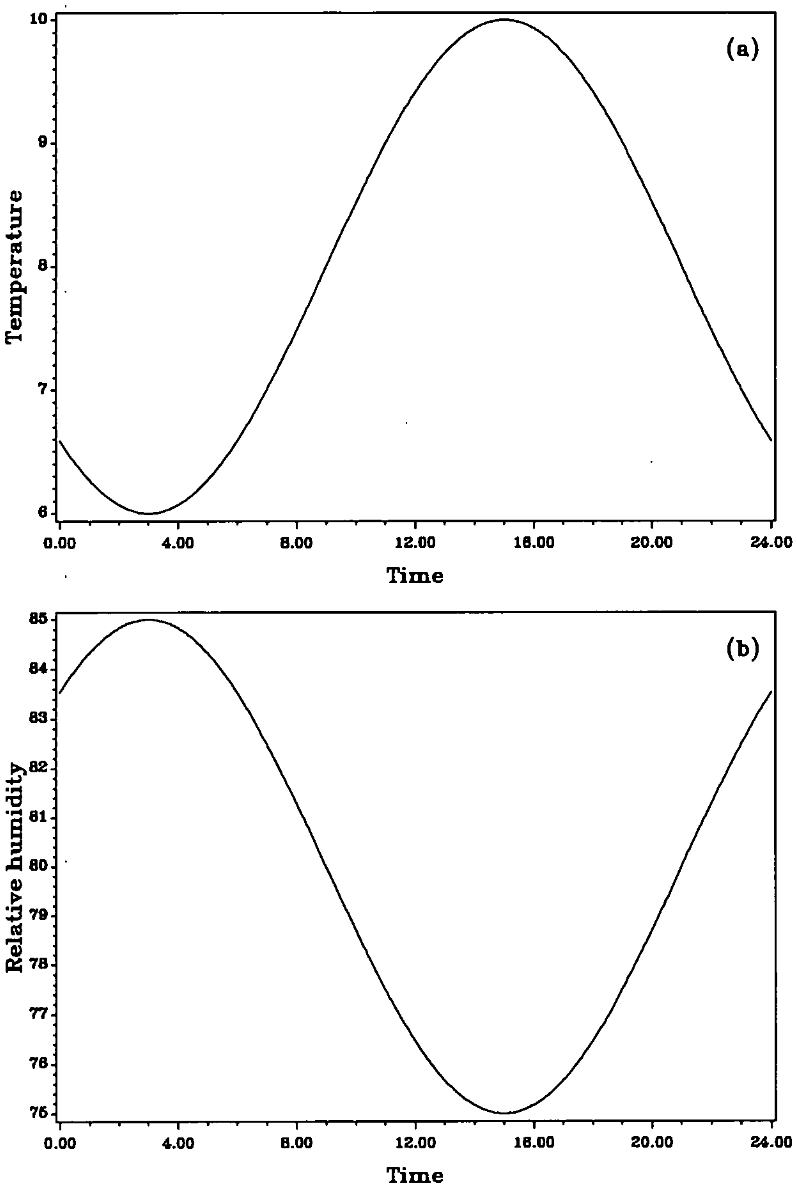


Figure 2.6. Temperature (a; °C) and humidity (b; % RH) settings of the wind tunnel control system during the wind treatment in March 1991.

by the season. The temperature peaked during the middle of the afternoon, at which time the humidity was at its lowest value (see FIGURE 2.6). For the winter treatment the wind tunnel was set to fluctuate around 8 °C and 80% RH. For the remaining treatments during the autumn of 1990, and in spring, summer and autumn 1991 these values were 16 °C and 70% respectively.

Halfway through the wind treatment, plants were moved to the position diagonally opposite in the working section of the wind tunnel, to avoid an effect of the position in the wind tunnel. For the same reason care was taken to ensure that successive plants from each open-top chamber were placed in different positions in the wind tunnel.

2.5.2. First year, 1990.

In 1990 two wind treatments were applied, a no-wind control and a treatment at 16 m s^{-1} in the wind tunnel for 48 hours. Half of the plants from each open-top chamber were treated, with the plants from the acid mist experiments treated during the second and third weeks of September, and the plants from the ozone experiments during the third week of September and first week of October.

2.5.3. Second year, 1991.

In 1991 the emphasis of the work was on the wind effects, and more treatments, all at 16 m s^{-1}, were applied. For this purpose the plants had been potted up in December 1990, and the first batch of trees were treated for 48 hours in the wind tunnel at the end of March, to simulate a winter storm, i.e. during the dormant period. This batch was only used in the ozone experiment. The second batch was treated at budburst, also for 48 hours, to simulate a spring storm. The plants were selected such that their buds had just started to burst (stage 2 to 3; Cannell & Smith, 1983) when they were placed into the wind tunnel. The third 48 hours treatment was applied during the third week of August, but only to plants treated with acid mist. Together with this treatment a few ozone treated plants were wind treated for 120 hours, to investigate a possible effect of the duration of the wind treatment. The last batch was treated towards the end of September, just before the start of the frost hardiness measurements on the plants from the ozone experiment.

2.6. Timing.

FIGURE 2.7 summarises the periods during which the acid mist, ozone and wind treatments were applied, together with the timing of the gas exchange and frost hardiness measurements. Some smaller experiments were also carried out, see Chapter 5, but these are not indicated in FIGURE 2.7. Budburst in 1990 occurred during the last week in April and the first week in May (I.D. Leith, pers. comm.), the same as in 1991, when the simulated spring storm, indicated in FIGURE 2.7, was applied to plants while their new growth needles were just appearing from the buds.

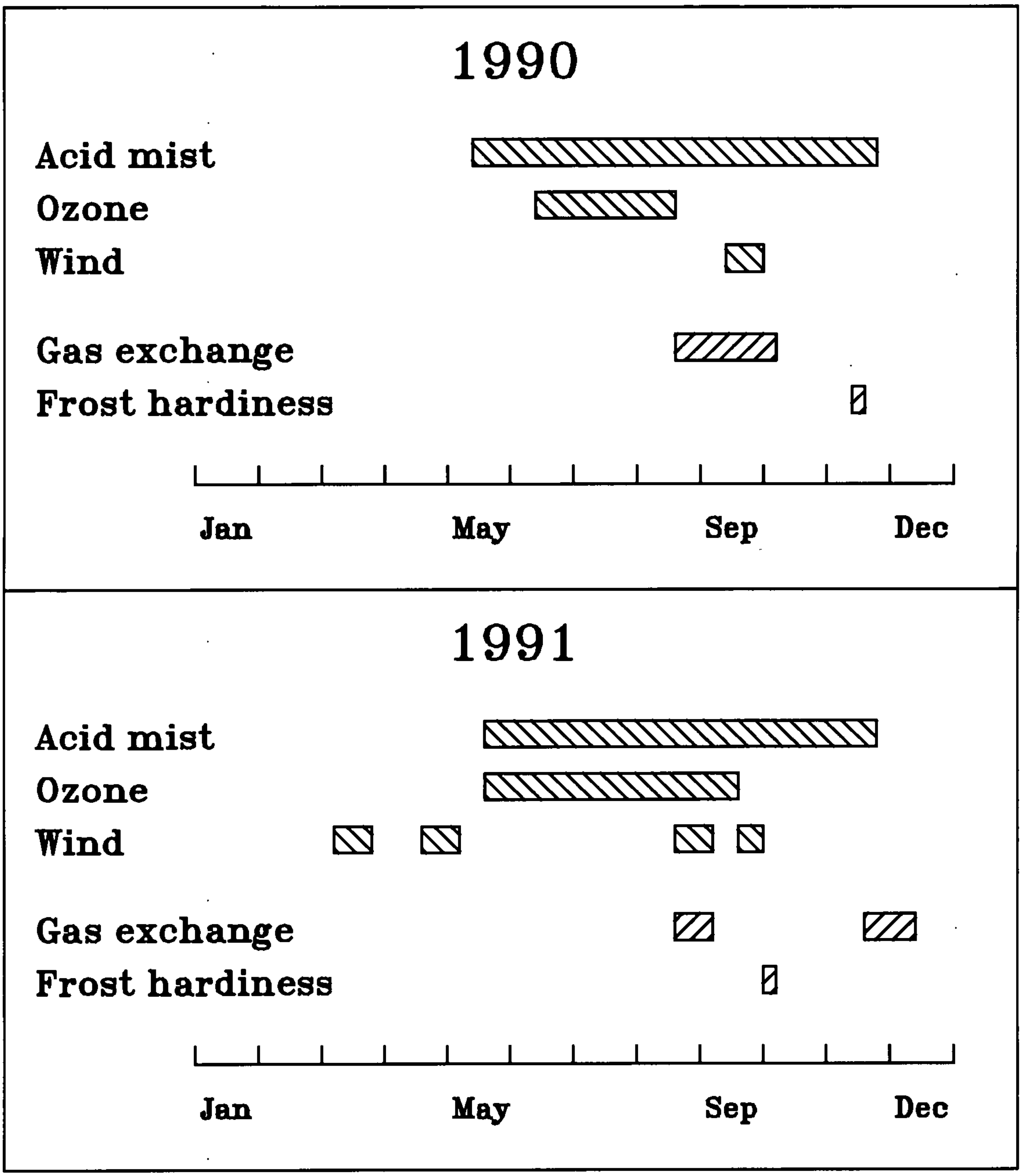


Figure 2.7. Timing of the acid mist, ozone and wind treatments in 1990 and 1991, together with that of the gas exchange and frost hardiness measurements.

Chapter 3.

Effects on Photosynthesis and Stomatal Conductance.

Air pollution has been shown to cause alterations to carbon assimilation and associated parameters such as stomatal conductance (Eamus & Fowler, 1990; Eamus & Murray, 1991), and because photosynthesis is the primary process on which increases in biomass in plants depend, this is an important area in which to seek evidence of critical effects of pollutants (Wolfenden & Mansfield, 1991). The sensitivity of the photosynthetic capacity to air pollutants varies widely with species, and also with environmental conditions (Darrall, 1989), but generally an inhibition of the net photosynthesis is reported after exposure to pollution, possibly due to increased respiration requirements (McLaughlin *et al.*, 1991). Similarly, the sensitivity of stomatal conductance to pollutants may influence plant growth. A reduced stomatal conductance will reduce assimilation rates, while an increase in stomatal conductance will render the plant susceptible to excess water loss in periods of drought. There is evidence that pollutants can affect the ability of both the stomata and other parts of the epidermal layer to regulate water loss (Wolfenden & Mansfield, 1991), but, as the cuticular conductance is two to three orders of magnitude smaller than the stomatal conductance (van Gardingen & Grace, 1991), only the latter has been measured in this study.

3.1. Gas Exchange System.

The measurements and results described in this chapter were obtained from a gas exchange system, specifically assembled for this project. The use of two cuvettes allowed simultaneous measurements of assimilation rates and stomatal conductances for two plants. The water vapour pressure was continuously monitored in both cuvettes, allowing the calculation of stomatal conductances for both trees throughout the measurement periods. The carbon dioxide (CO_2) concentration was monitored in only one cuvette at a time, due to the availability of only one infra-red gas analyzer. This allowed the monitoring of the CO_2 concentration in one cuvette, and the calculation of assimilation rates for one plant, more or less continuously, while for the plant in the other cuvette only steady state readings were taken.

The gas exchange system used was of the open path type as described by Jarvis & Čatský (1971), see FIGURE 3.1. Air was drawn from outside the building through two sets of 10 dm^3 glass bottles to buffer any sudden changes in the ambient CO_2 concentration. It was then drawn through a set of two humidifiers, of which the second was placed in a temperature controlled water bath, to provide the leaf chambers (see Section 3.1.1.) with air at a constant humidity (Jarvis *et al.*, 1971). After passing through the pump, the air was led back via a copper coil and water trap placed in the water bath, to avoid condensation in the air lines. As there was a slightly lower than ambient air pressure upstream of the pump, and a somewhat higher than ambient pressure downstream, and as the saturated vapour pressure of the air after passing through the humidifiers depended only on the temperature of the water bath, the vapour pressure

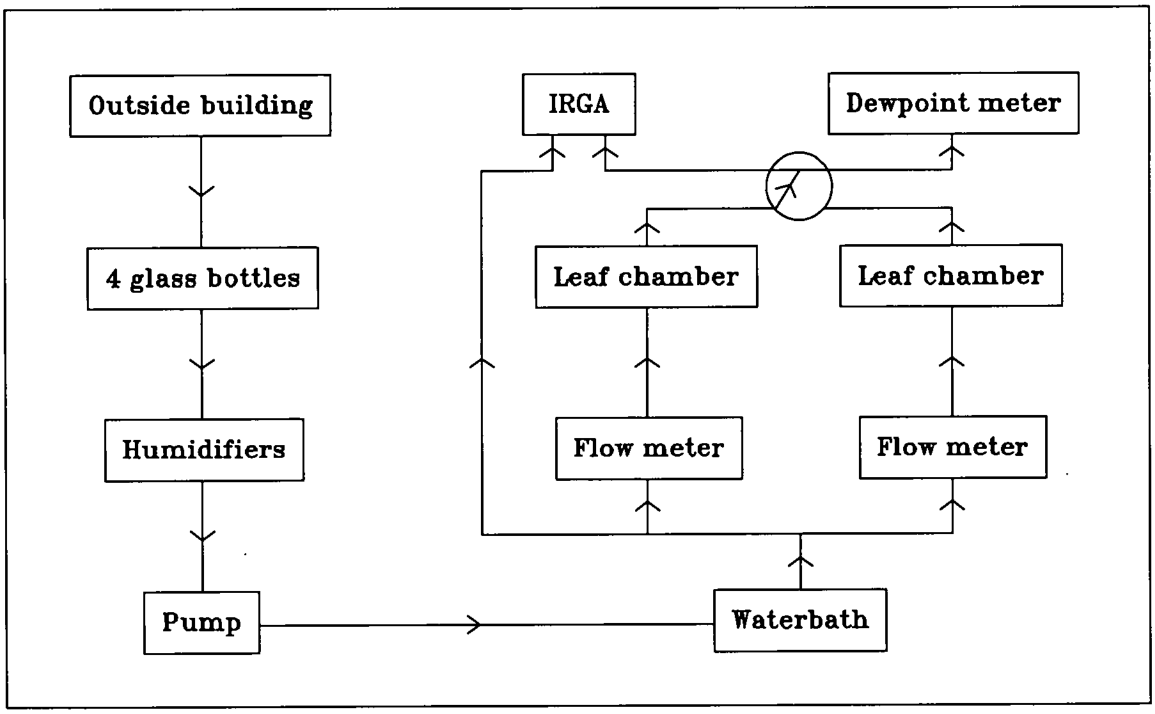


Figure 3.1. Diagrammatic representation of the gas exchange system.

downstream of the pump was higher than the initial saturated vapour pressure setting. This increased vapour pressure was enough to cause condensation in the lines at the temperature at which the room was kept, and a copper coil and water trap were therefore used. Measurements showed the humidity of the air after passing through this setup was virtually constant (water vapour pressure 1.2599 ± 0.0051 kPa, during a test run of 20 hours).

The air mixture was then fed into the two leaf chambers, through a mass flow meter for each chamber (Models 360 and 380, Tylan General (UK) Ltd., Westlea, Swindon, Wilts), using PTFE tubing (supplied by P.D. Marketing, Sidlesham, Chichester, West Sussex), with an in-line rotameter (variable-area flow meter, Field *et al.*, 1989) to each chamber controlling the air flow and serving as a secondary flow indicator. The flow meters were placed upstream of the leaf chambers, since it is usually not possible to completely seal the chambers (Pearcy *et al.*, 1989). Placing the meters downstream of the chambers would underestimate the flow rate, and therefore overestimate the assimilation rate and the stomatal conductance (Field *et al.*, 1989), which would be different for each shoot, as the entry point into the chamber for each shoot was the main problem point for sealing the chambers.

Air from one of the chambers was sampled continuously for 25 minutes at each photon flux, and at the end of this period the second chamber was sampled for three minutes. This sample air was fed through an infra-red gas analyzer (IRGA; Type 225-Mk3, Analytical Development Co. Ltd., Hoddesdon, Herts), to measure the difference in CO_2 concentration between air from the leaf chamber and ambient air, and a dew-point meter (Model 440, EG & G, Wattham, Massachusetts, U.S.A.), to measure the humidity of the sample. The measurements from the dew-point meter were used only as back-up, to check the data from the humidity sensors inside the leaf chambers. Output from the humidity sensors was used in the calculation of stomatal conductance, as these provided continuous data for both leaf chambers. Temperature and humidity probes (Model HMP 35A, Vaisala Oy, Helsinki, Finland), with the standard 0.2 μm membrane filter replaced by a plastic grid to improve the response time, were mounted on the leaf chambers, with only the thin-film capacitance humidity sensor and the platinum resistance thermometer encapsulated by the plastic grid inside the chambers.

Readings from the flow meters, IRGA, dew-point meter, and temperature and humidity probes were taken every second using a data logger (21X, Campbell Scientific Ltd., Shepshed, Leics.), the program for which is given in Appendix 2. Every 30 seconds the values were averaged, and the averages stored, together with a timer indicating the time elapsed since the last change in photon flux. These data were then transferred to a personal computer, and analyzed with purpose-written software (see Section 3.2).

Light to the leaf chambers was provided bilaterally (Leverenz & Jarvis, 1979), with metal halide lamps (Wotan HQI-R 250 W/NDL, supplied by Ian Fraser Lighting, Edinburgh), the light spectrum of which resembles the spectrum of natural sun light conditions (Bloom *et al.*, 1980). The distance to the chambers was adjusted to maintain a photon flux (photosynthetic photon flux density as defined by Shibles, 1976) incident on the chamber walls, of 600 - 650 μmol m^{-2} s^{-1}. This photon flux was reduced with one to four layers of neutral density filter (supplied by Strand Lighting Ltd., Isleworth, Middlesex), each reducing the photon flux by approximately 50 %, without changing the spectral composition (Bloom *et al.*, 1980). At the start of each measurement period a full set of photon flux measurements was made, i.e. at both walls of each of the two chambers, with zero to four neutral density filters in place, and with a sheet of black nylon cloth, which reduced the photon flux to virtually 0 μmol m^{-2} s^{-1} (six photon flux levels). Measurements were made with a home-made quantum sensor (F. Sinclair, pers. comm.) inside the leaf chamber, using the standard measurement routine of taking a reading every second, and storing 30 second averages on the data logger. After the first full set of measurements, readings were taken once every day, and only without the neutral density filters. The lower photon fluxes were calculated assuming a constant reduction due to the filters, which were always used in the same leaf chambers and in the same order. Ideally the photon flux should have been measured continuously together with the other sensors and analyzers, but no small quantum sensors were available. The incident photon flux on each shoot was assumed to be the sum of the two measured (or calculated) photon fluxes for the chamber that particular shoot was measured in, on that particular day.

3.1.1. Leaf chambers.

The leaf chambers, as presented diagrammatically in FIGURE 3.2, were built with a 2.0 mm copper sheet and 2 mm glass panes, materials chosen for their low water vapour absorbtion properties (Dixon & Grace, 1982). Their dimensions were 150 mm long, 84 mm wide and 75 mm high. The front, base and rear walls were made of copper, acting as a permanent cooling plate, as the room temperature was kept at about 4 °C below the set temperature inside the leaf chambers, so counteracting the heat input from the lamps. The two side walls and the top

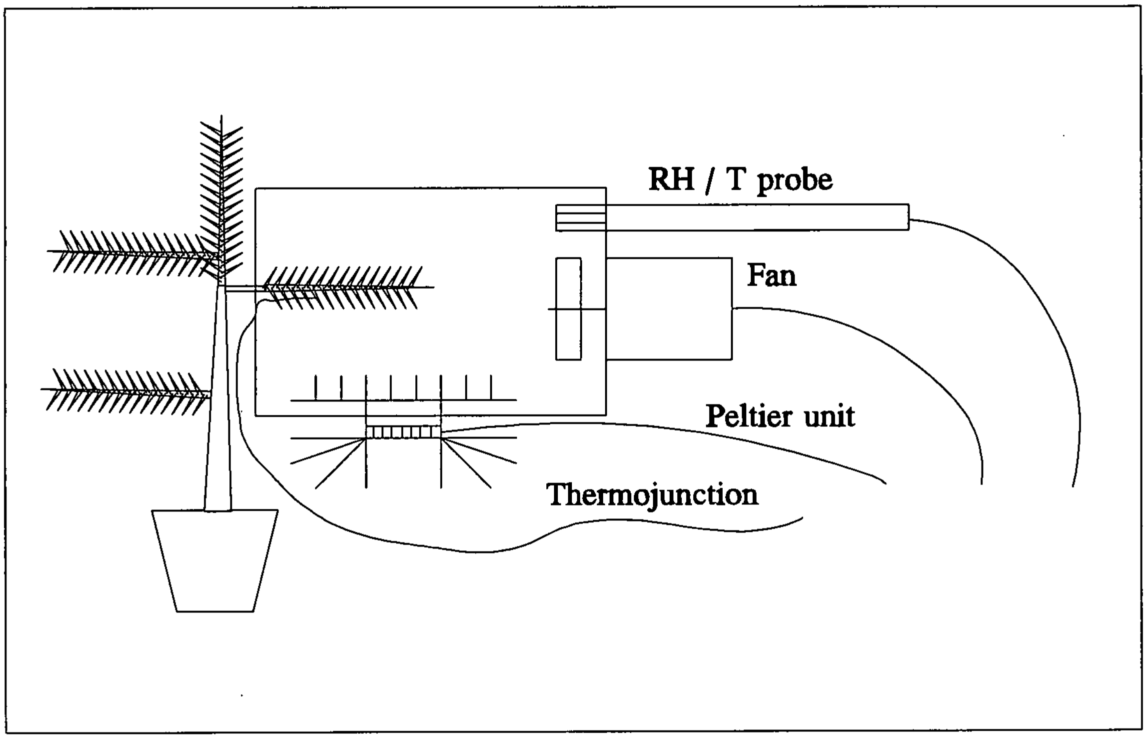


Figure 3.2. Diagrammatic representation of one of the leaf chambers, used for the gas exchange measurements.

consisted of glass panes, which allowed the chambers to be used with unilateral or bilateral lighting. The choice of materials was governed by the need to minimise the water uptake by the chamber walls, to avoid any influence of the walls on the humidity measurements (Bloom *et al.*, 1980; Dixon & Grace, 1982).

The air inside the chambers was stirred vigorously to maximise the boundary layer conductance (Field *et al.*, 1989), using small DC servo motors (Airpax, supplied by Farnell Electronic Components Ltd., Leeds), connected to the outside of the rear wall, and purpose made copper rotor blades (shaft 4.5 mm diameter and 21 mm long, with three twisted blades of 16 by 21 mm) inside the chambers. The rotor blades were encapsulated by plated steel finger guards (diam 92 mm, RS Components, Corby, Northants. Order no. 504-940), to protect the shoots.

3.1.2. Temperature control system.

The air temperature inside the chambers was controlled with Peltier units (thermoelectric heat pumps; Field *et al.*, 1989). A hole (50 by 50 mm) was cut out in the base of the leaf chambers, to allow the connection of heat sinks inside the chambers with Peltier units (40 by 40 mm, RS Components. Order no. 618-730). The heat sinks inside the chambers were made of 2.0 mm thick copper plates, 100 by 60 mm, with eight 60 by 8 mm ribs soldered onto them. To raise the heat sinks of the base of the chambers, to avoid heat transfer to and from the chamber walls, five pieces of copper (40 by 40 mm) were placed between the heat sinks and the Peltier units. On the other side of the Peltier units, underneath the chambers, commercially available heat sinks (RS Components. Order No. 403-140) were placed, to balance the heat sinks inside the chambers. All junctions between the heat sinks and the Peltier units were pasted with heat sink compound (RS Components. Order No. 554-311), to aid thermal conductivity. The Peltier unit and heat sinks were mounted onto the leaf chambers using 3 mm unplasticised thermoplastic (Vynalast, ICI Ltd, Hyde, Cheshire), to stop heat transfer, and all connections inside the chambers were sealed with silicone rubber compound.

The temperature inside the leaf chambers was controlled by a second Campbell Scientific 21X data logger. Ideally this should have been performed by the same logger that was used for the monitoring of the different analyzers and sensors, but it was found that the setting of control ports affected the readings of especially single ended channels. This was found to be unsatisfactory, as all signals were measured as single ended inputs, because of the number of channels involved. The program for this second data logger is also given Appendix 2. Every second the air temperature inside the chambers was measured with a thermojunction, and depending on these readings, digital control ports were set to adjust the temperature to a preset value. The control system, which is illustrated in FIGURE 3.3 for one of the chambers, basically consisted of two sub-systems. The first controlled the direction of the current through the Peltier unit, allowing it to both cool and heat the air inside the chambers. It is operated through the first digital control port on the 21X (DCP 1) for the first leaf chamber (DCP 3 for chamber 2), which activates a change-over relay (RS Components. Order No. 350-585), through

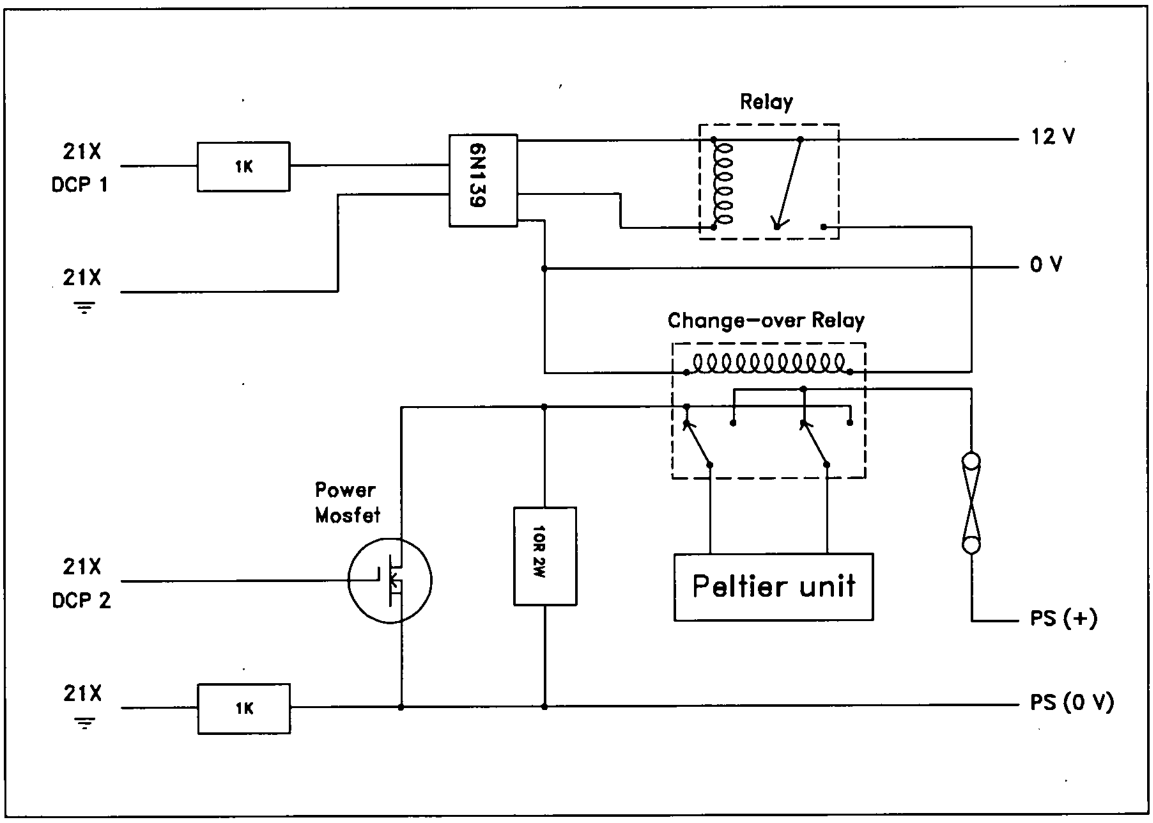


Figure 3.3. Electrical connections in the temperature control system.

a smaller relay (RS Components. Order No. 344-467), because of current consumption of the change-over relay, and an opto-isolator (6N139) to protect the data logger in case of a component failure. The second sub-system controlled the amount of current flowing through the Peltier unit, allowing cooling or heating to take place at either of two levels. It operates through DCP 2 (DCP 4), which, when set high, activated a power mosfet (RFP15N05L), allowing the higher current level to flow through the Peltier unit, causing a higher level of cooling or heating. This current level was set in such a way, that the cooling plate inside the chambers was not colder than the dew-point at the highest expected humidities, to avoid condensation, which would introduce errors in the humidity measurements. When DCP 2 (DCP 4) was set low, the power mosfet would not conduct, and only a smaller current would flow through the Peltier unit, through the resistor across the power mosfet. This served two purposes, it improved the temperature control, and increased the expected life-time of the Peltier unit.

If the air temperature inside the first leaf chamber was above 20.2 °C, DCP 1 was set high, and the Peltier unit would transfer heat from the chamber, thereby cooling the air inside the chamber. During the next execution of the program table (a second later) DCP 2 would also be set high, allowing a higher current to flow through the Peltier unit, increasing the rate at which the air was cooled. Once the temperature was reduced to below 20.0 °C, DCP 2 would be set low, reducing the current through the Peltier unit, and cooling would occur at the lower rate. This continued until the temperature would either be reduced to below 19.8 °C, in which case DCP 1 would be set low, and DCP 2 high, causing the unit to start heating the chamber at high level, or the temperature would rise again to above 20.2 °C, and DCP 2 would be set high again. A similar sequence would occur when starting from a temperature lower than 19.8 °C. The same system was used for the second leaf chamber, using DCP 3 and 4.

An example of the performance of this control system is given in FIGURE 3.4, which shows the 5-minutes averages, together with the maximum and minimum

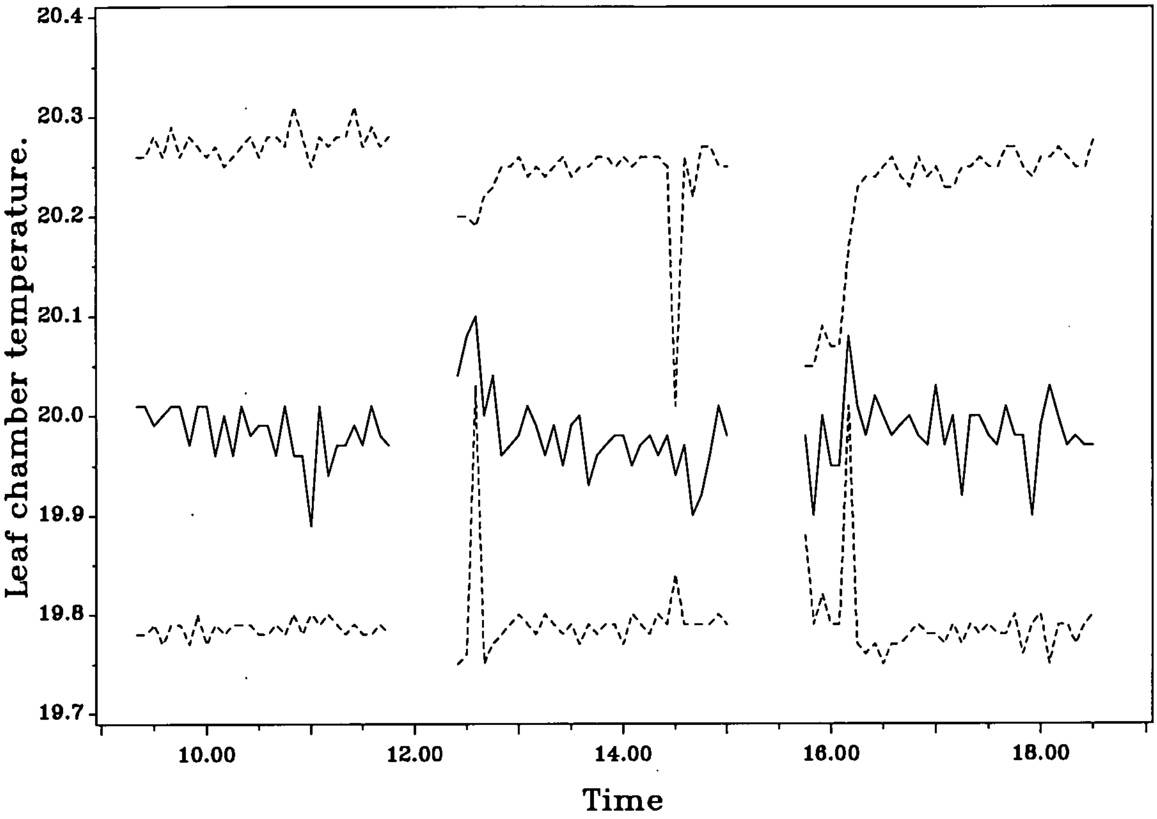


Figure 3.4. 5-minute average, maximum and minimum temperatures (°C) in leaf chamber 2 on 20 August 1991. Solid lines: average temperatures, broken lines: minima and maxima.

temperatures during those 5-minutes periods, of leaf chamber 2, for 20 August 1991. This figure shows the average temperatures during the times that shoots were in the chambers, were very close to the set temperature of 20 °C, and generally ranged between 19.8 and 20.3 °C, and at times this range was even narrower (peaks in the maximum or minimum temperatures).

The measurements were done at 20 °C, except in November 1991, when the ozone treated plants were measured at 15 °C. These temperatures were chosen to avoid large differences with the ambient conditions in which the plants were kept, while at the same time avoiding too low temperatures, which would reduce the assimilation rates (Ludlow & Jarvis, 1971).

3.1.3. Calibrations.

The Infra-Red Gas Analyzer (IRGA) was fully calibrated before every round of measurements, and quick calibration checks were carried out every other day during the measurement periods. A full calibration consisted of determining the response to changes in the CO_2 concentration in both the absolute and differential mode, measured at different absolute CO_2 concentrations. Air with an accurately known CO_2 concentration was produced by mixing CO_2-free air (ambient air drawn through columns of soda lime) and pure CO_2, using a set of three gas-mixing pumps (models 1 SA 18/3F, 1 SA 2 and 1 G 27/3F, Wosthoff, Bochum, Germany). The IRGA was calibrated for measuring absolute CO_2 concentrations, by taking the voltage output for three or four concentrations to the data logger, and measuring the signal using the normal measurement procedure of taking a reading every second, and storing an average every 30 s. A linear regression was then performed on these averages, and the resulting slope and offset were used in the program of the data logger. As the slope was found to be very stable, and only the offset fluctuated considerably (Bloom *et al.*, 1980), only the offset was checked and adjusted every other day during the measurement periods. Immediately following calibration of the IRGA in absolute mode, the CO_2 concentration of air from a cylinder of compressed ambient air was determined. This cylinder was then used for the calibration of the IRGA in differential mode, and for the quick checks afterwards. To determine the response of the IRGA in differential mode, various combinations of the cylinder air and the accurately known source produced by the pumps, were passed through the analyzer's analysis and reference cells, and the output signal was monitored by the data logger, again using the normal procedure. A straight line was then fitted to the resulting averages, and the slope and the offset were used in the data logger program. The calibration checks consisted of passing cylinder air through the analyzer's reference and analysis cells, and by making use of the division of both cells (the reference cell consists of two sub-cells, one making up 98% of the total length of the cell, and the other the remaining 2%; the analysis cell is split into

95% and 5%; Long & Hällgren, 1985).

The dew-point meter and the humidity sensors were calibrated by providing them with air from a humidification setup as described earlier on in this chapter. The output of the dew-point meter was calibrated in °C, the actual temperature at each measurement being the temperature of the water bath, as measured with a thermocouple. For the calibration of the humidity sensors inside the chambers, the saturated vapour pressure of the air was calculated by the data logger from the water bath temperature. Measurements were taken at a range of water bath temperatures, and a linear regression was performed on the averaged outputs. The resulting slopes and offsets were entered into the logger programme.

The flow meters were calibrated using a 2 dm^3 soap-film flow meter (Field *et al.*, 1989). At each of five flow rates, spanning the range used in the measurements, five readings were taken of the time needed for the soap-film to move between the 0 and 2 dm^3 gradation marks. These time were converted to flow rates, and a linear regression performed on these values plotted against the averaged voltage signals, to obtain a value for the gain and zero-offset for the flow meters. Ideally these meters should have been calibrated for molar flow, rather than volumetric flow, but this introduced a negligible error, compared to the sensitivity of the flow meters.

The platinum resistance thermometers of the Vaisala probes, were set up by placing the sensor in ice-water, to obtain a ratio of the resistances of the probe and an external resistor, placed across the terminal block of the logger (this ratio is needed for the logger programme). The sensors were set up before being built into the leaf chambers, and could therefore not be recalibrated, but as the resistances should not change over time, this was not expected to introduce any errors in the measurements.

The quantum sensor was calibrated against a factory calibrated sensor (Model

190SB, Licor Inc., Lincoln, Nebraska, U.S.A.), using the metal halide lamps of the gas exchange system as a light source.

3.2. Calculation of parameters.

The calculation of the assimilation rate (*A*) and stomatal conductance (g_s) was based on von Caemmerer & Farquhar (1981) and Long & Hällgren (1985). The flow rate (*f*) was calculated as:

$$f = \frac{f_v}{1000} \frac{1}{v_{mol,STP}} \frac{T_{STP}}{T} \frac{P}{P_{STP}} \frac{1}{60} \tag{3.1}$$

where f is the molar flow rate (mol s^{-1}), f_v is the volumetric flow rate (ml min^{-1}), $v_{mol,STP}$ is the volume of one mole of air at standard temperature and pressure (dm^3), T_{STP} is the standard temperature (273.15 K), T is the absolute temperature (K), P is the atmospheric pressure (kPa), and P_{STP} is the standard pressure (101.3 kPa).

The transpiration rate was derived from:

$$E = \frac{f}{s} \frac{v_o - v_e}{P - v_o} \tag{3.2}$$

where E represents the transpiration rate (mol m^{-2} s^{-1}), s is the leaf area (m^2), and v_e and v_o are the partial vapour pressures of the air entering and leaving the leaf chamber (kPa).

The assimilation rate was calculated as:

$$A = -\Delta c \frac{f}{s} \frac{P - v_e}{P - v_o} - E c_a \tag{3.3}$$

where A represents the assimilation rate (µmol m^{-2} s^{-1}), Δc is the difference in CO_2 concentration between ambient air and air leaving the leaf chamber (µmol mol^{-1}), and c_a is the CO_2 concentration in ambient air (µmol mol^{-1}).

Finally, the stomatal conductance was calculated as:

$$g_s \simeq E\frac{P}{v_{sat}-v_o}1000 \qquad (3.4)$$

where g_s represents the stomatal conductance (mmol m^{-2} s^{-1}), and v_{sat} is the saturated vapour pressure of air at the temperature inside the leaf chamber (kPa).

EQUATION 3.4 only approximates the stomatal conductance. It is assumed that the boundary layer conductance (g_b) is much higher than g_s, because the air inside the leaf chambers was stirred vigorously. However, no measurements of g_b were made inside the chambers. The cuticular conductance (g_c), operating in parallel with the stomatal conductance, is negligible compared to g_s (van Gardingen & Grace, 1991).

Ideally the temperature of the needles should have been measured, and the saturated vapour pressure at this temperature used, but this would inevitably have disturbed the micro-environment of several needles, and affected the measurements. As the air inside the leaf chambers was stirred vigorously, and the needles were small, and therefore well coupled to the air (van Gardingen & Grace, 1991), it was assumed the temperature of the needles was the same as that of the surrounding air.

In these calculations an allowance was made for the zero-drift of the IRGA, in both absolute and differential mode. Before and after each set of two plants were placed in the measuring system, the absolute CO_2 concentration in air was measured, together with zero-readings for the analyzer in differential mode, using the normal route for the air streams, i.e. ambient air through the reference cell, and air from the chambers through the analysis cell. This allows not only for the zero-drift of the IRGA, but also for the effects of any small pieces of plant material left unnoticed in the leaf chambers. In the computer program the drift in these three parameters, ambient CO_2 and a zero-reading for both chambers, was assumed to be constant.

The humidity of the air entering the leaf chambers was calculated in a similar way. The measurements from the two humidity sensors during these periods of ambient air passing through the chambers, were used to calculate the humidity inside the chambers without a shoot being present.

The projected needle areas were measured with an area meter (Model AMS, Delta-T Devices Ltd., Burwell, Cambridge). Two sets of pieces of green electrical wire, with different, accurately known projected areas, were measured regularly interspersed with the sets of needles. A linear regression line was fitted to the readings for the pieces of wire, and the slope and intercept of this regression were used to calculate the projected needle areas.

Values of A and g_s were calculated for each shoot at thirty second intervals during each photon flux. These were plotted against time and graphs like FIGURE 3.5 were produced for each individual set of measurements. These graphs were used to check for irregularities, and for further reference in subsequent analyses.

FIGURE 3.5 shows some cycling in the stomatal conductance, at varying degrees. This could be due to either stomatal cycling at low humidities (Long & Hällgren, 1985), or to some electrical instability of the humidity sensors inside the leaf chambers. Analysis of data collected for calibration of the humidity sensors revealed similar cycling patterns. The humidity probes used are designed to measure relative humidities, rather than water vapour pressures, and therefore slight changes in the air temperature will affect the output signals. An attempt was made to correct this, by adjusting the readings according to the saturated vapour pressures at the measured and set air temperatures. The voltage signals from the sensors were multiplied by the saturated vapour pressure at the measured air temperature, and divided by the saturated vapour pressure at the set temperature, before being converted into vapour pressure readings, but this

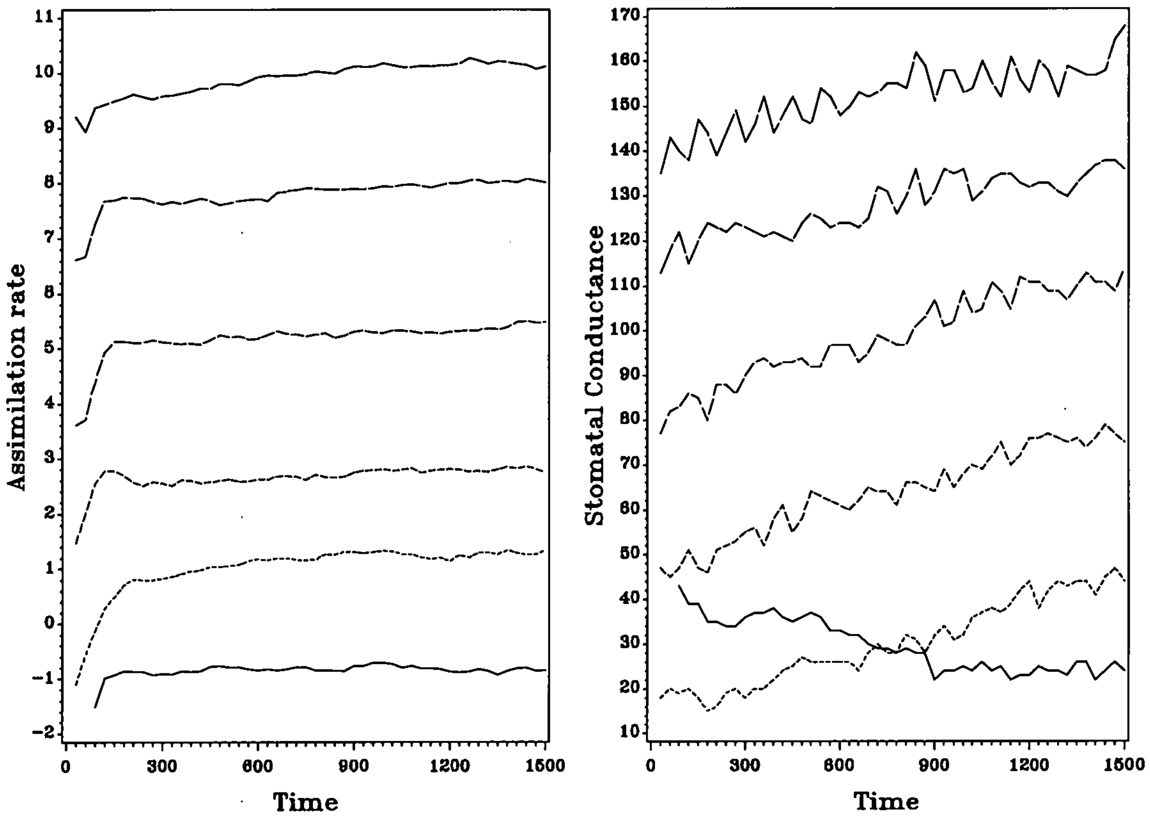


Figure 3.5. Graphical output of the analyzing computer program, for tree 70223, measured on 15 August 1991. It shows the assimilation rate (µmol m^{-2} s^{-1}) and stomatal conductance (mmol m^{-2} s^{-1}) plotted against time (s), at each of six photon flux densities (0, 78, 145, 288, 569, and 1176 µmol m^{-2} s^{-1}).

did not completely overcome the problem. However, as further calculations were performed on the average of the last ten data points at each light level, or on all data points at the highest photon flux, this cycling will have been averaged out, and is assumed to have little or no influence on the results.

For each plant measured in the gas exchange system, values were obtained for the assimilation rate at six photon fluxes. Long & Hällgren (1985) suggested that in many cases curves through the data points, plotting the assimilation rate against the photon flux, can be represented by a rectangular hyperbola:

$$A=\frac{A_{\max}Q}{k+Q} \qquad (3.5)$$

where A is the assimilation rate, A_{max} is the light saturated value for A, Q is the photon flux, and k the value of Q at which $A = A_{max} / 2$ (all in µmol m^{-2} s^{-1}).

This, however, represents the gross assimilation rate, i.e. the amount of CO_2 converted by the plant, and does not include the CO_2 that is respired at the same time. There are two ways to allow for this: 1. Add a term for the dark respiration (R_d) to EQUATION 3.5, effectively moving the curve downwards. 2. Add a term for the light compensation point ($Q_{A=0}$) to Q and k, moving the curve towards the right. The first method has the advantage that R_d is calculated as one of the primary parameters, and the main advantage for the second method is that the quantum yield (ϕ, the slope of the curve through the light compensation point) can be easily calculated mathematically, rather than using data points (e.g. Eamus & Fowler, 1990). As a value for R_d can still be simply obtained by calculating the value for A at Q=0, the second method was chosen, which resulted in the following equation:

$$A = \frac{A_{\max}(Q - Q_{A=0})}{(k - Q_{A=0}) + (Q - Q_{A=0})} \tag{3.6}$$

The parameters in EQUATION 3.6, A_{max}, $Q_{A=0}$ and k, were calculated by fitting a curve to the data points, using the least squares method described in Appendix 4.

The quantum yield is calculated as:

$$\phi = \frac{A_{\max}}{k - Q_{A=0}} \tag{3.7}$$

and the dark respiration as:

$$R_d = \frac{-A_{\max} Q_{A=0}}{k - 2Q_{A=0}} \tag{3.8}$$

For the analysis of the data collected on the effects on the stomatal conductance of the shoots, two parameters were calculated: 1. $g_{s,1200}$, the stomatal conductance at the highest photon flux, and 2. τ, a measure of the time needed to reach a new steady state after changing the light level to its highest value. τ represents the time needed by the shoots to achieve 0.632 (1 - 1/e) of the total response to changing the photon flux from about 600 to about 1200 μmol m^{-2} s^{-1} (Fritschen & Gay, 1979), and was calculated by fitting time response curves to the fifty values of g_s for the highest photon flux. The solution for the output of a sensor exposed to a step change in temperature is (Equation 3.6 in Fritschen & Gay (1979)):

$$T-T_a=(T_0-T_a)e^{-\frac{t}{\tau}} \tag{3.9}$$

where T is the temperature, T_0 is the initial temperature, T_a is the new temperature, t is the time, and τ represents the time constant (unit the same as that of t).

Replacing T by g_s, and rearranging gives:

$$\ln\left(\frac{g_{s,n}-g_s}{g_{s,n}-g_{s,o}}\right)=-\frac{t}{\tau} \tag{3.10}$$

where g_s is the stomatal conductance, $g_{s,o}$ is g_s at the time of changing the photon flux, $g_{s,n}$ is the new stabilised value of g_s.

By plotting the left-hand term of EQUATION 3.10 against t, and fitting a straight line to the data, τ can be calculated from the slope (α) of this line:

$$\tau=-\frac{1}{\alpha} \tag{3.11}$$

In these calculations, $g_{s,o}$ was estimated as the smallest calculated g_s-value, reduced by 1 mmol m^{-2} s^{-1}, and the initial $g_{s,n}$ was estimated as the largest g_s-value, increased by 1 mmol m^{-2} s^{-1}, to avoid mathematical problems of attempting to take the logarithm of zero. As the stomatal conductance did not normally stabilise within the 25-minute measuring period, the initial $g_{s,n}$ was increased by 0.05, 0.1, 0.2, 0.3, 0.5, 0.75 and 1.0 times the difference between $g_{s,n}$ and $g_{s,o}$. Linear regressions were performed for each estimate of $g_{s,n}$, and that value of τ was used where the linear regression gave the smallest error sum of squares.

3.3. Acid Mist Experiments.

3.3.1. First year, 1990.

In 1990 four open top chambers were used for the acid mist experiments. The acidity of the applied mist was pH = 2.5 in two chambers, and pH = 5.0 in the other two chambers acting as a control (see Chapter 2). In each chamber 10 Norway spruce (*Picea abies* (L.) Karst.) seedlings were used for the gas exchange measurements. Five of these received a 48 hours treatment in the wind tunnel, at 16 m s^{-1}. The other five plants in each chamber were used as a no-wind control. Before the plants were placed in the wind tunnel for 48 hours, they were all subjected to gas exchange measurements, between 22 August and 1 September, to allow more accurate analyses on the data collected after the wind treatment. Measurements were done on shoots from the top whirl of the seedlings, i.e. current year foliage. The results, obtained using the analyzing computer program (see Section 3.2), indicating effects of 14 weeks of acid mist treatment (equivalent to a deposition of 24 kg ha^{-1} N and 27 kg ha^{-1} S), are presented in TABLE 3.1. An analysis of variance (GENSTAT, 1987) on the results is provided in TABLE 3.2.

TABLE 3.2 shows there was a significant effect ($P \leq 0.05$) on the maximum assimilation rate (A_{max}), of the acid mist treatment (pH = 2.5) compared with the pH = 5.0 control (10 plants each). The average A_{max} was reduced from 13.04 to 9.97 μmol m^{-2} s^{-1} (see TABLE 3.1). An even stronger effect was found on the dark respiration rate, which was reduced very significantly ($P \leq 0.01$) from -1.28 μmol m^{-2} s^{-1} for the control plants to -0.91 μmol m^{-2} s^{-1} for the plants treated with mist at pH = 2.5. This means that the curves for the individual trees were flatter on average, i.e. showed smaller responses to changes in photon flux. This was also shown by the effect on the apparent quantum efficiency (ϕ), which was reduced from 0.024 to 0.020, although this effect was not significant ($P > 0.05$). The light

Table 3.1. Averages for the gas exchange parameters, calculated for the acid mist treated plants, measured between 22 August and 1 September, before the wind treatment in 1990. (A_{max}, $Q_{A=0}$, k and R_d in µmol m^{-2} s^{-1}, ϕ in mol mol^{-1}, $g_{s,1200}$ in mmol m^{-2} s^{-1}, and τ_{1200} in s).

	acid mist	
	pH = 5.0	pH = 2.5
A_{max}	13.04	9.97
$Q_{A=0}$	47.9	45.6
k	616	571
ϕ	0.0236	0.0200
R_d	-1.28	-0.91
$g_{s,1200}$	214	143
τ_{1200}	1884	1792

Table 3.2. Results from the analysis of variance on the calculated gas exchange parameters of the acid mist treated plants, before the wind treatment in 1990. The tabulated values represent the F-probabilities from the analysis of variance.

	acid mist
A_{max}	0.040
$Q_{A=0}$	0.787
k	0.666
ϕ	0.142
R_d	0.006
$g_{s,1200}$	0.135
τ_{1200}	0.848

compensation point ($Q_{A=0}$) and k, the photon flux at which the assimilation rate is half the maximum rate, were not affected.

There were no significant effects on any of the parameters associated with the stomatal conductance. However, the stomatal conductance at the maximum photon flux, $g_{s,1200}$, was reduced from 214 to 143 mmol m^{-2} s^{-1}, when comparing the plants treated with pH = 2.5 with plants from the control chambers. The stomatal

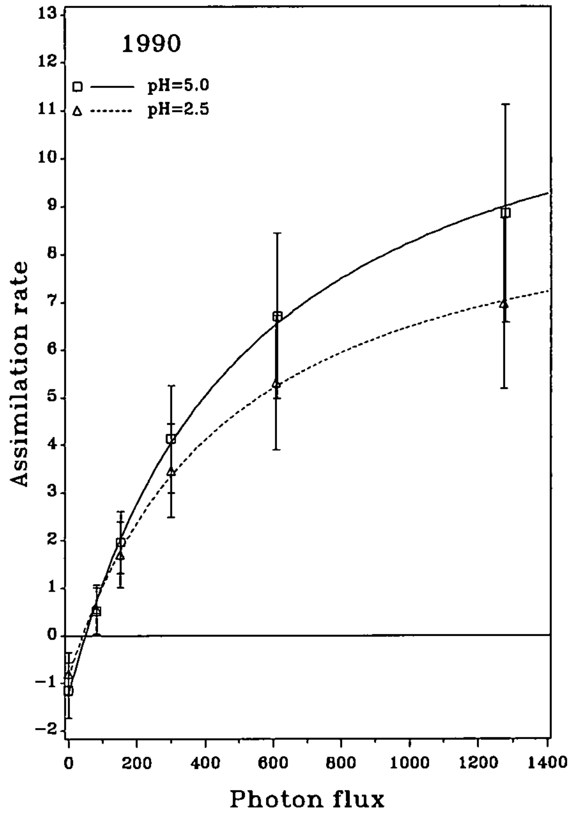


Figure 3.6. Assimilation rate (μmol m^{-2} s^{-1}) as a function of the photon flux density (μmol m^{-2} s^{-1}), for the acid mist treated plants measured between 22 August and 1 September, before the wind treatment in 1990. The figure shows the average data points (± 1 s.d.), together with the curves fitted to the data sets.

Table 3.3. Combined curve analysis performed on the lines fitted for the shoots from the acid mist experiment, measured between 22 August and 1 September 1990.

Comparison	F-probability
pH = 5.0 ↔ pH = 2.5	$P \leq 0.001$

response time to changes in the photon flux to the highest light level, τ_{1200}, was not altered by the treatment, but a similar calculation on the second highest photon flux showed a nearly significant effect ($P = 0.070$), τ_{600} being reduced from 1843 to 1408 s.

The data points for the two acid mist treatments were then pooled, and plotted together with curves (rectangular hyperbolas) fitted for each of the treatments, see FIGURE 3.6. A combined curve analysis (see Appendix 4) was performed on these fitted lines, the results of which are presented in TABLE 3.3. This showed the fitted curves were significantly different ($P \leq 0.05$).

After treating half the plants from each of the chambers in the wind tunnel for 48 hours, between 7 September and 15 September, all the plants were again subjected to gas exchange measurements. Shoots from the top whirl of the seedlings were used again, but not the same shoots as during the first round of measurements. The wind treated plants were measured within 8 hours of being removed from the wind tunnel, and the others (wind control plants) interspersed with these. Nine plants, evenly spread across treatments and chambers, were not measured again due to the need for extensive repair work to one of the leaf chambers.

The same analytical procedure was followed again, assimilation rates and stomatal conductances were calculated for individual branches, curves were fitted for each shoot, and the resulting parameters were subjected to an analysis of variance (GENSTAT allows for missing values). The averages for the gas parameters are given in TABLE 3.4, and the results of the analysis of variance are given in TABLE 3.5.

TABLE 3.5 shows three columns of F-probabilities, the first giving the likelihood of the results for the acid mist treatments, across the wind treatments, the second that of the wind treatments pooled across the mist treatments, and the third column gives the results for the interaction between the two treatments.

TABLE 3.5 shows that both the acid mist and the wind treatment significantly effected the light compensation point ($Q_{A=0}$), and the wind treatment significantly reduced the dark respiration (R_d), while the effect of the acid mist on R_d was almost significant. The acid mist treatment at pH = 2.5 on average reduced the light compensation point from 45.5 to 34.9 μmol m^{-2} s^{-1} (see TABLE 3.4), and the dark respiration from -1.14 to -0.86 μmol m^{-2} s^{-1}, as compared with the pH = 5.0 control, apparently making the plants more efficient at lower light intensities. The

Table 3.4. Averages for the gas exchange parameters, calculated for the acid mist and wind treated plants, measured between 7 and 15 September 1990. (A_{max}, $Q_{A=0}$, k and R_d in µmol m^{-2} s^{-1}, ϕ in mol mol^{-1}, $g_{s,1200}$ in mmol m^{-2} s^{-1}, and τ_{1200} in s).

	pH = 5.0		pH = 2.5	
	without wind	with wind	without wind	with wind
A_{max}	11.58	12.50	11.95	10.93
$Q_{A=0}$	47.7	43.2	42.2	27.5
k	566	579	564	535
ϕ	0.0226	0.0235	0.0234	0.0224
R_d	-1.19	-1.09	-1.06	-0.66
$g_{s,1200}$	167	176	170	142
τ_{1200}	1410	1635	1304	1466

Table 3.5. Results from the analysis of variance on the calculated gas exchange parameters of the acid mist and wind treated plants in 1990. The tabulated values represent the F-probabilities from the analysis of variance.

	acid mist	wind	interaction
A_{max}	0.217	0.953	0.251
$Q_{A=0}$	0.012	0.013	0.154
k	0.285	0.803	0.500
ϕ	0.906	0.984	0.614
R_d	0.052	0.021	0.160
$g_{s,1200}$	0.455	0.555	0.282
τ_{1200}	0.758	0.313	0.869

wind treatment had a similar effect. When compared with the no-wind control plants, it reduced $Q_{A=0}$ from 45.0 to 35.4 µmol m^{-2} s^{-1}, and R_d from -1.13 to -0.88 µmol m^{-2} s^{-1}. For both the light compensation point and the dark respiration there was a slight synergistic interaction between the two treatments, in that the combined effect was stronger than the added individual effects, all compared to the double control plants (plants not treated in the wind tunnel, and receiving mist at a pH = 5.0). Individual effects were about 10 %, whereas in both cases the combined effect was a reduction of more than 40 %. There were no effects of either acid mist or wind, nor an interaction between the two treatments, on A_{max}, k or ϕ.

TABLE 3.5 shows no effects nor interaction on the stomatal conductance parameters, $g_{s,1200}$ and τ_{1200}. There was, however, a highly significant wind effect ($P \leq 0.001$) on τ_{600} (data not presented). The average response time was reduced from 1932 s for the no-wind control plants to 1152 s for the plants treated in the wind tunnel, although τ_{1200} was slightly longer for the wind treated plants than the control plants.

Because the same plants had been measured before the wind treatment, it was possible to take the first round measurements into account at the analysis of the results of the second round, by doing an analysis of covariance (GENSTAT, 1987). This would take out any genetic variability, which was most likely to be present, as the plants that were used were seedlings, and not clonal material. The results for this analysis of covariance are given in TABLE 3.6.

TABLE 3.6 show that the results of the analysis of covariance were broadly in line with results of the analysis of variance. There was no effect on A_{max}, k or ϕ, nor an

Table 3.6. Results from the analysis of covariance on the calculated gas exchange parameters of the acid mist and wind treated plants in 1990. The tabulated values represent the F-probabilities from the analysis of covariance.

	acid mist	wind	interaction
A_{max}	0.638	0.425	0.545
$Q_{A=0}$	0.025	0.031	0.184
k	0.536	0.710	0.644
ϕ	0.404	0.417	0.451
R_d	0.654	0.017	0.163
$g_{s,1200}$	0.507	0.356	0.454
τ_{1200}	0.717	0.209	0.903

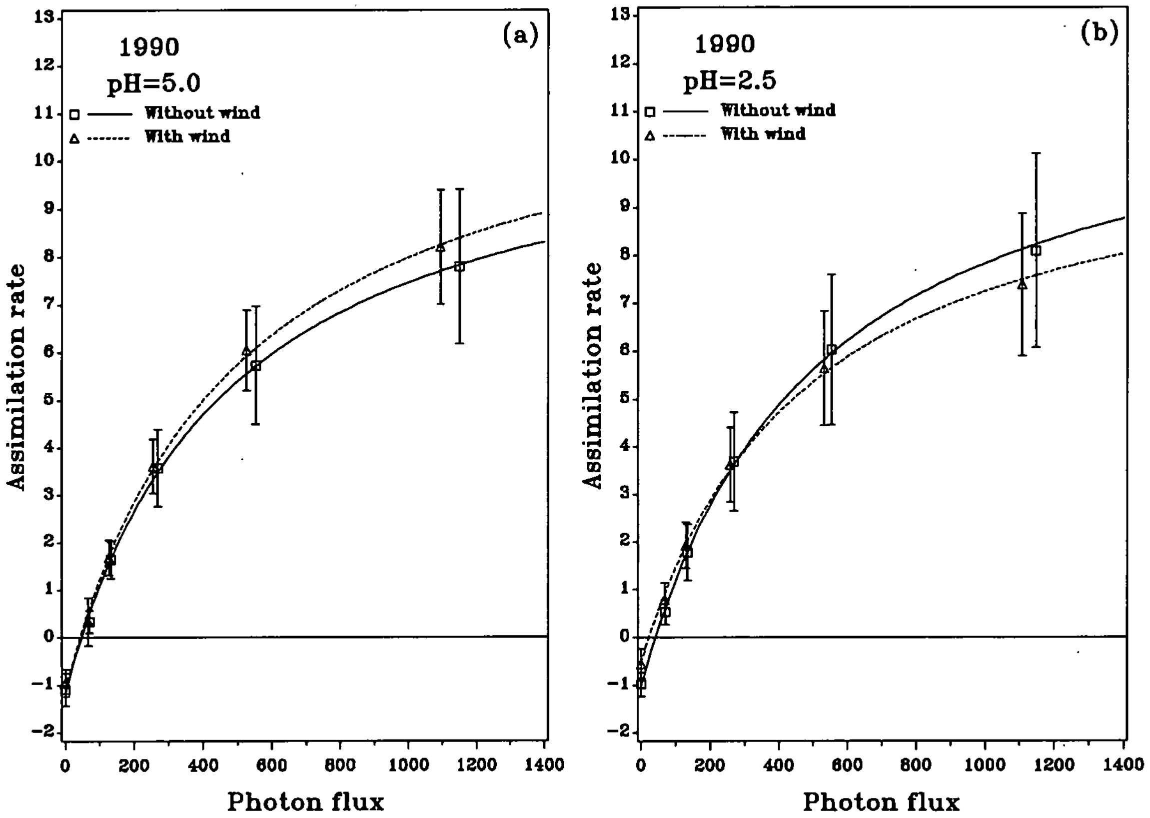


Figure 3.7. Assimilation rate (μmol m^{-2} s^{-1}) as a function of the photon flux density (μmol m^{-2} s^{-1}), for the acid mist and wind treated plants measured between 7 and 15 September 1990. The figures show the average data points (± 1 s.d.), together with the curves fitted to the data sets.

Table 3.7. Combined curve analysis performed on the lines fitted for the shoots from the acid mist experiment, measured between 7 and 15 September 1990.

Comparison	Link	F-probability
No wind ↔ wind	pH = 5.0	$P \leq 0.50$
No wind ↔ wind	pH = 2.5	$P \leq 0.50$
pH = 5.0 ↔ pH = 2.5	Combined curves	$P \leq 0.75$

interaction between the acid mist and wind treatments on these parameters. The effects of both mist and wind on the light compensation point were somewhat reduced, but still significant ($P \leq 0.05$). The wind effect on the dark respiration was slightly stronger, but the acid mist effect completely disappeared. The effect of wind on the stomatal response to changes in the photon flux from 300 to 600 μmol m^{-2} s^{-1}, τ_{600}, was still highly significant (not shown), but the analysis of covariance could also not detect an effect on τ_{1200}.

The data points of the individual trees were then pooled across the mist and wind treatments, and rectangular hyperbolas were fitted to the resulting data sets. These curves, together with the average data points at each photon flux and the standard deviation, are presented in FIGURE 3.7. FIGURE 3.7a shows the curves and data for the two wind treatments at pH = 5.0, whereas those for the pH = 2.5 treatment are shown in FIGURE 3.7b. Clearly, the significant acid mist effect found two weeks earlier, had disappeared, which was confirmed by a combined curve analysis, presented in TABLE 3.7. Two more combined curve analyses revealed the wind treatment had no effect either on the plants' response to changes in the photon flux.

3.3.2. Second year, 1991.

In 1991 the same acid mist treatments, pH = 2.5 and pH = 5.0, were applied again, but were combined with different wind treatments, see Chapter 2. An explanation for the relatively small effect of the wind treatment on the gas exchange parameters was found in the 1990 experiment, could be that by the time the wind treatment was applied, the plants had produced a sufficient epicuticular wax thickness to protect them even from a wind treatment as severe as 48 hours in a wind tunnel at about 16 m s^{-1}. Therefore, the wind treatments applied in 1990, were repeated, but a third treatment was added. Some plants were placed in the wind tunnel at the time when on average their bud scales were just breaking open, and the new needles were exposed. For each of these three wind treatments, no wind control, "spring storm" and "summer storm", two plants were kept in each of eight open top chambers, four sprayed with acid mist at pH = 2.5, and the other four with the acid mist control at pH = 5.0.

The "spring storm" treatment, plants treated at budburst, was applied between 26 April and 8 May 1991. The plants were taken from the standing out area, and were placed back there until the open top chambers were ready later in May. The "summer storm" treatment was applied between 13 August and 23 August 1991,

Table 3.8. Averages for the gas exchange parameters, calculated for the acid mist and wind treated plants, measured between 13 and 23 August, 1991 (A_{max}, $Q_{A=0}$, k and R_d in µmol m^{-2} s^{-1}, ϕ in mol mol^{-1}, $g_{s,1200}$ in mmol m^{-2} s^{-1}, and τ_{1200} in s; * and ** indicate significant effects by individual wind treatments ($P < 0.05$ and $P < 0.01$ respectively), non-marked values are not significantly different from the relevant control).

	pH = 5.0			pH = 2.5		
	no wind	budburst	summer	no wind	budburst	summer
A_{max}	12.41	13.81	12.69	11.80	12.50	12.14
$Q_{A=0}$	41.4	34.9	28.3**	39.3	36.5	31.2
k	460	453	419	435	448	442
ϕ	0.0296	0.0333	0.0330	0.0296	0.0304	0.0294
R_d	-1.37	-1.25	-0.98*	-1.29	-1.23	-1.02
$g_{s,1200}$	132	133	129	128	105	109
τ_{1200}	863	1169	1547	1271	959	1477

Table 3.9. Results from the analysis of variance on the calculated gas exchange parameters of the acid mist and wind treated plants in 1991. The tabulated values represent the F-probabilities from the analysis of variance.

	acid mist	wind	interaction
A_{max}	0.590	0.632	0.932
$Q_{A=0}$	0.781	0.002	0.628
k	0.920	0.731	0.680
ϕ	0.549	0.582	0.692
R_d	0.921	0.021	0.878
$g_{s,1200}$	0.267	0.509	0.523
τ_{1200}	0.858	0.149	0.459

and the plants receiving this treatment were subjected to the gas exchange measurements within 24 hours of being removed from the wind tunnel. The other plants, those treated at budburst, and the controls, were measured interspersed with the newly treated trees. By the end of August 1991 considerable visible damage occurred on the seedlings (see Section 5.1.), and gas exchange measurements were done on shoots from the second whorl of the seedlings, in

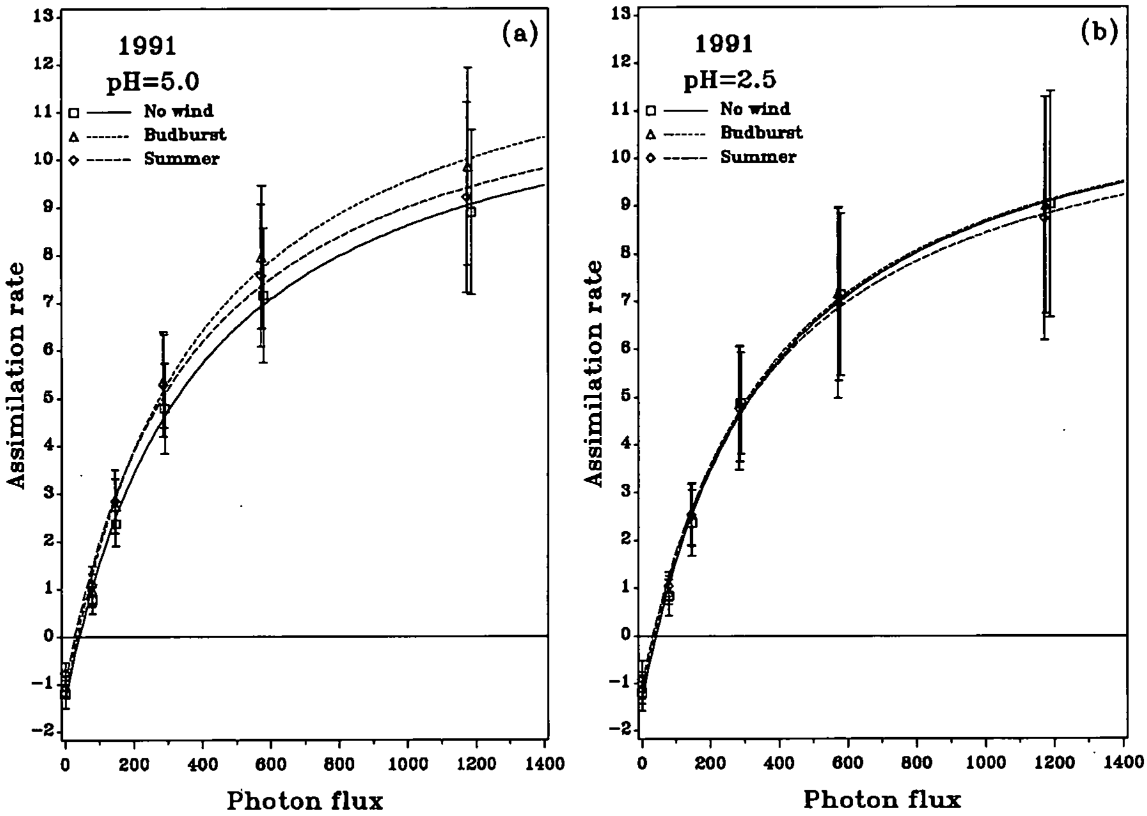


Figure 3.8. Assimilation rate (μmol m^{-2} s^{-1}) as a function of the photon flux density (μmol m^{-2} s^{-1}), for the acid mist and wind treated plants measured between 13 and 23 August 1991. The figures show the average data points (± 1 s.d.), together with the curves fitted to the data sets.

Table 3.10. Combined curve analysis performed on the lines fitted for the shoots from the acid mist experiment, measured between 13 and 23 August 1991.

Comparison	Link	F-probability
No wind ↔ budburst	pH = 5.0	$P \leq 0.10$
No wind ↔ summer	pH = 5.0	$P \leq 0.25$
No wind ↔ budburst	pH = 2.5	$P > 0.75$
No wind ↔ summer	pH = 2.5	$P > 0.75$
pH = 5.0 ↔ pH = 2.5	Combined curves	$P \leq 0.50$

order to use current-year foliage that was not visibly damaged. As in 1990, an initial analysis of variance was performed, the results from which are presented in TABLE 3.9, while the averages for the parameters are shown in TABLE 3.8.

TABLE 3.9 shows that contrary to the results in 1990, there were no differences for any of the parameters associated with the assimilation rate and the stomatal conductance, between the plants treated with acid mist at pH = 2.5 and pH = 5.0, although the plants had been sprayed for almost as long as in 1990 (12 weeks). The only significant effects found in 1991 were caused by the wind treatments. As in 1990, the light compensation point ($Q_{A=0}$) was reduced, this time very significantly ($P \leq 0.01$), by both wind treatments. For those plants treated when their buds were beginning to burst, $Q_{A=0}$ was reduced from 40.3 μmol m^{-2} s^{-1} for the control plants to 35.7 μmol m^{-2} s^{-1}, on the plants treated just before the gas exchange measurements $Q_{A=0}$ was as low as 29.7 μmol m^{-2} s^{-1}. The only other significant effect ($P \leq 0.05$) found was on the dark respiration (R_d), which was also reduced by the two wind treatments. The average R_d for the control plants was -1.33 μmol m^{-2} s^{-1}, and those for the spring and summer storms -1.24 and -1.00 μmol m^{-2} s^{-1} respectively. Again there were no interactions between the wind and acid mist treatments.

Curves were fitted to the combined data sets of trees having received the same combination of acid mist and wind treatments, see FIGURE 3.8, and to allow combined curves analyses. FIGURE 3.8a shows small differences between the wind treatments when combined with acid mist at pH = 5.0, with the plants from both wind treatments showing higher assimilation rates than the no wind control, but these differences were not significant, see TABLE 3.10. For the plants sprayed with acid mist at pH = 2.5 the wind treatments had virtually no effect, see FIGURE 3.8b and TABLE 3.10. The acid mist treatments were compared by using a combined curve analysis on the curves fitted to the combined data sets for the two mist treatments, and this analysis showed there was very little effect of the acid mist treatment on the assimilation rates, confirming what was found on individual parameters.

3.4. Ozone Experiments.

3.4.1. First year, 1990.

As with the acid mist experiment, space was limited in the chambers designated to the ozone fumigation experiments, and for the gas exchange measurements only ten Norway spruce seedlings could be accommodated in each of four open-top chambers. Two chambers were provided with charcoal filtered air only, to serve as a control, while the other two were fumigated with ozone at times when the weather conditions were favourable for natural ozone production (see Chapter 2). Five of the plants in each open-top chamber received a 48 hrs treatment in the wind tunnel, at 16 m s^{-1}. The other five plants in each chamber were used as a no-wind control. Before the plants were wind treated, they were all subjected to gas exchange measurements, between 1 and 8 September, to investigate ozone treatment responses with 10 replicate plants in each chamber, and more accurate analyses on the data collected after the wind treatment. Current-year foliage was used for the gas exchange measurements, on shoots from the top whirl of the seedlings. On the results from the data analysis, for which the averages for the gas exchange parameters are presented in TABLE 3.11, an analysis of variance was performed (GENSTAT, 1987), in order to detect any effects of 200 hours of ozone treatment on the various parameters calculated for individual shoots. The results of this ANOVA are given in TABLE 3.12.

The only significant effect found (TABLE 3.12), was on the stomatal conductance at the maximum photon flux ($g_{s,1200}$), which was reduced from 158 to 143 mmol m^{-2} s^{-1} (see TABLE 3.11) by the ozone treatment, as compared to the charcoal filtered control ($P \leq 0.05$). There was also a slight indication that the ozone treatment affected the light compensation point ($Q_{A=0}$; reduced from 36.1 µmol m^{-2} s^{-1} for the control plants to 33.2 µmol m^{-2} s^{-1} for the ozone treated plants) and the dark respiration (R_d; reduced from -0.89 to -0.80 µmol m^{-2} s^{-1}), but neither of these effects were significant.

Table 3.11. Averages for the gas exchange parameters, calculated for the ozone treated plants, measured between 1 and 8 September, before the wind treatment in 1990. (A_{max}, $Q_{A=0}$, k and R_d in µmol m^{-2} s^{-1}, ϕ in mol mol^{-1}, $g_{s,1200}$ in mmol m^{-2} s^{-1}, and τ_{1200} in s).

	ozone	
	filtered air	140 ppb ozone
A_{max}	10.40	9.38
$Q_{A=0}$	36.1	33.2
k	503	460
ϕ	0.0230	0.0221
R_d	-0.89	-0.80
$g_{s,1200}$	158	143
τ_{1200}	1242	1186

Table 3.12. Results from the analysis of variance on the calculated gas exchange parameters of the ozone treated plants, before the wind treatment in 1990. The tabulated values represent the F-probabilities from the analysis of variance.

	ozone
A_{max}	0.295
$Q_{A=0}$	0.141
k	0.290
ϕ	0.360
R_d	0.126
$g_{s,1200}$	0.040
τ_{1200}	0.800

The data points were then pooled for the two ozone treatments, and rectangular hyperbolas fitted to the data points for the two treatments. A combined curve analysis performed to test for an ozone effect on the combined light response curves for each of the two treatments. The fitted curves are presented in FIGURE 3.9, together with the average assimilation rates at each photon flux and their standard deviation. The results from the combined curve analysis are shown in TABLE 3.13.

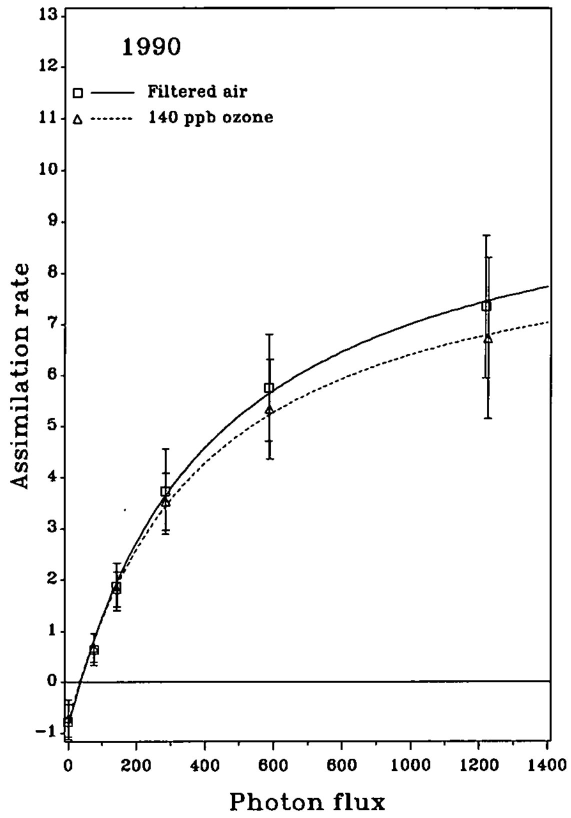


Figure 3.9. Assimilation rate (µmol m^{-2} s^{-1}) as a function of the photon flux density (µmol m^{-2} s^{-1}), for the ozone treated plants measured between 1 and 8 September, before the wind treatment in 1990. The figure shows the average data points (± 1 s.d.), together with the curves fitted to the data sets.

Table 3.13. Combined curve analysis performed on the lines fitted for the shoots from the acid mist experiment, measured between 1 and 8 September 1990.

Comparison	F-probability
Filtered air ↔ 140 ppb ozone	$P \leq 0.05$

It was found that the two fitted lines were significantly different from each other ($P \leq 0.05$). This shows that although none of the individual parameters associated with the assimilation rates showed significant effects, due to the considerable variation between the trees, combining the data points indicated that 200 hours of ozone fumigation at 140 nmol mol^{-1} did have an effect on the plants. Overall, assimilation rates were decreased by approximately 10%.

Five plants from each of the four chambers were then placed in the wind tunnel,

Table 3.14. Averages for the gas exchange parameters, calculated for the ozone and wind treated plants, measured between 29 September and 5 October 1990. (A_{max}, $Q_{A=0}$, k and R_d in µmol m^{-2} s^{-1}, ϕ in mol mol^{-1}, $g_{s,1200}$ in mmol m^{-2} s^{-1}, and τ_{1200} in s).

	filtered air		140 ppb ozone	
	without wind	with wind	without wind	with wind
A_{max}	11.95	10.65	8.99	10.72
$Q_{A=0}$	33.7	35.9	34.7	40.5
k	555	542	435	568
ϕ	0.0235	0.0206	0.0218	0.0206
R_d	-0.84	-0.82	-0.81	-0.90
$g_{s,1200}$	159	128	140	139
τ_{1200}	1494	1569	1175	1295

Table 3.15. Results from the analysis of variance on the calculated gas exchange parameters of the ozone and wind treated plants in 1990. The tabulated values represent the F-probabilities from the analysis of variance.

	ozone	wind	interaction
A_{max}	0.249	0.843	0.162
$Q_{A=0}$	0.379	0.429	0.721
k	0.196	0.202	0.125
ϕ	0.461	0.078	0.467
R_d	0.654	0.758	0.629
$g_{s,1200}$	0.677	0.170	0.191
τ_{1200}	0.124	0.602	0.904

between 29 September and 5 October, for a treatment of 48 hours at a wind speed at plant level of 16 m s^{-1}. All the plants were then measured again, the wind treated plants during the same day they were removed from the wind tunnel. Shoots from the top whorl of the seedlings were used again, but different shoots from those used for the first series of measurements. The data were analyzed in the same way as before, the different parameters were calculated for each individual shoot, the averages for which are presented in TABLE 3.14, and an

analysis of variance was done to check for any treatment effects, see TABLE 3.15.

TABLE 3.15 shows there were no significant effects. There were, however, again a few indications that the two treatments did alter the gas exchange mechanisms to some extent. The apparent quantum efficiency (ϕ) was reduced by the wind treatment (on average from 0.0227 to 0.0206; see TABLE 3.14), the wind also affected the stomatal conductance, measured at the highest photon flux ($g_{s,1200}$), which was reduced from 150 to 134 mmol m^{-2} s^{-1}, and the time needed to respond to the change in photon flux (τ_{1200}) was reduced by the ozone treatment, from 1532 to 1235 s. But none of these effects were significant.

As with the 1990 acid mist experiment, an analysis of covariance was performed on the data collected after the wind treatment of the plants from the ozone experiment, using the data set from the first round of measurements as a reference for the plants' responses in the gas exchange system. The results are presented in TABLE 3.16.

This table shows an effect of wind on the apparent quantum efficiency, which was reduced. The analysis of variance already showed some indication of this effect,

Table 3.16. Results from the analysis of covariance on the calculated gas exchange parameters of the ozone and wind treated plants in 1990. The tabulated values represent the F-probabilities from the analysis of covariance.

	ozone	wind	interaction
A_{max}	0.640	0.463	0.191
$Q_{A=0}$	0.295	0.546	0.470
k	0.280	0.349	0.683
ϕ	0.309	0.035	0.194
R_d	0.358	0.929	0.424
$g_{s,1200}$	0.062	0.125	0.601
τ_{1200}	0.149	0.912	0.633

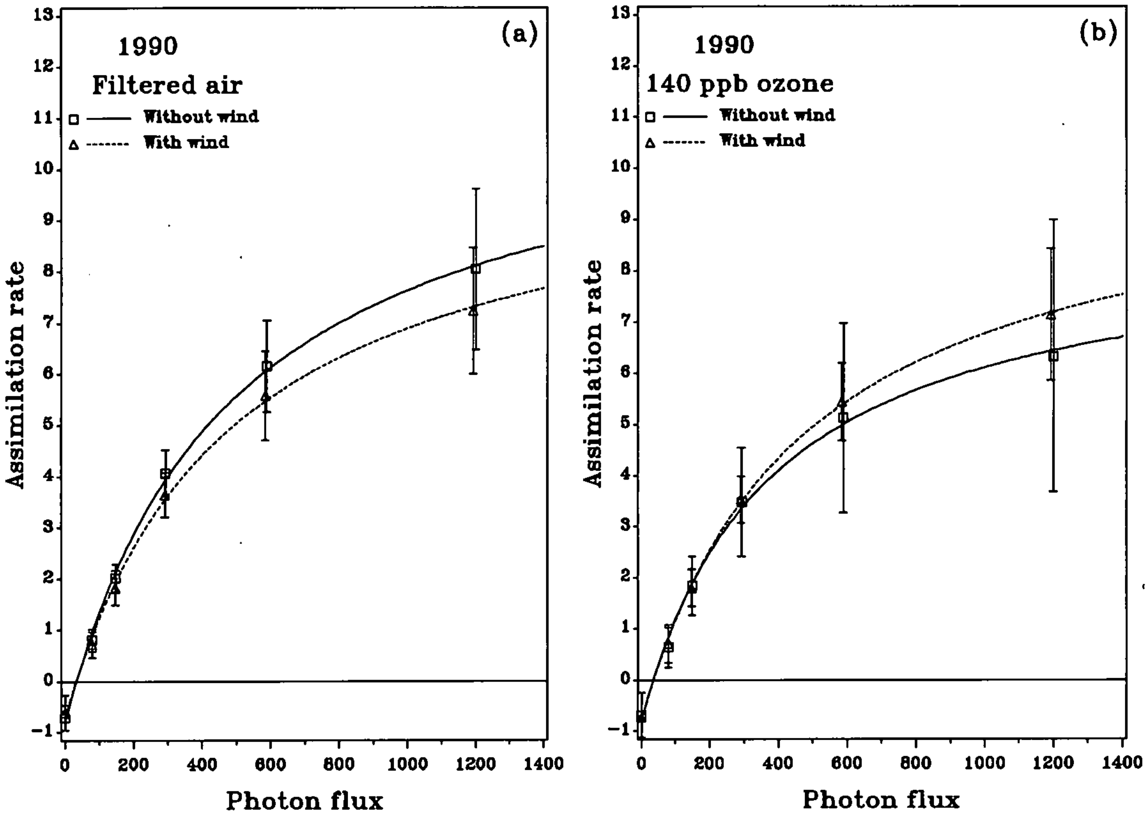


Figure 3.10. Assimilation rate (μmol m^{-2} s^{-1}) as a function of the photon flux density (μmol m^{-2} s^{-1}), for the ozone and wind treated plants measured between 29 September and 5 October 1990. The figures show the average data points (± 1 s.d.), together with the curves fitted to the data sets.

Table 3.17. Combined curve analysis performed on the lines fitted for the shoots from the acid mist experiment, measured between 29 September and 5 October 1990.

Comparison	Link	F-probability
No wind ↔ wind	Filtered air	$P \leq 0.025$
No wind ↔ wind	140 ppb ozone	$P \leq 0.25$
Filtered air ↔ 140 ppb ozone	Combined curves	$P \leq 0.01$

but taking the first round of measurements into account, this effect was significant ($P \leq 0.05$). The wind treatment also appeared to have a lowering effect on the highest stomatal conductance ($g_{s,1200}$), but this was again not significant. One other effect that was nearly significant (P = 0.062), was that of the ozone treatment on $g_{s,1200}$, which appeared to be increased by the ozone treatment as compared to the charcoal filtered control. The analysis of variance showed no effect (P = 0.677), but taking the previous history of the plants into account, an analysis of covariance interpreted this as an effect opposite to that in the first round.

Rectangular hyperbolas were fitted for each of the ozone and wind treatment combinations, after pooling the data for each of the four combinations of wind and ozone treatments. The curves fitted to the data points are shown in FIGURE 3.10, together with the average and the standard deviation of the data points at each photon flux. FIGURE 3.10a presents the wind treatments combined with a treatment in the open-top chambers with charcoal filtered air, and the wind treatments combined with the 140 nmol mol^{-1} ozone treatment are given in FIGURE 3.10b.

The combined curve analysis, presented in TABLE 3.17, showed the two lines fitted to the data points for the two wind treatments combined with charcoal filtered air were significantly different ($P \leq 0.05$), the wind treatment reducing the assimilation rates (see FIGURE 3.10a). FIGURE 3.10b shows the wind effect was reversed when the plants were also treated with 140 nmol mol$_{-1}$, but this effect was not significant (see TABLE 3.17). The combined curve analysis on the combined data sets for the two ozone treatments showed the plants' assimilation response to changes in the photon flux was very significantly reduced by the ozone treatment ($P \leq 0.01$).

3.4.2. Second year, 1991.

In 1991 fewer experiments were carried out on ozone out at the Institute of Terrestrial Ecology, and therefore more space was available for this project. This gave the opportunity to increase the number of wind treatments (see Chapter 2). In addition to the no-wind control, four different wind treatments were applied: 1. During the dormant period, before the 1991 growing season, applied between 20 and 28 March. 2. At budburst, applied between 26 April and 6 May. 3. Towards the end of the growing season, between 26 and 30 September, repeating

Table 3.18. Averages for the gas exchange parameters, calculated for the ozone and wind treated plants, measured between 18 November and 11 December, 1991 (A_{max}, $Q_{A=0}$, k and R_d in µmol m^{-2} s^{-1}, ϕ in mol mol^{-1}, $g_{s,1200}$ in mmol m^{-2} s^{-1}, and τ_{1200} in s; *** indicates a significant effect by individual wind treatments ($P < 0.001$), non-marked values are not significantly different from the relevant control).

	filtered air				
	no wind	winter	budburst	autumn	120 hrs
A_{max}	10.02	11.22	10.68	11.76	10.39
$Q_{A=0}$	21.6	18.4	12.3	21.6	24.2
k	351	362	377	399	368
ϕ	0.0308	0.0336	0.0308	0.0315	0.0305
R_d	-0.72	-0.66	-0.49	-0.72	-0.83
$g_{s,1200}$	113	104	110	114	115
τ_{1200}	1153	1112	1181	1166	2262^{***}
	140 ppb ozone				
	no wind	winter	budburst	autumn	120 hrs
A_{max}	10.52	12.47	12.28	9.49	8.15
$Q_{A=0}$	16.0	29.5	20.9	24.5	22.7
k	406	478	427	338	306
ϕ	0.0314	0.0281	0.0306	0.0304	0.0278
R_d	-0.63	-0.80	-0.69	-0.80	-0.73
$g_{s,1200}$	130	110	116	108	94
τ_{1200}	1009	1071	971	1032	1177

the 1990 treatment. 4. A 120 hours treatment, applied between 13 and 23 August. The ozone treatments were the same as in 1990, a charcoal filtered control, and a treatment applied under the meteorological conditions specified in Chapter 2, aimed at fumigating the plants with 140 nmol mol^{-1}, in each of two replicate chambers. The plants received 265 hours of ozone fumigation.

From each open-top chamber, four plants were used for each of the five wind treatments, except for the 120 hours treatment, for which only two plants were

Table 3.19. Results from the analysis of variance on the calculated gas exchange parameters of the ozone and wind treated plants in 1991. The tabulated values represent the F-probabilities from the analysis of variance.

	ozone	wind	interaction
A_{max}	0.993	0.157	0.158
$Q_{A=0}$	0.263	0.555	0.508
k	0.594	0.457	0.227
ϕ	0.693	0.882	0.412
R_d	0.514	0.805	0.887
$g_{s,1200}$	0.835	0.804	0.798
τ_{1200}	0.015	0.016	0.058

available from each chamber, due to operational problems with the wind tunnel. These plants were subjected to gas exchange measurements between 18 November and 11 December 1991, at a reduced temperature inside the leaf chambers of 15 °C. As no visible injury occurred on the seedlings from the the ozone experiment, no change was made in the choice of shoots, contrary to the acid mist experiment in 1991. Current-year shoots from the top whirl of the seedlings were used for the gas exchange measurements.

The data were analyzed as before, and the averages for the individual parameters are given TABLE 3.18, while the results of the analysis of variance are presented in TABLE 3.19.

TABLE 3.19 shows a significant effect ($P \leq 0.05$) of both the ozone and wind treatments, and an almost significant ($P = 0.058$) interaction between the two treatments, on the shoots' time needed to respond to the photon flux changing to its highest level (τ_{1200}). The ozone treatment on average reduced τ_{1200} from 1276 s to 1038 s (see TABLE 3.18), while the wind effect found was entirely due to the 120 hours treatment, for which the response time was 1720 s on average, compared with 1076 to 1099 s for the other four wind treatments. The same 120

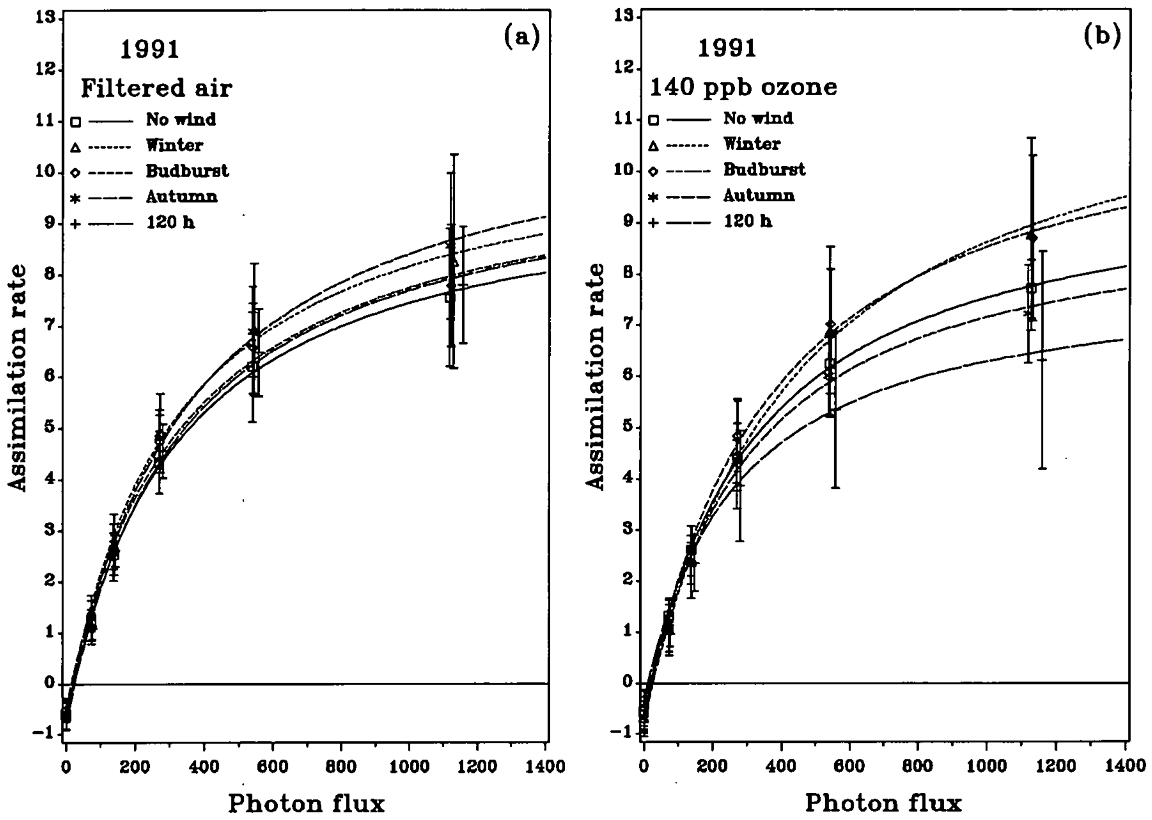


Figure 3.11. Assimilation rate (μmol m^{-2} s^{-1}) as a function of the photon flux density (μmol m^{-2} s^{-1}), for the ozone and wind treated plants measured between 18 November and 11 December 1991. The figures show the average data points (± 1 s.d.), together with the curves fitted to the data sets.

Table 3.20. Combined curve analysis performed on the lines fitted for the shoots from the ozone experiment, measured between 18 November and 11 December 1991.

Comparison	Link	F-probability
No wind ↔ winter	Filtered air	$P \leq 0.25$
No wind ↔ budburst	Filtered air	$P \leq 0.50$
No wind ↔ autumn	Filtered air	$P \leq 0.025$
No wind ↔ 120 hrs	Filtered air	$P > 0.75$
No wind ↔ winter	140 ppb ozone	$P \leq 0.05$
No wind ↔ budburst	140 ppb ozone	$P \leq 0.025$
No wind ↔ autumn	140 ppb ozone	$P \leq 0.25$
No wind ↔ 120 hrs	140 ppb ozone	$P \leq 0.01$
Filtered air ↔ 140 ppb ozone	Combined curves	$P \leq 0.50$

hours wind treatment was also responsible for the interaction found: while the ozone treatment slightly reduced τ_{1200} when combined with the other four wind treatments, combined with the 120 hours treatment the effect was a reduction of almost 50% (from 2262 to 1177 s). Another effect, although not significant, was that of the wind treatments on the maximum assimilation rate (A_{max}). The three 48 hours wind treatments all increased A_{max}, the later the treatment was applied, the smaller the increase was, and the 120 hours wind treatment reduced A_{max}, when compared to the no-wind control. The interaction between the ozone and wind treatments on A_{max} consisted of varying responses within the wind treatments to ozone.

The data points were then pooled for the wind and ozone treatments, and rectangular hyperbolas were fitted to the combined data sets. These data sets (averages and standard deviations) and fitted curves are presented in FIGURE 3.11a for wind treatments combined with charcoal filter air, and in FIGURE 3.11b for the wind treatments combined with 140 nmol mol^{-1} ozone.

The combined curve analysis, presented in TABLE 3.20, showed there was virtually no difference between the two combined lines for the ozone treatments, and that there were significant ($P \leq 0.05$) differences between the wind treatments and the relevant controls. For the plants treated with charcoal filtered air a wind treatment at the end of the growing season increased the assimilation rates, while the other three wind treatments had little effect. For plants from the ozone fumigated chambers the autumn storm had no effect, while the other three wind treatments did affect the plants' response to changes in the photon flux. The winter and budburst storms significantly increased the assimilation rates, and the 120 hrs treatment in the wind tunnel very significantly ($P \leq 0.01$) reduced the assimilation rate.

3.5. Discussion.

3.5.1. Maximum assimilation rate.

Numerous reports are available on the effects of air pollutants on the maximum assimilation rate (A_{max}) of plants, especially on the effects of ozone. In general the application of ozone to plants has been shown to result in reduced photosynthetic rates, as shown in TABLE 3.21, which summarises a number of reports found in the literature. Frequently, the reductions were closely correlated with the absorbed dose, or the total exposure (cumulative average concentration over time). Some workers found no effect of ozone on A_{max}, but this is probably related to the amount of ozone absorbed through the stomata (Reich, 1987), as some species have relatively low stomatal conductances, which renders them less susceptible to ozone fumigation (e.g. Foster *et al.*, 1990). There are also a few reports of increased maximum assimilation rates, all by Eamus and co-workers, working with Norway spruce (Eamus *et al.*, 1989*a*; Eamus *et al.*, 1990; Eamus & Murray, 1991). This apparent discrepancy was explained by the timing of the measurements. Eamus and co-workers did their experiment in early spring, while most other reports are on measurements carried out in autumn. Eamus & Murray (1991) suggested that ozone fumigated trees were photosynthetically more active, and responded faster to changes in temperature in spring, in parallel with their reduced frost hardiness. However, Sasek *et al.* (1991) found a reduction in maximum assimilation rates, even though they did some of their measurements in May 1988, before any 1988 flush had been produced, but they used loblolly pine (*Pinus taeda* L.) seedlings, a species adapted to warmer climates than Norway spruce (Dallimore & Jackson, 1966), and it seems likely the dormancy necessary for a sufficient frost hardiness, is not quite as deep as in Norway spruce, and does not affect the assimilation as much in loblolly pine.

The data collected for the ozone treated plants in this study agree reasonably well with the reported literature. Before the wind treatment in September 1990 there

Table 3.21. Effects of ozone, acid mist and wind on the maximum assimilation rate (A_{max}) of plants, as reported in the literature. (1 ppb ozone = 1 nmol mol^{-1} ozone)

Source	**Treatment**	**Species**	**Effect**
Ozone:			
Temple *et al.*, 1988	111 ppb	*Gossypium hirsutum*	decreased
Rowland-Bamford *et al.*, 1989	150 ppb	*Hordeum vulgare*	decreased
McLaughlin & McConathy, 1983	200 ppb	*Phaseolus vulgaris*	decreased
Barnes & Pfirrmann, 1992	73 ppb	*Raphanus sativus*	decreased
Retzlaff *et al.*, 1991	117 ppb	Several fruit and tree species	decreased
Eamus & Murray, 1991	140 ppb	*Fagus sylvatica*	no effect
Leonardi *et al.*, 1990	110 ppb	*Fagus sylvatica*	decreased
Tjoelker & Luxmoore, 1991	108 ppb	*Liriodendron tulipifera*	no effect
Keller & Matyssek, 1990	100 ppb	*Picea abies*	decreased in fertilised plants, larger decrease in non-fertilised plants
Wallin *et al.*, 1990	50 ppb	*Picea abies*	decreased
Keller & Häsler, 1987	150 ppb	*Picea abies*	decreased
Wallin *et al.*, 1992*b*	50 ppb	*Picea abies*	decreased
Eamus *et al.*, 1989*a*	Ambient + 50 ppb	*Picea abies*	increased
Barnes *et al.*, 1990*b*	100 ppb	*Picea abies*	no effect
Eamus *et al.*, 1990	100 ppb	*Picea abies*	increased
Eamus & Murray, 1991	140 ppb	*Picea abies*	increased
Skärby *et al.*, 1987	200 ppb	*Pinus sylvestris*	no effect
Wiselogel *et al.*, 1991	320 ppb	*Pinus taeda*	decreased
Tjoelker & Luxmoore, 1991	108 ppb	*Pinus taeda*	no effect
Sasek *et al.*, 1991	130 ppb	*Pinus taeda*	decreased
Sasek & Richardson, 1989	92 ppb	*Pinus taeda*	decreased
Richardson *et al.*, 1992	124 ppb	*Pinus taeda*	decreased
Spence *et al.*, 1990	120 ppb	*Pinus taeda*	decreased
Foster *et al.*, 1990	150 ppb	*Quercus alba*	no effect

Table 3.21. Continued.

Source	**Treatment**	**Species**	**Effect**
Acid mist:			
Eamus & Murray, 1993	pH = 2.5	*Picea abies*	increased
van Elsacker & Impens, 1989	pH = 4.0	*Picea abies*	decreased
Eamus & Fowler, 1990	pH = 2.5	*Picea rubens*	increased per unit leaf area, decreased per unit chlorophyll
McLaughlin & Tjoelker, 1992	rain pH = 3.8, mist pH = 3.0	*Picea rubens*	decreased in natural soil, increased in fertilised soil
Sasek *et al.*, 1991	pH = 3.3	*Pinus taeda*	no effect
Richardson *et al.*, 1992	pH = 3.5	*Pinus taeda*	no effect
Neufeld *et al.*, 1985	pH = 2.0	*Platanus occidentalis*	decreased
Martens *et al.*, 1989	pH = 4.0	*Populus*	decreased
Wind:			
Russell & Grace, 1978*b*	7.4 m s^{-1}	*Festuca arundicacea*	no effect
Russell & Grace, 1978*b*	7.4 m s^{-1}	*Lolium perenne*	no effect

was no significant difference in the maximum assimilation rates, but there was a 10% reduction in A_{max}, and when curves, fitted to the combined data sets were compared, the difference between them was statistically significant ($P \leq 0.05$). Four weeks later, after the wind treatment, there were again only non-significant differences in A_{max}, calculated for the individual seedlings, but the combined curves for the ozone treatments were very significantly different ($P \leq 0.01$). In 1991 the measurements were carried out at the end of the November and early December, and no differences due to the ozone treatment were found, even though the ozone exposure period, and presumably the absorbed dose, was about 30% larger than in 1990. This could indicate a combination of two factors: damage caused by the ozone treatment resulted in a reduced maximum assimilation rate, while at the same time A_{max} of the control plants was reduced due to the deeper dormancy, necessary for a sufficient frost hardiness. This will be discussed in Chapter 6, when the results from the frost hardiness measurements will also be taken into account.

The effects of acid mist on the maximum assimilation rate are not as clear cut as those by ozone, and appear to be influenced by other parameters, such as plant nutrition. Damage often occurs (Neufeld *et al.*, 1985; van Elsacker & Impens, 1989; Martens *et al.*, 1989), but acid mist can also provide the plants with an additional source of nutrients, especially nitrogen, which, when other nutrients such as calcium and magnesium are available in sufficient amounts, may result in increases in A_{max} (McLaughlin & Tjoelker, 1992), probably due to increased chlorophyll levels (Eamus & Fowler, 1990).

The results from this study appear to follow this apparent general pattern. Although the plants in 1990 were supplied with sufficient amounts of fertilizer, this was later found to result in unbalanced nutrient levels in the plant tissues. This caused A_{max} to be significantly reduced by the acid mist treatment before the wind treatment in 1990, by some 24%, and the difference between the rectangular hyperbolas fitted to the combined data sets was highly significant ($P \leq 0.001$). In 1991 a better balanced fertilizer was used, and A_{max} was not significantly affected. The slight reduction caused by the acid mist treatment at pH = 2.5 was regarded as random variation. The lack of an effect by the acid mist treatment after exposure of half the plants in the wind tunnel in 1990, may be related to the onset of the winter hardening process in the acid mist control plants, but this will be further discussed in Chapter 6.

Exposure to high wind speeds is not expected to alter maximum assimilation rates directly, and any effects reported in the literature are usually attributed to stomatal closure (Caldwell, 1970), increased mesophyll resistance due to a reduced water content (Grace & Thompson, 1973), or to changes in the orientation of foliage with respect to available radiation (Caldwell, 1970). This was also found in this study, as the wind treatments in both 1990 and 1991, in both the acid mist and ozone experiments had no effect on A_{max}.

3.5.2. Light compensation point.

The light compensation point, $Q_{A=0}$, is closely correlated with the dark respiration (R_d) and the apparent quantum efficiency (ϕ), and is therefore not often reported in studies on air pollution effects on plants. Foster *et al.* (1990) did mention $Q_{A=0}$ in a study on white oak (*Quercus alba* L.) seedlings, exposed to a varying ozone regime, but found no significant differences. They also did not detect any significant differences in light saturated net photosynthesis or stomatal conductance, dark respiration, or apparent quantum yield, and suggested that white oak was relatively insensitive to ozone due to its low stomatal conductance. Reich (1983) investigated the effects of chronic exposure of hybrid poplar (*Populus deltoides x trichocarpa*) to 125 nmol mol^{-1} of ozone, and found that the light compensation point was only temporarily increased, during the leaf expansion. Hanson *et al.* (1988) found no effects after exposing loblolly pine seedlings to acid mist at pH = 3.3 or ozone at 160 nmol mol^{-1}.

The reason that the light compensation point is not usually calculated or reported

Table 3.22. Effects of ozone, acid mist and wind on the light compensation point ($Q_{A=0}$) of plants, as reported in the literature. (1 ppb ozone = 1 nmol mol^{-1} ozone)

Source	**Treatment**	**Species**	**Effect**
Acid mist:			
Hanson *et al.*, 1988	pH = 3.3	*Pinus taeda*	no effect
Ozone:			
Barnes *et al.*, 1990*b*	100 ppb	*Picea abies*	no effect
Wallin *et al.*, 1992*b*	50 ppb	*Picea abies*	increased in current-year, no effect in older needles.
Hanson *et al.*, 1988	160 ppb	*Pinus taeda*	no effect
Reich, 1983	125 ppb	*Populus deltoides x trichocarpa*	no effect on fully expanded leaves
Foster *et al.*, 1990	150 ppb	*Quercus alba*	no effect

is presumably because it is closely linked with the dark respiration and apparent quantum yield, although there is no direct relationship due to the occurrence of the 'Kok effect' under normal conditions (Sharp *et al.*, 1984). In this study the light compensation point was calculated from the rectangular hyperbolas fitted to the data points for each individual shoot. This implies that the 'Kok effect' was assumed to be negligible, as there were not enough data to estimate the effect.

In the acid mist experiment of 1990 no effects by the mist treatment were found on the light compensation point before the wind treatment, but $Q_{A=0}$ was significantly reduced by both the acid mist and wind treatments three weeks later, while there also appeared to be a synergistic interaction, although this was not significant. In 1991 the acid mist treatment had no effect on $Q_{A=0}$, but the effect of the wind treatments was very significant. Both the treatment during the previous dormant period, and especially the wind treatment during the summer reduced $Q_{A=0}$.

Before the wind treatment in 1990, the ozone treatment at 140 nmol mol^{-1} caused a small, but not significant reduction in the light compensation point, compared to the charcoal-filtered control. After the wind treatment there was no longer any effect, by either treatment, and any differences were random variation. In 1991 the same result was obtained, there were small differences, but these had to be regarded as coincidental after an analysis of variance.

The results from the ozone experiments in this study appear to agree reasonably well with reports found in the literature, while the sometimes significant reductions by the acid mist treatment can only be compared with the work by Hanson *et al.* (1988). The results from the acid mist experiment are contrary to what was expected, for both the acid mist and wind treatments. None of the applied treatments was thought to be beneficial to trees, and any effects were expected to be increases in light compensation points, but this will be discussed in the section on the dark respiration rates.

3.5.3. Apparent quantum yield.

The apparent quantum yield (ϕ) of plants after ozone fumigation is usually decreased or not affected. Foster *et al.* (1990) found no effects on ϕ after exposing white oak seedlings to ozone for two growing seasons, while several other workers reported reductions in the apparent quantum yield. In contrast, Eamus *et al.* (1990) reported an increased ϕ after two summers of ozone fumigation of Norway spruce. Similarly, Eamus & Fowler (1990) and Eamus & Murray (1993) found increases in ϕ after applying acid mist at pH = 2.5 to red spruce (*Picea rubens* Sarg.) and Norway spruce, respectively, while Barnes *et al.* (1990*b*) found no effect on Norway spruce after a treatment with acid mist at pH = 3.6.

Before the wind treatment in 1990, spraying with acid mist appeared to reduce the apparent quantum yield somewhat in this study, although this was not significant (P = 0.142). After the wind treatment in 1990, and also in the 1991 experiment acid

Table 3.23. Effects of ozone, acid mist and wind on the apparent quantum yield (ϕ) of plants, as reported in the literature. (1 ppb ozone = 1 nmol mol^{-1} ozone)

Source	**Treatment**	**Species**	**Effect**
Acid mist:			
Barnes *et al.*, 1990*b*	pH = 3.6	*Picea abies*	no effect
Eamus & Murray, 1993	pH = 2.5	*Picea abies*	increased
Eamus & Fowler, 1990	pH = 2.5	*Picea rubens*	increased
Ozone:			
Rowland-Bamford *et al.*, 1989	150 ppb	*Hordeum vulgare*	decreased
Barnes *et al.*, 1990*b*	100 ppb	*Picea abies*	no effect
Eamus *et al.*, 1990	100 ppb	*Picea abies*	increased
Wallin *et al.*, 1992*b*	50 ppb	*Picea abies*	decreased in older needles
Reich, 1983	125 ppb	*Populus deltoides x trichocarpa*	decreased
Foster *et al.*, 1990	150 ppb	*Quercus alba*	no effect

mist at pH = 2.5 did not affect ϕ. The wind treatment had no effect on ϕ in either of these experiments. In both 1990 and 1991 ozone had no effect on the apparent quantum yield, and the same is true for the wind treatments in the ozone experiment of 1991. In 1990, however, the wind treatment did reduce the apparent quantum yield, although not quite significantly (P = 0.078).

The results form this study agree with what is most commonly found in the literature, i.e. either no effect or a reduction in apparent quantum yield, while the actual values calculated for the seedlings used in this study, fall well within the range reported previously. On average the values ranged from just over 0.020 mol mol^{-1} in 1990 and around 0.030 mol mol^{-1} in 1991, while for the same species Barnes *et al.* (1990*b*) reported averages between 0.012 and 0.020 mol mol^{-1}, and the averages reported by Wallin *et al.* (1992*b*) ranged from 0.022 to 0.049 mol mol^{-1}.

3.5.4. Dark respiration.

If plant material is damaged by air pollutants or high wind speeds, it seems likely that the dark respiration (R_d) would increase, reflecting repair mechanisms. This has been found after a treatment of perennial ryegrass (*Lolium perenne* L.; Russell & Grace, 1978*a*), and is also usually reported in experiments involving ozone effects on plants, although there are some reports without significant effects (Hanson *et al.*, 1988; Wallin *et al.*, 1992*a*), possibly due to a relatively low absorbed dose (Reich, 1987). Some authors found reductions (Yang *et al.*, 1983), although this reduction was only found in an ozone-sensitive clone of eastern white pine (*Pinus strobus* L.), while two other less sensitive clones showed no change in dark respiration. Acid mist appears to have relatively little effect on the dark respiration. Several authors found no effect, while Eamus & Fowler (1990) presented data indicating a reduction in R_d, although this was not commented on, and it is therefore not clear whether this reduction was significant.

In the experiments conducted in this study the dark respiration was found to be

Table 3.24. Effects of ozone, acid mist and wind on the dark respiration (R_d) of plants, as reported in the literature. (1 ppb ozone = 1 nmol mol^{-1} ozone)

Source	**Treatment**	**Species**	**Effect**
Acid mist:			
Barnes *et al.*, 1990*b*	pH = 3.6	*Picea abies*	no effect
Eamus & Fowler, 1990	pH = 2.5	*Picea rubens*	decreased
Hanson *et al.*, 1988	pH = 3.3	*Pinus taeda*	no effect
Ozone:			
Rowland-Bamford *et al.*, 1989	150 ppb	*Hordeum vulgare*	increased
Barnes *et al.*, 1990*b*	100 ppb	*Picea abies*	increased
Wallin *et al.*, 1990	50 ppb	*Picea abies*	increased
Wallin *et al.*, 1992*a*	50 ppb	*Picea abies*	no effect
Yang *et al.*, 1983	300 ppb	*Pinus strobus*	decreased
Skärby *et al.*, 1987	200 ppb	*Pinus sylvestris*	increased
Hanson *et al.*, 1988	160 ppb	*Pinus taeda*	no effect
Reich, 1983	125 ppb	*Populus deltoides x trichocarpa*	increased
Foster *et al.*, 1990	150 ppb	*Quercus alba*	no effect
Wind:			
Russell & Grace, 1978*a*	1.7 m s^{-1}	*Lolium perenne*	increased

either reduced or not affected, by both the pollutant and wind treatments. The ozone treatment did not significantly affect the dark respiration in either 1990 or 1991, although before the wind treatment in 1990 there was small, non-significant reduction in R_d. Acid mist very significantly ($P \leq 0.01$) reduced the dark respiration in the measurement series before the wind treatment in 1990, an effect which was still apparent, but not quite significant ($P = 0.052$) three weeks later, after the wind treatment. In the 1991 experiment acid mist had no effect on R_d.

When combined with the ozone treatment, the exposure of seedlings to high wind speeds in the wind tunnel did not affect the dark respiration, but when combined with the acid mist treatments, significant ($P \leq 0.05$) reductions were found. In 1991 both wind treatments reduced R_d, especially the exposure to high wind speeds during the summer. In 1990 there also appeared to be a synergistic interaction between the wind and acid mist treatment, although this was not significant.

It has already been mentioned that the light compensation point is closely linked with the dark respiration and the apparent quantum yield, on theoretical grounds, and this is also found in the present study. In the ozone experiments no effect was found on ϕ, and the effects on $Q_{A=0}$ exactly follow the effects on R_d. The same is true in the acid mist experiments, with one exception. Before the wind treatment in 1990, $Q_{A=0}$ was not changed, while R_d was significantly reduced. However, in this case ϕ was also reduced, albeit not significantly. Even the considerable, although non-significant, synergistic interaction between the wind and acid mist treatments in 1990 is found in both $Q_{A=0}$ and R_d. It seems likely, therefore, that the assumption of a negligible 'Kok effect' was warranted, and that the light response curves approximate a rectangular hyperbola throughout the range of photon fluxes, which in turn means that the combined curve analyses used throughout this chapter are valid.

3.5.5. Stomatal conductance.

The literature shows a varied response of the stomatal conductance (g_s) to pollutants, especially acid mist. Both decreases and increases have been reported, and the differences can not be simply attributed to differences in sensitivity of species, as Norway spruce has been shown to be both positively (Eamus & Fowler, 1990; Eamus & Murray, 1993) and negatively (van Elsacker & Impens, 1989) affected. Differences in treatment regimes between studies can also be ruled out as the only cause for the varied response of g_s, as Neufeld *et al.* (1985) studied several species, and found a reduction in g_s in sweetgum (*Liquidambar styraciflua* L.), but no effect on American plane (*Platanus occidentalis* L.). Acid mist can therefore affect the stomatal conductance of trees, but the extend and nature of the effect depends on other factors.

Wind appeared to have a similar effect, both increases and decreases in g_s have been found in response to a treatment with high wind speeds, while there are also some reports of no change in g_s, even after a quite high wind speed of 15 m s^{-1} (Caldwell, 1970). In a recent review of the effects of wind on plants, van Gardingen & Grace (1991) concluded that there is no direct effect of wind on the stomatal conductance, but that any changes found in studies on the effect of wind treatment on the stomatal conductance were caused by changes in the water vapour deficit. Bunce (1985) demonstrated that changes in the stomatal conductance can be explained by an increase in the vapour pressure gradient

Table 3.25. Effects of ozone, acid mist and wind on the stomatal conductance (g_s) of plants, as reported in the literature. (1 ppb ozone = 1 nmol mol^{-1} ozone)

Source	**Treatment**	**Species**	**Effect**
Acid mist:			
Neufeld *et al.*, 1985	pH = 2.0	*Liquidambar styraciflua*	decreased
Eamus & Fowler, 1990	pH = 2.5	*Picea rubens*	increased
van Elsacker & Impens, 1989	pH = 4.0	*Picea abies*	decreased
Eamus & Murray, 1993	pH = 2.5	*Picea abies*	increased
Neufeld *et al.*, 1985	pH = 2.0	*Platanus occidentalis*	no effect
Martens *et al.*, 1989	pH = 4.0	*Populus*	decreased
Wind:			
Dixon & Grace, 1984	5 m s^{-1}	*Fagus sylvatica*	no effect
Caldwell, 1970	15 m s^{-1}	*Pinus cembra*	no effect
Dixon & Grace, 1984	5 m s^{-1}	*Pinus sylvestris*	decreased
Dixon & Grace, 1984	5 m s^{-1}	*Quercus robur*	increased
Caldwell, 1970	15 m s^{-1}	*Rhododendron ferrugineum*	decreased
Dixon & Grace, 1984	5 m s^{-1}	*Sorbus aucuparia*	increased

Table 3.25. Continued.

Source	Treatment	Species	Effect
Ozone:			
Temple, 1986	92 ppb	*Gossypium hirsutum*	decreased
Temple *et al.*, 1988	111 ppb	*Gossypium hirsutum*	decreased
Rowland-Bamford *et al.*, 1989	150 ppb	*Hordeum vulgare*	decreased
Barnes *et al.*, 1988	75 ppb	*Pisum sativum*	decreased
Barnes & Pfirrmann, 1992	73 ppb	*Raphanus sativus*	decreased
Retzlaff *et al.*, 1991	117 ppb	Several fruit and tree species	decreased
Leonardi *et al.*, 1990	110 ppb	*Fagus sylvatica*	transient increase first, then decreased
Eamus & Murray, 1991	140 ppb	*Fagus sylvatica*	decreased
Havranek *et al.*, 1989	120 ppb	*Picea abies*	increased
Barnes *et al.*, 1990*a*	100 ppb	*Picea abies*	decreased
Eamus *et al.*, 1990	100 ppb	*Picea abies*	increased
Skärby *et al.*, 1987	200 ppb	*Pinus sylvestris*	decreased during the day, increased at night
Sasek & Richardson, 1989	92 ppb	*Pinus taeda*	no effect
Tjoelker & Luxmoore, 1991	108 ppb	*Pinus taeda*	decreased
Foster *et al.*, 1990	150 ppb	*Quercus alba*	no effect

associated with an increased boundary layer conductance at higher wind speeds.

On the whole, ozone appears to induce stomatal closure, as most reports of effects of ozone fumigation on the stomatal conductance indicate a reduction in g_s in response to the ozone treatment. There are exceptions, however. White oak was found to be not affected (Foster *et al.*, 1990), and there are also some reports of increases in g_s. The increased stomatal conductance found by Eamus *et al.* (1990) fits in well with the increase in maximum assimilation rate also found by these authors, and the increased g_s at night shown by Skärby *et al.* (1987) could indicate a stomatal sluggishness in response to darkness as reported by Keller and Häsler (1984, 1987), but this will be discussed in Section 3.5.6.

In the acid mist experiments in this study the stomatal conductance was not significantly affected. Although there was a considerable reduction in the measurements before the wind treatment in 1990, from 214 to 143 mmol m^{-2} s^{-1}, this was not significant ($P = 0.135$), while in the other series of measurements the differences were much smaller and due to random variation. Similarly, the wind treatments, when combined with the acid mist treatment in both 1990 and 1991, had no effects on the stomatal conductance.

In the ozone experiments the measurements before the wind treatment in 1990 showed a significant reduction in stomatal conductance ($P \leq 0.05$), from 158 to 143. However, this reduction was not found again four weeks later after the wind treatment, while there were also no effects in the 1991 experiment. As in the acid mist experiment, the wind treatments, when combined with the ozone fumigation, did not significantly alter g_s.

The wind treatments did not alter the stomatal conductance when combined with either pollutant. This result fits in well with the conclusion by van Gardingen & Grace (1991), as the measurements were not done during the high wind treatments. All plants were measured in the same leaf chambers, and therefore no differences in g_s were expected.

3.5.6. Stomatal response to changes in the photon flux.

Very little is known about the speed of responses of stomata to changes in light levels. No reports were found of effects of acid mist or wind on the response time, while only three studies are available on the effects of ozone fumigation on stomatal response to changing light conditions. Temple (1986) exposed field-grown cotton (*Gossypium hirsutum* L.) to 92 nmol mol^{-1} of ozone throughout the growing season, but found no differences in opening and closing time in response to changes in light intensity. Keller and Häsler (1984, 1987) reported stomatal sluggishness in Norway spruce as a result of ozone fumigation, i.e.

stomata reacted slower, which resulted in higher transpiration rates after a short period of darkness. Using the same species, Barnes *et al.* (1990*d*) found slower stomatal closure in response to increasing water deficit in the needles, after exposing them to 78 nmol mol^{-1} of ozone for 8 hours per day over three consecutive summers, although this does not necessarily mean that the same mechanism was affected, and as already mentioned Skärby *et al.* (1987) found increases in stomatal conductance in Scots pine (*Pinus sylvestris* L.) at night.

In contrast to the results of Keller and Häsler (1984, 1987), this study showed an improved stomatal response to changes in light conditions after fumigation with ozone, although the response time was calculated for changes in the photon flux from about 600 to about 1200 µmol m^{-2} s^{-1}, and not from light to darkness. τ_{1200}, the time needed by the stomata to change about two thirds (1 - 1/e = 0.632) of the difference between the stomatal conductance at 600 and 1200 µmol m^{-2} s^{-1}, for the ozone treated plants ranged from 79 to 96% of the control values, with one exception for the plants also treated in the wind tunnel for 120 hours, when the ozone treated plants only needed 52% of the time needed by the charcoal-filtered control plants to respond to the change in photon flux. In summary, the ozone treated plants always responded faster than their counterparts treated with charcoal-filtered air only, although this was not always statistically significant.

For the plants from the acid mist experiments τ_{1200} for the plants treated with mist at pH = 2.5 ranged from 82 to 147% of their pH = 5.0 treated controls, but the differences were due to random variation, as indicated by the analyses of variance ($P > 0.75$ in all three measurement series).

In 1990 the wind treatment had no significant effect on the response time of the stomata to changes in the light intensity, for plants from the acid mist experiments, with the wind treated plants needing only slightly longer than the no-wind control plants. In the acid mist experiment in 1991 there was slight, non-significant wind effect ($P = 0.149$), but overall the results were highly variable, with the wind treated plants requiring between 75 and 170% of the time needed by the control plants, to respond to increases in light levels. In the 1990 ozone experiment the wind treatment had no effect, while in 1991 the wind treatments had a significant effect on τ_{1200}. However, this was entirely due to the 120 hours treatment, which increased the response time by almost 100% for the charcoal-filtered air treated plants, and 17% for the ozone fumigated plants. The response times for the remaining wind treatments (during the previous dormant period, at budburst, and in autumn) ranged from 96 to 106% of their respective controls. On the whole, 48 hour wind treatments caused some damage to the stomatal control mechanisms, but only the very severe treatment of 120 hours at 16 m s^{-1} during the summer had a significant effect in increasing the time needed by the stomata to respond to altering light conditions.

Chapter 4.

Effects on Frost Hardiness.

Winter injury has been suggested as one the most important causal factors leading to the current decline of red spruce (*Picea rubens* Sarg.) in the high elevation forests of the eastern United States (Friedland *et al.*, 1984). Although winter injury to natural stands of red spruce occurred occasionally in the past, it has become relatively common during the last three decades. Anthropogenic pollutants have frequently been linked with the observed reduction in frost tolerance, although in the field only circumstantial evidence of increases in damage coinciding with increased pollution emission is available (DeHayes, 1992). Similarly, damage to Norway spruce (*Picea abies* (L.) Karst.) trees in central Europe, suddenly became evident in the early 1980s after a series of severe frosts between 1979 and 1983, when severe air frosts occurred after a series of extended periods of mild weather (Rehfuess, 1987). There are now a number of reports from controlled experiments, which show that air pollutants, such as ozone and acid mist, increase the susceptibility of Norway and red spruce to freezing temperatures (Barnes & Davison, 1988; Fincher *et al.*, 1989; Fowler *et al.*, 1989; Sheppard *et al.*, 1993*b*). In this study the frost hardiness of the Norway spruce seedlings, previously exposed to several combinations of acid mist, ozone and wind, was measured during the hardening period in autumn.

4.1. Techniques.

The measurements described in this chapter largely follow the techniques for determining frost hardiness, developed by Murray *et al.* (1989) and Sheppard *et al.* (1989). Essentially this technique relies on the tendency of damaged cells to leak electrolytes. Thus bathing shoots in deionised water and measuring the conductivity of the resulting solution provides an indication of the cell wall integrity of the plant material.

At the start of a series of measurements, a sufficient number of shoots were cut from seedlings for which the frost hardiness was to be determined. Only current-year shoots were used, and individual shoots were assigned at random to freezing temperatures. The shoots were kept in an unlit cold-store at 2 °C for a maximum of 7 days, in sealed polyethylene bags, until they were used. Two or three of the shoots from each of the trees were subjected to a particular freezing temperature from a range of temperatures, chosen to span and preferably exceed the hardiness range at the time of year when the measurements were made. Freezing was done in a cabinet (Cannell & Sheppard, 1982) at I.T.E., purpose built to simulate air frosts. The temperature was lowered at 10 degrees C per hour to 2 °C, and then at 5 deg C h^{-1} to a preset temperature, which was held for 3 hours. The temperature was then raised again to 2 °C, at 10 deg C h^{-1}, and held there until the shoots were removed. The lowest temperature in the freezing cabinet was recorded with two maximum-minimum recording thermometers, and as these thermometers measured the actual temperatures in the cabinet, their values were used in subsequent analyses. It turned out that the freezing cabinet did not quite reach a temperature as low as it was set for.

After the freezing treatment the shoots were removed from the freezing cabinet, and the central 1 cm section of each shoot was placed in a 20 ml polypropylene vial. Any buds or dead needles were removed first. 15 ml of deionised water was added, using a zippette (Dispensette, Rudolf Brand GmbH + Co, Wertheim, Germany), sufficient to cover the shoots and, more particularly, the electrode of the platinum electrode water analyzer (model PWA1, Jencons (Scientific) Ltd., Leighton Buzzard, Beds). The time the water was added, was recorded for each vial using a datalogger (21X, Campbell Scientific Ltd., Shepshed, Leics.), and used as the starting time in the calculation of the electrolyte leakage rate. The conductivity of the solution was measured after about one hour, the exact time (in minutes) and the conductivity being recorded by the datalogger. The vials were then capped and stored in the dark at 2 °C in a cold room, until the next set of readings were taken the following day, after first allowing the vials to warm up to room temperature before measurements were made. This was repeated after about five days, and the exact times were again recorded by the datalogger. The vials and shoots were then autoclaved for 4 minutes at 104 °C, in order to obtain a value for the maximum leakage, thus standardizing for differences in shoot size and the (small) differences in amounts of deionized water added.

During the conductivity measurements, the excess water on the electrode was removed with paper tissue. The sensor head was not rinsed with deionised water, as any residue of the previous sample left after wiping, would introduce a smaller error than a residue of deionised water, as the difference in conductivity between samples was generally less than that compared with deionised water. Absorbing the excess water introduced a loss of 0.060 ± 0.048 g, which is negligible compared to the 15 ml of water that was added originally. Another potential source of error was autoclaving, when evaporating water might leave a more concentrated solution, which would lead to an overestimate of the maximum conductivity. However, measurements showed the loss of water due to autoclaving was 0.035 ± 0.010 g, i.e. even less than due to the conductivity measurements.

4.2. Calculation of parameters.

Purpose written software was used to estimate the electrolyte leakage coefficient and the initial conductivity, using EQUATION 4.1, based on equation 3 from Murray *et al.* (1989):

$$C_t - C_0 = (C_{auto} - C_0)(1 - e^{-k_e t}) \qquad (4.1)$$

where C_t is the conductivity at time t, C_0 is the initial conductivity which results from the removal of deposited material on the cuticle, C_{auto} the conductivity after autoclaving (all in µS), k_e the electrolyte leakage coefficient (which has units of time^{-1}), and t the time (hours).

C_0 was estimated along with k_e, rather than taking the conductivity as a spot reading after 1 hour as an initial value. This allows the first reading to be used in the curve fitting process, increasing the accuracy to which k_e is estimated. The curve fitting technique is described in Appendix 4.

The values of k_e were multiplied by 2400, in order to express k_e in % day^{-1}, to facilitate comparisons with other experiments reported in the literature, which are expressed in similar units.

Before further analysis, the estimated k_e-values were ln-transformed, as their distribution was approximately ln-normal (Fowler *et al.*, 1989).

Plotting the ln-transformed k_e-values for the two extreme freezing temperatures, i.e. the unfrozen control and the coldest temperature, pooled across the pollution and wind treatments, in a histogram, normally reveals two distinct populations

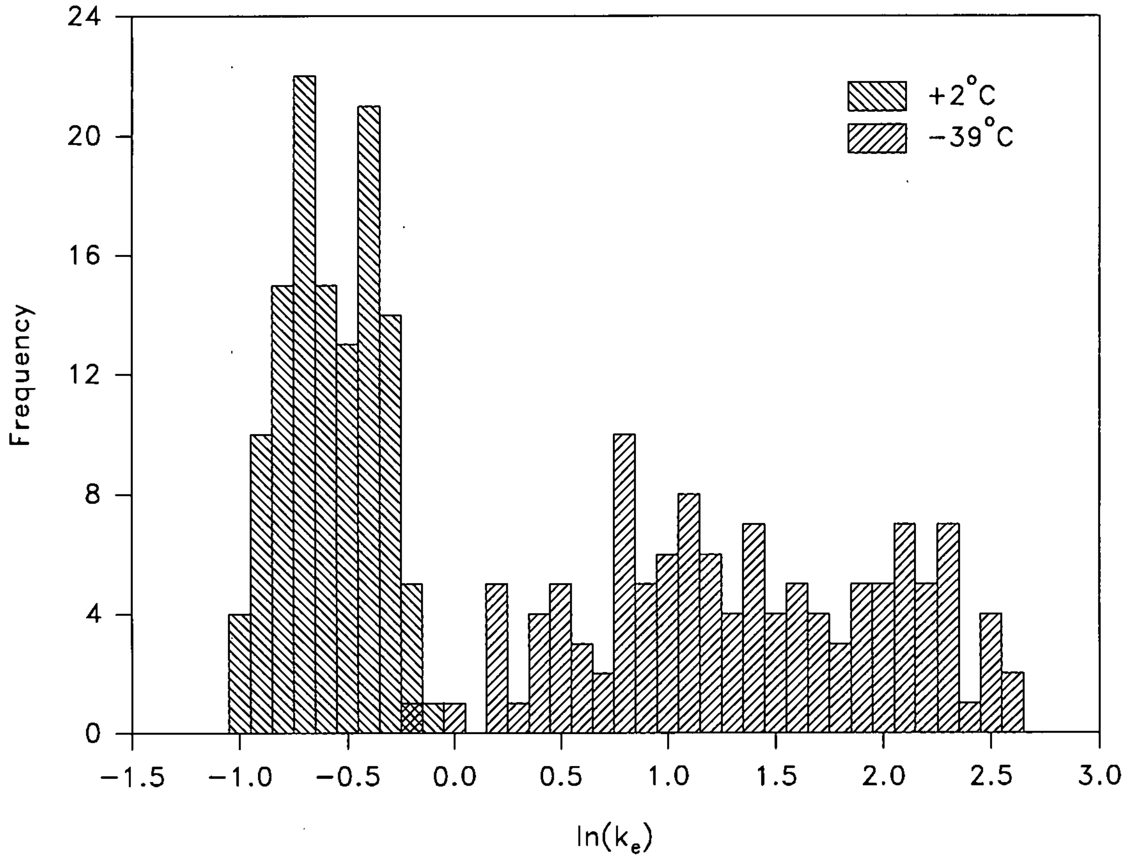


Figure 4.1. Histogram showing the distribution of ln-transformed k_e-values in the two extreme temperatures.

of values (see FIGURE 4.1). The population of the lower ln-transformed k_e-values belongs to the unfrozen shoots, while the higher k_e-values identify the shoots that were killed. This method of determining a critical k_e-value was not evaluated against visual scoring of the shoots. However, in previous freezing experiments, where the two methods have been compared, a good agreement between them has been found (Fowler *et al.*, 1989). Adopting the value of k_e separating the two populations as the lethal temperature, all individual shoots were determined to be either dead or alive following the freezing treatment. In theory all ln-transformed k_e-values of the shoots from all freezing treatments should be plotted in the histogram. However, the distinction between the two populations of values, those calculated for the shoots that would have survived the temperature they were subjected to, and the values for the killed shoots, tends to be blurred. This makes the choice of a critical k_e-value, the value separating the two populations, more subjective, and one of the main advantages of the electrolyte leakage method over the conventional visual scoring method is its objectivity.

The proportion of shoots killed was then plotted against the freezing temperature, and a modified logistic function was fitted to the data points. The logistic function is represented by (equation 9.37 from Causton, 1983):

$$y=\frac{a}{1+be^{-kt}} \tag{4.2}$$

which is often used to describe the mass of microbial culture (y) against time (t). a, b and k are constants.

This equation was modified by substituting R_{dead} (proportion of shoots killed) for y, and temperature (T) for time. A term (T_{corr}) was added to allow the inflexion point to move freely along the x-axis, and the negative-sign was removed to give a maximum value for R_{dead} (a) at low temperatures, and a minimum value at the control temperature. A value of 1 was substituted for a, because, as the curves were fitted to the proportions of shoots killed, the maximum value would be 1.

A subscript (l) was added to b and k, to distinguish them from other parameters used in this work. This gives the following equation:

$$R_{dead}=\frac{1}{1+b_l e^{k_l(T-T_{corr})}} \tag{4.3}$$

EQUATION 4.3 was still not found to be satisfactory, as it gave a poor relationship with the data points, when some shoots were killed without the freezing treatment, or when freezing at the lowest temperature did not kill all the shoots. Therefore two more terms were added to EQUATION 4.3, a_l (the maximum value for R_{dead}) was reintroduced, and $R_{control}$ was added, to allow the curve to move away from the x-axis:

$$R_{dead}=\frac{a_l}{1+b_l e^{k_l(T-T_{corr})}}+R_{control} \tag{4.4}$$

Using purpose written software, a_l, b_l, k_l, T_{corr} and $R_{control}$ were estimated in the same way as k_e and C_0 were estimated when fitting data points to EQUATION 4.1. In the curve fitting process no constraints were placed on the values for a_l and $R_{control}$, although theoretically a_l should have been forced to be less than or equal to unity, and $R_{control}$ should be positive. It was found, however, that this caused the computer program to go around in indefinite loops, and in practice omitting the constraints resulted in negligible errors.

Using the combined curve technique, described in Appendix 4, analyses of variance were used to test for treatment effects on the fitted curves (Ross, 1981).

From the parameters obtained from fitting data sets to EQUATION 4.4, the temperature at which 50% of the shoots were killed (*LT50*) can be calculated by substituting 0.5 for R_{dead}, which after rearranging gives:

$$LT50=\frac{\ln\left(\frac{\frac{a_l}{0.5-R_{control}}-1}{b_l}\right)}{k_l}+T_{corr} \qquad (4.5)$$

4.3. Acid Mist Experiments.

4.3.1. First year, 1990.

Three shoots from each of five Norway spruce seedlings for each of the two wind treatments, from the two replicate chambers for each of the two acid mist treatments, were sampled on 12 November, and frozen during the third week of November in 1990. Freezing temperatures were chosen on the basis of past experience, and ranged from -15 °C to -39 °C (in steps of 4 °C), together with an unfrozen control at +2 °C.

The initial conductivity (C_0) and the electrolyte leakage rate (k_e) were estimated for each shoot, and the average values for each combination of acid mist and wind treatments, as affected by the freezing temperature, are presented in FIGURE 4.2. An analysis of variance performed on the estimated C_0-values showed a highly

Table 4.1. Results from the analysis of variance on the initial conductivity (C_0) and the electrolyte leakage rate (k_e) of the plants from the acid mist experiment in 1990. The tabulated values represent the F-probabilities from the analysis of variance.

	C_0	$\ln(k_e)$
acid mist	< 0.001	0.020
wind	0.039	0.857
interaction	0.038	0.754
temperature	0.116	< 0.001

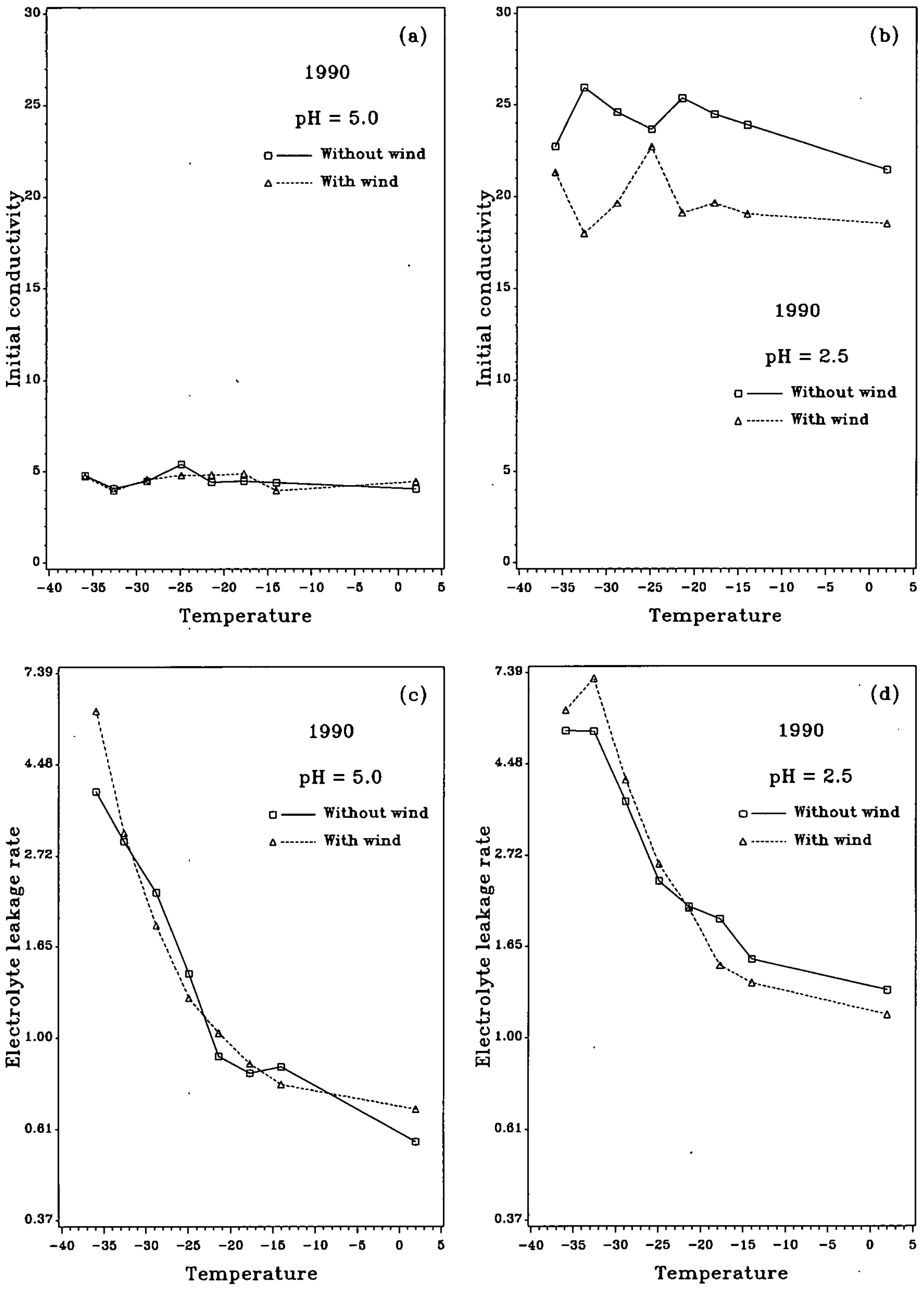


Figure 4.2. Effects of freezing temperature (°C) on the initial conductivity (C_0, in µS; (a) pH = 5.0 treated plants, and (b) pH = 2.5 treated plants) and the electrolyte leakage rates (k_e, in % day^{-1} (ln-scale); (c) pH = 5.0 treated plants, and (d) pH = 2.5 treated plants) of plants from the acid mist experiment in 1990.

significant ($P \leq 0.001$) acid mist effect, and a significant ($P \leq 0.05$) wind effect, see TABLE 4.1. The initial conductivity, averaged across the two wind treatments, was increased to 21.9 µS for the shoots treated with the pH = 2.5 mist, as compared to 4.5 µS for the acid mist control shoots (pH = 5.0). C_0 for the wind treated plants, averaged across the two acidity levels, was 12.1 µS, compared to 14.3 µS for the no-wind controls. There was also a significant interaction between the wind and acid mist treatment: the wind effect occurred only on plants from the chambers sprayed with acid mist at pH = 2.5 (see FIGURE 4.2a and b). There was no effect of the freezing temperature on the initial conductivity.

The estimated k_e-values for each shoot were ln-transformed before any further analysis. An analysis of variance, performed on the ln-transformed electrolyte leakage rates, showed that acid mist significantly increased electrolyte leakage rates, but there clearly was no wind effect nor an interaction between the two. The acid mist treatment at pH = 2.5 increased the ln-transformed k_e-value (averaged across the wind treatments and the freezing temperatures) to 0.951 compared to 0.300 for the control. These values correspond to electrolyte leakage rates of 2.59 and 1.35 % day^{-1}. As could be expected in an experiment with a wide enough range of freezing temperatures, there was a highly significant effect of temperature on the ln-transformed k_e-values, with the corresponding k_e-values ranging from 0.9 % day^{-1} for the unfrozen control to over 4.3 % day^{-1} for the two lowest temperatures, averaged across the acid mist and wind treatments.

The ln-transformed k_e-values for the two extreme temperatures, -39 °C and +2 °C, were then plotted in a histogram as in FIGURE 4.1, to obtain a critical value for $\ln(k_e)$, differentiating between alive and dead shoots. There was some overlap between values from the two temperatures, as some of the shoots were apparently damaged before a freezing treatment was even applied, although none of the shoots showed any visual damage. For this reason a critical value of 0.60 was chosen for $\ln(k_e)$, where only one shoot treated with acid mist at pH = 2.5 was determined to have survived the lowest freezing temperature, and none of

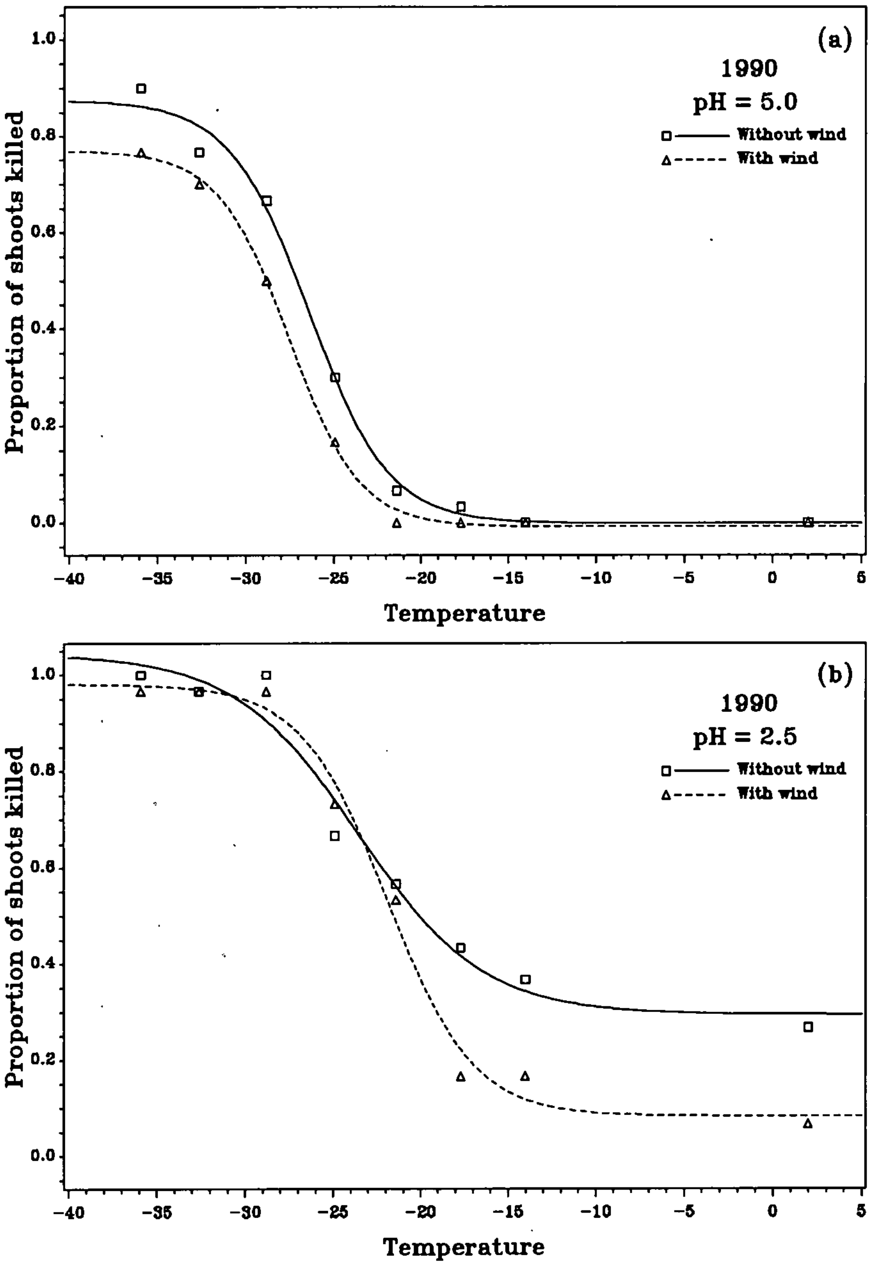


Figure 4.3. The proportion of the shoots killed as a function of the freezing temperature (°C), for the (a) pH = 5.0 and (b) pH = 2.5 treated plants from the acid mist experiment in 1990.

Table 4.2. Combined curve analyses performed on the lines fitted for the shoots from the acid mist experiment in 1990.

Comparison	Link	F-probability
No wind ↔ wind	pH = 5.0	$P \leq 0.025$
No wind ↔ wind	pH = 2.5	$P \leq 0.25$
pH = 5.0 ↔ pH = 2.5	Combined curves	$P \leq 0.001$

pH = 5.0 control shoots were killed without freezing. The proportion of dead shoots, determined from this critical ln(k_e)-value, was plotted against the freezing temperatures (FIGURE 4.3), together with curves according to EQUATION 4.4 fitted to each of the data sets. Combined curve analyses were performed on these fitted curves, and the results are given in TABLE 4.2. These analyses showed the curves fitted for the two wind treatments on the plants treated with acid mist at pH = 5.0 were significantly different ($P \leq 0.05$), while the two lines for the pH = 2.5 treated plants were not significantly different ($P > 0.05$). The relative effect of the acid mist treatments was established by performing a combined curve analysis on the combined curves for the two wind treatments for both acid mist treatments (R. I. Smith, pers. comm.). This analysis showed that the curves fitted for the two acid mist treatments were highly significantly different ($P \leq 0.001$), but it does not distinguish between effects on the overall frost hardiness and the damage which occurred without a freezing treatment.

Table 4.3. Temperature at which 50 % of the shoots were killed (*LT50*; °C), for the trees from the acid mist experiment in 1990.

	pH = 5.0	pH = 2.5
without wind	-27.1	-20.1
with wind	-28.8	-21.5

Using EQUATION 4.5 the temperature at which 50% of the shoots were killed (*LT50*) for each of the acid mist and wind treatment combinations were calculated, see TABLE 4.3. This table shows that the acid mist treatment at pH = 2.5 reduced the frost hardiness by about 7 °C as compared to the pH = 5.0 control, which suggests that the results from the combined curve analysis were at least partly due to effects of the acid mist treatment on the overall frost hardiness. The wind treatment increased the frost hardiness slightly, but the effect was only 25% of the acid mist effect.

4.3.2. Second year, 1991.

In 1991 measurements of frost hardiness were not possible on the trees from the acid mist experiment. By early autumn the plants receiving mist at an acidity of pH = 2.5, were severely damaged, see section 1 of chapter 5 for visible injury assessments. This meant that only enough shoots were available on the control (pH = 5.0) plants, and although it would have been possible to obtain some indication of a wind effect, no measurements were done.

4.4. Ozone Experiments.

4.4.1. First year, 1990.

The experimental freezing regime was the same as that used for the 1990 acid mist experiment. Three shoots from each of five seedlings, taken from each combination of wind and ozone (two chamber replicates), were frozen with those from the acid mist experiment, during the third week of November 1990, to temperatures ranging from -19 °C to -39 °C (in steps of 4 °C). Curves were fitted to data sets for each shoot, and the initial conductivity (C_0) and the electrolyte leakage rate (k_e) were estimated using EQUATION 4.1. The averages of C_0 and k_e are presented in FIGURE 4.4. Analyses of variance were performed on the estimated initial conductivities and the ln-transformed electrolyte leakage rates

Table 4.4. Results from the analysis of variance on the initial conductivity (C_0) and the electrolyte leakage rate (k_e) of the plants from the ozone experiment in 1990. The tabulated values represent the F-probabilities from the analysis of variance.

	C_0	$\ln(k_e)$
ozone	0.580	0.603
wind	0.368	0.311
interaction	0.107	0.234
temperature	< 0.001	< 0.001

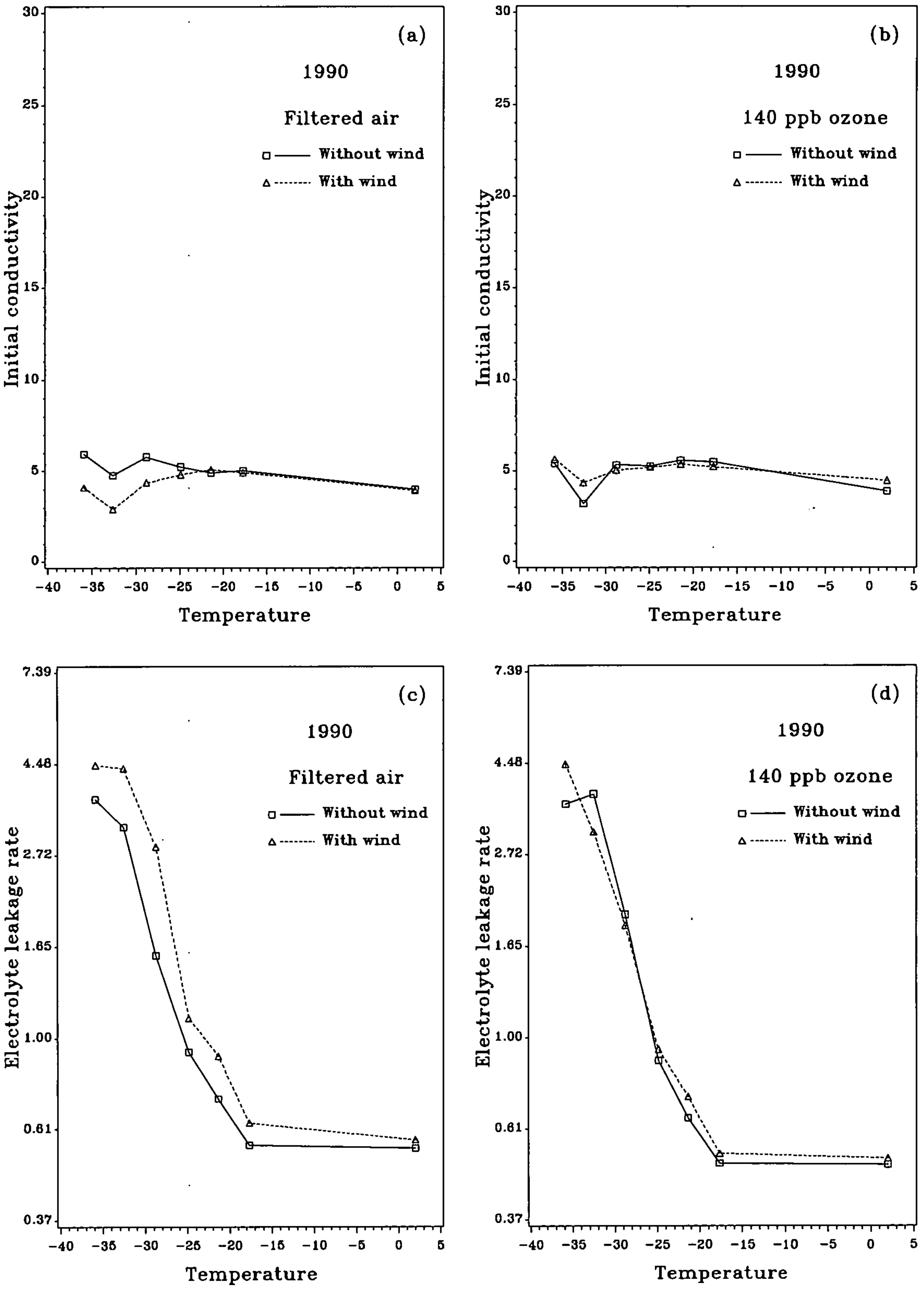


Figure 4.4. Effects of freezing temperature (°C) on the initial conductivity (C_0, in µS; (a) filtered air treated plants, and (b) 140 nmol mol^{-1} ozone treated plants) and the electrolyte leakage rates (k_e, in % day^{-1} (ln-scale); (c) filtered air treated plants, and (d) 140 nmol mol^{-1} ozone treated plants) of plants from the ozone experiment in 1990.

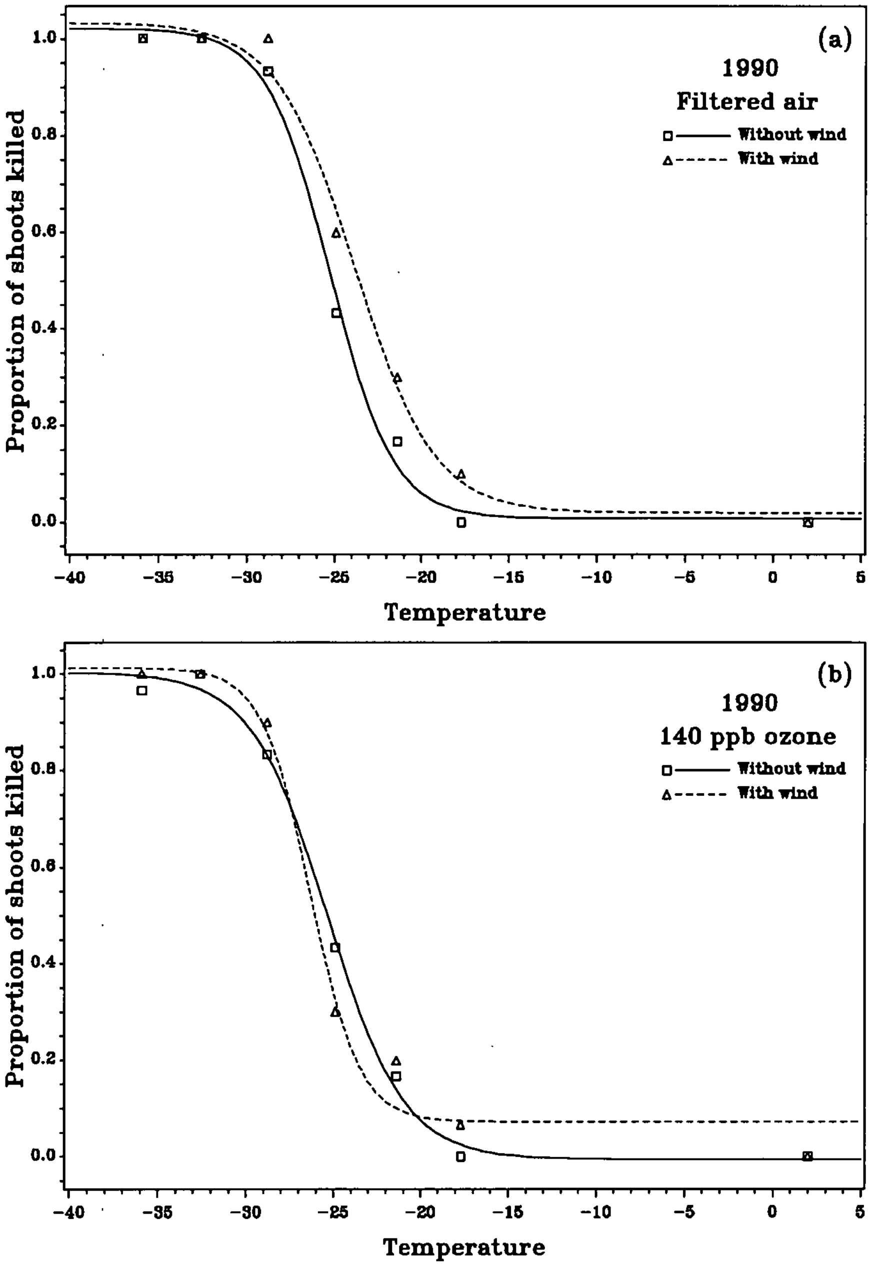


Figure 4.5. The proportion of the shoots killed as a function of the freezing temperature (°C), for the (a) filtered air and (b) 140 nmol mol^{-1} ozone treated plants from the ozone experiment in 1990.

Table 4.5. Combined curve analyses performed on the lines fitted for the shoots from the ozone experiment in 1990.

Comparison	Link	F-probability
No wind ↔ wind	Filtered air	$P \leq 0.5$
No wind ↔ wind	140 ppb ozone	$P > 0.75$
Filtered air ↔ 140 ppb ozone	Combined curves	$P \leq 0.25$

(see TABLE 4.4).

Neither wind nor the ozone treatment had an effect on C_0, nor was there an interaction between the two (TABLE 4.4). There was, however, a highly significant effect ($P \leq 0.001$) of freezing temperature on C_0: average values ranged from 3.8 µS to 5.3 µS (FIGURE 4.4). The analysis of variance on ln-transformed k_e-values showed the expected highly significant effect of freezing temperature, but as with C_0, there was no effect of, nor an interaction between, the wind and ozone treatments.

Plotting the ln-transformed k_e-values from the coldest freezing temperature (-39 °C) and the unfrozen shoots (+2 °C) in a histogram (FIGURE 4.1), showed the critical value for $\ln(k_e)$ to be -0.10, which corresponds to a k_e-value of 0.90 % day^{-1}. In this experiment with ozone, in contrast to the treatment with acid mist, the difference between the values from the two temperatures was quite clear cut: only one of the shoots frozen at -39 °C was determined to have survived the freezing treatment. Using the critical $\ln(k_e)$-value, the proportion of dead shoots was calculated for each freezing temperature, and for each combination of ozone and wind treatments, and plotted in FIGURE 4.5, together with the curves fitted to these data points.

The combined curve analyses, presented in TABLE 4.5, showed that neither the two comparisons for the wind treatments (at both levels of ozone exposure), nor the comparison of the two combined curves to check for an ozone effect, revealed any differences between the fitted curves.

Differences were also absent between the *LT50* values for the ozone and wind treatment combinations, presented in TABLE 4.6. Both the ozone treatment at 140 nmol mol^{-1} and the wind treatment at 16 m s^{-1} had little effect on the *LT50*, when compared to their respective controls, the differences being less than 2.5 °C.

Table 4.6. Temperature at which 50 % of the shoots were killed (*LT50*; °C), for the trees from the ozone experiment in 1990.

	Filtered air	140 ppb ozone
without wind	-25.1	-25.3
with wind	-23.6	-26.0

4.4.2. Second year, 1991.

In 1991 more wind treatments were applied than in the previous year, with unequal numbers of plants in each treatment due to problems with the wind tunnel. The ozone treatments were the same as in 1990. Four open-top chambers were used, two received filtered air only (ozone controls), and two were supplied with ozone to 140 nmol mol^{-1} during periods of favourable weather conditions. In each chamber six plants received no wind treatment (wind controls), six had been treated during the previous dormant period (winter storm), six were treated when the buds were bursting (spring storm), two were treated for five days during the summer (120 hrs), and four plants were treated just before the frost hardiness measurements (autumn storm).

From each seedling, two shoots were subjected to one of seven freezing temperatures ranging from -5 °C to -19 °C (in 2 °C steps, excluding -17 °C), during the first week of October 1991. The average initial conductivities and ln-transformed k_e-values are presented in FIGURE 4.6, and the corresponding probabilities, as calculated using analyses of variance (GENSTAT, 1987), are presented in TABLE 4.7.

The analyses of variance showed highly significant effects ($P \leq 0.001$) of ozone and temperature on the initial conductivity, and a significant wind effect ($P \leq 0.05$) on C_0 (see TABLE 4.7). The average C_0 for the trees from the ozone treated chambers was 3.9 µS, averaged across wind treatments and freezing temperatures, as compared to 5.1 µS for the ozone controls (FIGURE 4.6). The average C_0 for the

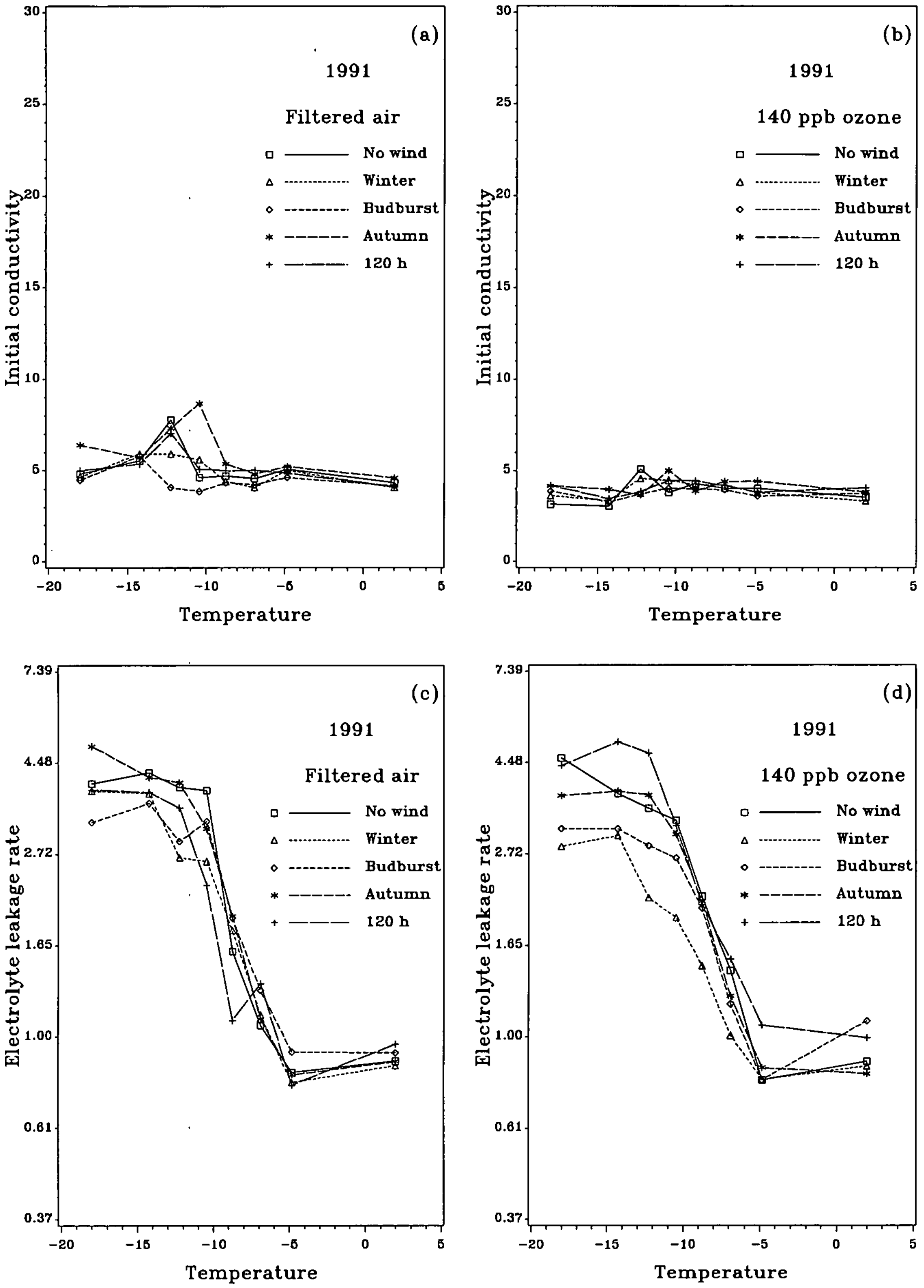


Figure 4.6. Effects of freezing temperature (°C) on the initial conductivity (C_0, in µS; (a) filtered air treated plants, and (b) 140 nmol mol^{-1} ozone treated plants) and the electrolyte leakage rates (k_e, in % day^{-1} (ln-scale); (c) filtered air treated plants, and (d) 140 nmol mol^{-1} ozone treated plants) of plants from the ozone experiment in 1991.

Table 4.7. Results from the analysis of variance on the initial conductivity (C_0) and the electrolyte leakage rate (k_e) of the plants from the ozone experiment in 1991. The tabulated values represent the F-probabilities from the analysis of variance.

	C_0	$\ln(k_e)$
ozone	< 0.001	0.374
wind	0.014	0.044
interaction	0.285	0.305
temperature	< 0.001	< 0.001

wind treatments ranged from 4.1 µS for the plants treated at budburst to 5.1 µS after the autumn treatment, while the three other wind treatments showed initial conductivities of around 4.5 µS. For the different temperatures C_0 ranged from 3.9 to 5.3 µS. There was no interaction between wind and ozone.

The only effects found by the analysis of variance performed on the ln-transformed k_e-values (see TABLE 4.7) were a temperature effect (highly significant, indicating a good range of freezing temperatures), and a wind effect (significant). The values for the wind treatments, averaged across the ozone treatments and freezing temperatures, ranged from 0.53 for the winter treatment (average back-transformed k_e-value 1.7 % day^{-1}), to 0.65 (1.9 % day^{-1}) for the spring storm treated trees, to around 0.73 for the other three wind treatments (the equivalent back-transformed k_e-value being 2.1 % day^{-1}).

A critical ln-transformed k_e-value was determined by plotting the two extreme temperatures (+2 °C and -19 °C) in a histogram, and this critical value was set at 0.47 (back-transformed k_e-value 1.6 % day^{-1}). This resulted in five shoots frozen at -19 °C being classified as alive, and two temperature control shoots as dead. For each combination of the two ozone and five wind treatments the proportion of dead shoots at each freezing temperature was determined, and plotted in FIGURE 4.7. Curves (EQUATION 4.4) were fitted to each of the data sets, and these are also presented in FIGURE 4.7.

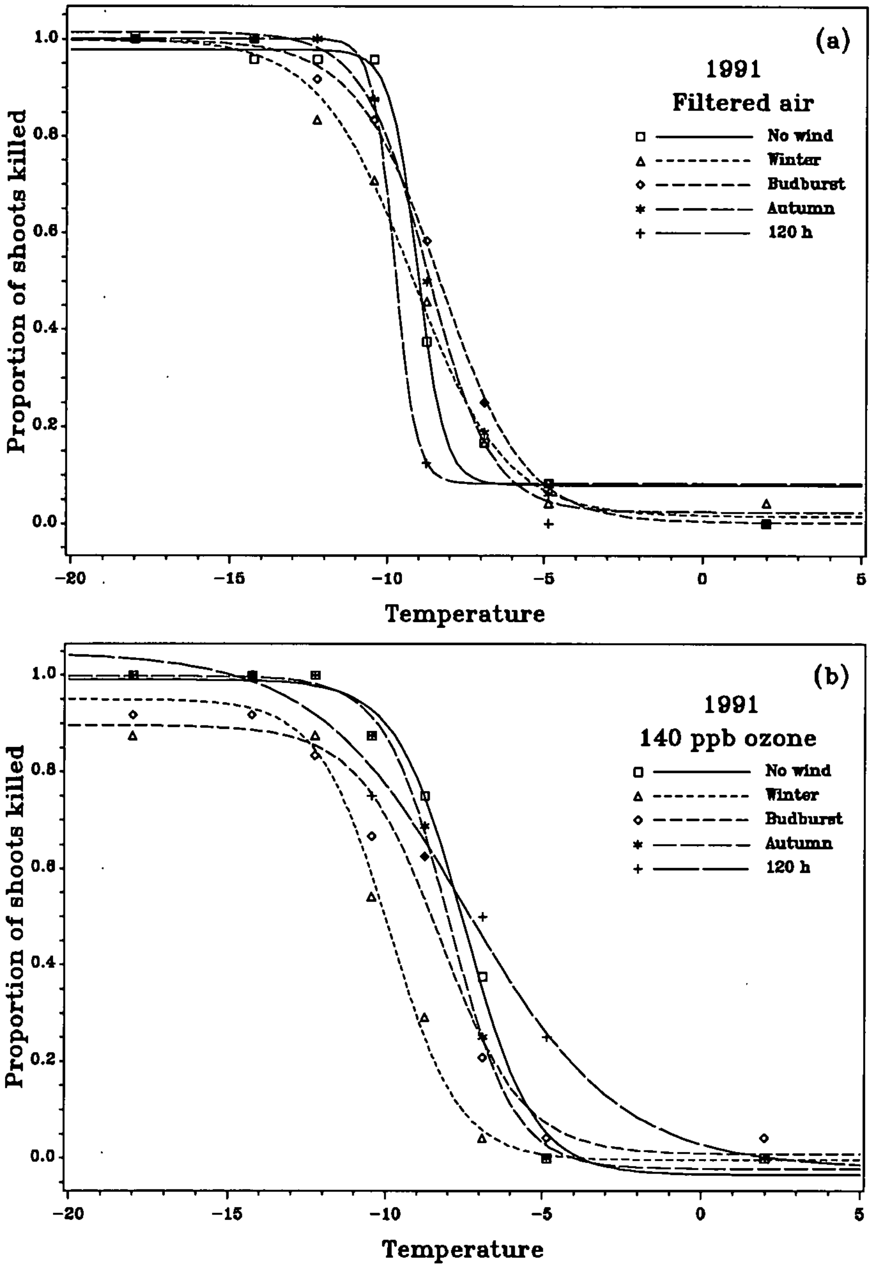


Figure 4.7. The proportion of the shoots killed as a function of the freezing temperature (°C), for the (a) filtered air and (b) 140 nmol mol^{-1} ozone treated plants from the ozone experiment in 1991.

The results from combined curve analyses performed on these fitted curves are presented in TABLE 4.8. For both ozone treatments, the curve for each wind treatment was compared with the one for the no-wind control, which resulted in the eight combined curve analyses for wind treatments given in TABLE 4.8. Only the winter storm treatment had an effect, the frost hardiness of the plants

Table 4.8. Combined curve analyses performed on the lines fitted for the shoots from the ozone experiment in 1991.

Comparison	Link	F-probability
No wind ↔ winter	Filtered air	$P \leq 0.25$
No wind ↔ budburst	Filtered air	$P \leq 0.25$
No wind ↔ autumn	Filtered air	$P \leq 0.50$
No wind ↔ 120 hrs	Filtered air	$P \leq 0.75$
No wind ↔ winter	140 ppb ozone	$P \leq 0.005$
No wind ↔ budburst	140 ppb ozone	$P \leq 0.25$
No wind ↔ autumn	140 ppb ozone	$P \leq 0.75$
No wind ↔ 120 hrs	140 ppb ozone	$P \leq 0.25$
Filtered air ↔ 140 ppb ozone	Combined curves	$P \leq 0.25$

receiving 140 nmol mol^{-1} was very significantly reduced ($P \leq 0.01$), when compared with the no-wind control at 140 nmol mol^{-1}. A test for an ozone effect was done by fitting curves to all 40 data points for both treatments, and subjecting these to a combined curve analysis. This analysis also showed no significant effect.

The calculation of the temperatures at which 50% of the shoots were killed (*LT50*), for each of the ozone and wind treatment combinations (TABLE 4.9), shows there was very little difference between the wind treatments when the trees were kept in charcoal filtered air, and somewhat larger differences when the trees were also

Table 4.9. Temperature at which 50 % of the shoots were killed (*LT50*; °C), for the trees from the ozone experiment in 1991.

	Filtered air	140 ppb ozone
no wind	-9.00	-7.56
winter	-9.17	-10.02
budburst	-8.32	-8.53
autumn	-8.65	-8.01
120 hrs	-9.74	-7.28

fumigated with 140 nmol mol^{-1} of ozone, but as in 1990, there was no profound effect of either the ozone or wind treatments on the frost hardiness of the trees.

4.5. Discussion.

4.5.1. Initial conductivity.

The initial conductivity (C_0), as estimated by EQUATION 4.1, represents the solutes readily washed from the needle surfaces (Murray *et al.*, 1989). C_0 is expected to increase when plants are misted with solutions of increasing concentrations of solutes (Fowler *et al.*, 1989), and this study as expected showed a highly significant ($P \leq 0.001$) effect of the acid mist treatment on C_0. The measurements on the seedlings from the acid mist experiment in 1990 also showed a significant ($P \leq 0.05$) wind effect, and an interaction between the two treatments. This indicated that the wind effect only occurred in combination with the acid mist treatment at pH = 2.5. In the presence of pH = 2.5 mist, the wind treatment reduced the initial conductivity. This suggests that at least part of the deposited ions were removed from the needles by the wind treatment some ten weeks before the frost hardiness assessments. As fumigation with ozone does not involve any deposition of chemicals, no effect on the initial conductivity was expected, and in the 1990 experiment neither the ozone nor the wind treatment had any effect on C_0, nor was there a significant interaction between the two treatments. However, in 1991 the ozone fumigation resulted in a statistically highly significant reduction in initial conductivity, although differences were only found at two of the eight freezing temperatures, and only for the plants treated with charcoal-filtered air. This also showed up as a significant wind effect, but no explanation could be found for this discrepancy.

4.5.2. Electrolyte leakage rate.

Significant differences in the electrolyte leakage rate (k_e) were found for the acid mist treatment in 1990, and the wind treatments in the 1991 ozone experiment, but as the k_e-values formed the basis on which the *LT50*'s (temperature at which 50% of the shoots were killed) were calculated, these will be discussed together with the overall frost hardiness of the seedlings, as determined by using EQUATION 4.4.

4.5.3. Overall frost hardiness.

No reports were found in the literature on effects of high wind speeds on the frost hardiness of plants, while the effects of air pollutants have been investigated extensively. Treatment with acid mist has produced varying effects in different experiments, but on the whole reductions in frost hardiness were found (e.g. Fowler *et al.*, 1989). Mists containing sulphate have been shown to be more damaging than mists containing nitrate (Cape *et al.*, 1990; Cape *et al.*, 1991), and the presence of ammonium ions has been shown to be deleterious to red spruce (Cape *et al.*, 1991), but had no effect on Norway spruce (Cape *et al.*, 1990). Not all workers agree on the effects of individual ions, however, as some studies have produced conflicting results. L'Hirondelle *et al.* (1992) found higher conductivities relative to autoclaved values for red spruce, frozen at several temperatures, when the needles were exposed to acid mist containing predominantly nitrate, compared to needles previously exposed to acid mist containing predominantly sulphate. This, however, was not linked to needles being killed by the freezing treatments or not, and the authors also found that the high-sulphate mist caused much more visible injury than the high-nitrate mist (L'Hirondelle *et al.*, 1992), which would suggest that physiological processes in the needles were more perturbed by the mist containing relatively high sulphate concentrations. Sheppard *et al.* (1993*a*) found a significant reduction in frost hardiness of trees which had been sprayed with 8 mm precipitation equivalent of acid mist per week (equimolar sulphuric acid and ammonium nitrate), but when this treatment was reduced to only 2 mm equivalent per week, no effects were found, either for mist of the same chemical composition, or for mist with sulphuric acid only. It appears therefore, that the

Table 4.10. Effects of ozone and acid mist on the frost hardiness of plants, as reported in the literature. (1 ppb ozone = 1 nmol mol^{-1} ozone)

Source	**Treatment**	**Species**	**Effect**
Acid mist:			
Cape *et al.*, 1990	pH = 2.5	*Picea abies*	reduced by sulphate ions, no effect by ammonium or nitrate ions
Fowler *et al.*, 1989	pH = 2.5	*Picea rubens*	reduced
Cape *et al.*, 1991	pH = 2.5	*Picea rubens*	reduced by sulphate or ammonium ions, no effect by nitrate ions
L'Hirondelle *et al.*, 1992	pH = 3.0	*Picea rubens*	high-nitrate misted plants gave higher relative conductivities than high-sulphate misted plants
Sheppard *et al.*, 1993*a*	pH = 2.7	*Picea rubens*	reduced at 8 mm $week^{-1}$ acid mist, no effect at 2 mm $week^{-1}$
Sheppard *et al.*, 1993*b*	pH = 2.5	*Picea rubens*	reduced at 2 mm acid mist twice per week, more reduced at 1 mm four times per week
Ozone:			
Eissenstat *et al.*, 1991	86 ppb	*Citrus paradisi*	reduced
Eissenstat *et al.*, 1991	108 ppb	*Persea americana*	reduced
Barnes & Davison, 1988	120 ppb	*Picea abies*	reduced
Cape *et al.*, 1990	140 ppb	*Picea abies*	reduced
Fincher *et al.*, 1989	102 ppb	*Picea rubens*	no overall effect
Lucas *et al.*, 1988	170 ppb	*Picea sitchensis*	temporarily reduced
Lucas & Penuelas, 1990	70 ppb	*Picea sitchensis*	no effect
Edwards *et al.*, 1990	90 ppb	*Pinus taeda*	varying during dormant season, differences small
Barnes *et al.*, 1988	75 ppb	*Pisum sativum*	reduced
Combined treatments:			
Senser, 1990	50 ppb / pH = 3.0	*Picea abies*	no effect
Vann *et al.*, 1990	ambient pollution excluded	*Picea rubens*	plants treated with filtered air and deionised mist showed improved frost hardiness

total dose of pollution is as important as the concentrations and chemical composition. The frequency of misting is also important. Four applications of 1 mm equivalent per week were more damaging than two applications of 2 mm (Sheppard *et al.*, 1993*b*).

In this study only the frost hardiness of seedlings treated with acid mist in 1990 was determined. The plants were sprayed twice a week to provide 4 mm precipitation equivalent per week. The differences between the curves fitted to the data for the two acid mist treatment were highly significant ($P \leq 0.001$), with a difference in *LT50* of about 7 °C. The plants were sprayed with mists containing equimolar concentrations of ammonium nitrate and sulphuric acid, and it is therefore not possible to contribute the results to a particular ion, or combination of ions. Similarly, because the plants in 1991, which were sprayed four times a week, with the same weekly dose as in 1990, were seriously damaged before frost hardiness measurements could be carried out, no further indication of the effects of misting frequency on the frost hardiness is available, but it is clear that more serious damage was caused by the higher misting frequency, as was shown by Sheppard *et al.* (1993*b*).

The effects of ozone on the frost hardiness of trees appear to be negative on the whole. Plant material subjected to higher concentrations of ozone during the growing season, is frequently less hardy during the autumn and winter than material that experienced lower concentrations. There are, however, exceptions, as several authors found no effect (Fincher *et al.*, 1989; Lucas & Penuelas, 1990). Lucas *et al.* (1988) found a temporary reduction in frost hardiness of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) was present only in November, and absent in shoots sampled four weeks later. These authors attributed the reduction in hardiness to a delay in the development of the hardening process, rather than an impairment, by the application of ozone during the previous summer. Edwards *et al.* (1990) reported varying rankings in the hardiness of loblolly pine (*Pinus taeda* L.) seedlings, exposed to subambient, ambient or twice ambient concentrations of ozone. Differences were only in the order of a few degrees C, and as the *LT50's* were about 15 °C below the lowest recorded temperatures at all three measuring dates, the effects of the ozone treatments would appear to be academic.

The frost hardiness of the seedlings used in this study was not affected by the ozone treatments, in either 1990 or 1991, when comparing the curves relating the proportion of dead shoots to the freezing temperature (EQUATION 4.4). In 1990 the measurements were carried out during the third week of November, while in 1991 the frost hardiness of the seedlings was determined in the first week of October. If ozone fumigation affects Norway spruce in the same way as Sitka spruce as reported by Lucas *et al.* (1988), by delaying the onset of frost hardiness, it should have shown up in either 1990 or 1991. The absence of a significant ozone effect on the frost hardiness in both years shows therefore that the delay in frost hardening did not occur in Norway spruce in this study. Reductions in frost hardiness following ozone fumigation during the growing season have been found for Norway spruce (Barnes & Davison, 1988; Cape *et al.*, 1990), using similar concentrations, which suggests that other factors, which have not been reported, may interact with ozone fumigation in eliciting a response in the frost hardiness of Norway spruce.

When the results from all reported experiments are combined, it appears that ozone fumigation influences the frost hardiness of different species at different concentrations. The faster growing, physiologically more active, species are susceptible to ozone at lower concentrations (Barnes *et al.*, 1988; Edwards *et al.*, 1990; Eissenstat *et al.*, 1991), while several spruce species remain relatively unaffected at higher concentrations (Lucas *et al.*, 1988; Fincher *et al.*, 1989).

The wind treatment significantly ($P \leq 0.05$) increased the frost hardiness of seedlings from the acid mist experiment in 1990, but only when combined with pH = 5.0 control, with the *LT50* reduced by just under 2 °C. For the plants from the high-acid (pH = 2.5) treatment the only effect appeared to be at warmer temperatures, with less damage occurring on the wind treated plants, but this was not statistically significant. It is not clear why the wind treatment improved the hardiness of trees, but as the difference was small, this is likely to be of little importance in the field.

The wind treatment had no effect on the seedlings from the ozone experiment in 1990. A very significant ($P \leq 0.01$) effect was observed in 1991, when wind exposure during the previous dormant season, enhanced the frost hardiness of plants subsequently fumigated with ozone, with the *LT50* lowered by 2.5 °C compared to the no-wind control at 140 nmol mol^{-1}. This result is again contrary to expectation, as it was believed that if the wind treatment affected the foliage, it would be a negative effect, and the frost hardiness was expected to be reduced by the high wind speeds. It can not be explained by experimental error, together with the discrepancy in the initial conductivities, as these were only found on the plants from the charcoal-filtered air chambers, and the unexpected results in overall hardiness were on plants previously fumigated with 140 nmol mol^{-1} ozone.

Chapter 5.
Effects on Other Physiological Parameters.

5.1. Visible Injury.

Several recent studies have shown visible injury occurring on plants treated with air pollutants, especially acid rain or mist (Percy, 1983; Skeffington & Roberts, 1985; Leith *et al.*, 1989), providing a useful indication of a direct pollution effect. In this study, Norway spruce (*Picea abies* (L.) Karst.) seedlings used for frost hardiness measurements in 1990 (see Chapter 4) were assessed for visible injury prior to sampling. In 1991 the plants from the acid mist experiment were scored at the end of August, as by this time, after 15 weeks of treatment with acid mist, severe injury became evident.

5.1.1. Methods and results.

The damage to the current year shoots was assessed on a scale of 0 to 11. Scores 0 to 10 estimated the damage to individual needles in 10% intervals, while a score of 11 indicated dead shoots, as described by Evans & Dougherty (1986) and Leith *et al.* (1989). Only current year shoots were assessed, as it was not possible to determine if damage on older needles was caused by the treatments, or by some factor from earlier years.

On 12 November 1990 10 plants from each of the open-top chambers used for the acid mist (26 weeks of treatment with 4 mm precipitation equivalent of acid mist at pH = 5.0 or pH = 2.5) and ozone (filtered air at all times, or 200 hours of ozone fumigation at 140 nmol mol^{-1} under favourable meteorological conditions, and filtered air at other times) experiments, were scored for visible injury. Five plants from each chamber had also received a treatment in a controlled environment wind tunnel (see Chapter 2.5.2), for 48 hours at 16 m s^{-1}, while the other five served as a no-wind control.

As no visible injury was found on the plants from the ozone experiment, only the data collected for the plants treated with acid mist at pH = 2.5 and pH = 5.0 will be discussed.

Before further analysis, the visible foliar injury scores were expressed as percentage damage, and subjected to an angular transformation (Sokal & Rohlf, 1969). The results are presented as percentage damage again, using the averages of the transformed data, converted back to percentage damage. The average percentage damage for each combination of acid mist and wind treatments is given in TABLE 5.1, together with the distribution range. The results from the analysis of variance on the transformed data are presented in TABLE 5.2.

Table 5.1. Averages for the visible injury scores, expressed as percentage damage for current year needles, for acid mist and wind treated plants, scored on 12 November, 1990. (Minimum and maximum scores in brackets.)

	pH = 5.0	pH = 2.5
Without wind	0.0 (0 - 0)	23.5 (10 - 50)
With wind	0.4 (0 - 10)	18.7 (10 - 40)

Table 5.2. Results from the analysis of variance on the visible injury scores, after an angular transformation, of the acid mist and wind treated plants in 1990. The tabulated values represent the F-probabilities from the analysis of variance.

	Acid mist	Wind	Interaction
Visible injury	0.012	0.956	0.124

TABLE 5.1 shows there was a clear effect of the acid mist treatments on the visible injury on the current year needles (statistically significant ($P \leq 0.05$), see TABLE 5.2). There was no direct effect by the wind treatment, but there was a slight interaction between the treatments, although not significant ($P = 0.124$), as the acid mist treatment appeared to do less damage when combined with a treatment in the wind tunnel.

In 1991 the first foliar injury was recorded in the middle of July, just over two months after the start of the experiment. On 29 August 1991 nine plants from each of the eight open-top chambers used for the acid mist experiment in 1991 were scored for visible injury, after 15 weeks of treatment with acid mist. Of these, three plants from each chamber had not been treated in the wind tunnel, three others had been placed in the wind tunnel for 48 hours at 16 m s^{-1} at the time of budburst, and the last three had been placed in the wind tunnel the week before (see Chapter 2.5.3), repeating the 1990 wind treatment. The data were analyzed as in 1990, and the back-transformed averages are presented in TABLE 5.3.

Table 5.3. Averages for the visible injury scores, expressed as percentage damage for current year needles, for acid mist and wind treated plants, scored on 29 August, 1991. (Minimum and maximum scores in brackets.)

	pH = 5.0	pH = 2.5
No wind	0.0 (0 - 0)	22.9 (0 - 90)
Wind at budburst	0.0 (0 - 0)	40.1 (0 - 90)
Wind in summer	0.0 (0 - 0)	15.7 (0 - 70)

As the summer treatment in the wind tunnel was only applied a week before the assessments, no effect on the visible injury scores was expected. An initial analysis showed the scores for the plants which had received this treatment were not significantly different from those for the no-wind control plants. Therefore, the scores from the plants treated with a summer storm were combined with the control plants' scores to allow a more accurate analysis of the effects of the wind treatment at budburst (TABLE 5.4).

This table shows the acid mist effect was the same as observed in 1990, a significant increase in the visible injury on current year shoots ($P \leq 0.05$). This year, however, an effect by the wind treatment was found: the simulated storm at budburst also significantly increased the visible injury as scored in August. There was also a significant interaction between the two treatments, the wind treatment at budburst caused no damage when combined with the pH = 5.0 acid

Table 5.4. Results from the analysis of variance on the visible injury scores, after an angular transformation, of the acid mist and wind treated plants in 1991. The tabulated values represent the F-probabilities from the analysis of variance.

	Acid mist	Wind	Interaction
Visible injury	0.012	0.012	0.012

mist control, but did exacerbate the effect of the pH = 2.5 acid mist treatment (see TABLE 5.3).

5.1.2. Discussion.

In both 1990 and 1991 the acid mist treatment had a significant effect on the visible injury observed on the first year needles. Acid mist at pH = 5.0 caused (virtually) no damage, while the plants sprayed with acid mist at pH = 2.5 damaged up to 90% of the current year needles in 1991. This finding is in line with Leith *et al.* (1989), who showed that red spruce (*Picea rubens* Sarg.) seedlings, sprayed with pH = 2.5 acid mist were significantly more damaged than those sprayed with pH = 5.0, after 10 weeks of treatment. Work by Chen *et al.* (1991) has shown that this species displays similar visible damage to that of Norway spruce.

The wind treatments in August of both 1990 and 1991 did not significantly alter the visible injury scores in the two acid mist treatments, but when plants were placed in the wind tunnel at the time of budburst, the acid mist treatment at pH = 2.5 was significantly more damaging than when applied without a wind treatment. The wind treatment at budburst probably affected the formation of the cuticle on the young needles, as has been shown in other studies (Hadley & Smith, 1986; van Gardingen *et al.*, 1991). This effect was not strong enough to influence the visible injury scores later during the year, as shown by the lack of damage on plants treated with acid mist at pH = 5.0 and a wind tunnel treatment at budburst. Similar results were found by Wilson (1984), who reported microscopic damage to sycamore (*Acer pseudoplatanus* L.) leaves without any visible discoloration. However, the wind treatment at budburst did increase the damage done by the pH = 2.5 treatment, confirming that the soft material of expanding needles is more easily damaged by wind than mature foliage (Wilson, 1980).

The wind treatment in August 1990 did not significantly damage the current year needles, as scored in November of that year, while the treatment in August 1991 could not realistically be expected to show an effect so soon after treatment, except when the wind treatment was excessive. It is interesting to note, however, that in both years the plants treated in the wind tunnel in August, showed less damage when combined with the pH = 2.5 acid mist treatment than the no-wind control. Jacobson *et al.* (1990) investigated the effect of drying periods between the acid mist treatments on the foliar injury of red spruce, and found increasing levels of damage with longer drying periods, due to evaporation of water from the needles, thereby increasing the concentration of solutes (Unsworth, 1984; Milne *et al.*, 1988). It is possible that a wind treatment at that time somehow reduced the effect by the acid mist treatment, for example by removing deposited pollutants from the needle surfaces.

Leith *et al.* (1989) hypothesized that a series of low night temperatures, below 5 °C over a 6-day period with an observed minimum of 1.8 °C, initiated the development of visible injury. This is not supported by the data from this study, as in 1991 the first symptoms were found as early as in the middle of July, and the lowest recorded temperature before 29 August 1991 was 7.5 °C. Similar results have also been found in later comparable experiments at the Institute of Terrestrial Ecology (I. D. Leith, pers. comm.).

In 1990 no visible injury was found on plants from the ozone experiment. Similar results were found in other studies (Sutinen *et al.*, 1990; Fincher & Alscher, 1992). Sutinen *et al.* (1990) reported that visible damage was only observed on Norway spruce clones at the end of the third summer of ozone fumigation. Fincher &

Alscher (1992) observed lesions on red spruce seedlings after two years of ozone fumigation, but these were attributed to either high temperatures during the growing season, or low temperatures during the winter. Visible injury caused by ozone, similar to that found for the plants from the acid mist experiment, was reported by Skeffington & Roberts (1985), but they applied ozone continuously, and their results can therefore not be directly compared with the results reported here.

No visible injury on Norway spruce, attributable to air pollution treatments, was found even after five years of exposure by Arndt *et al.* (1990). However, their treatment regimes were less severe than the ones used in this project (acid mist at pH = 4.0 and ozone fumigation at 90 nmol mol^{-1}). Leith *et al.* (1989) found that the approximate threshold concentration for damage to red spruce foliage was at a mist acidity of pH = 3.5. Arndt *et al.* (1990) did find an effect on beech (*Fagus sylvatica* L.) and silver fir (*Abies alba* Mill.). This means that Norway spruce is less susceptible than beech and fir, but the exact threshold in concentration and duration of treatments remains to be established. The results from this study, and that by Chen *et al.* (1991) indicate that the threshold concentration is above acid mist at pH = 2.5, and may well be similar to that of red spruce.

5.2. Whole Plant Transpiration Rate.

Effects on the transpiration from plant foliage have been shown after treatments with acid mist (Eamus *et al.*, 1989*b*), ozone (Reich *et al.*, 1985; Temple, 1986) and wind (Caldwell, 1970; Dixon and Grace, 1984). These effects are important, because if the evapotranspiration from foliage exceeds water uptake through the roots, desiccation will occur, resulting in plant death, as their water reserves are generally small (Wolfenden & Mansfield, 1991). It seemed therefore appropriate to investigate the water loss of the seedlings used in this study. The stomatal conductance was measured together with the assimilation rates (see Chapter 3), but another simple method to study the water status of whole seedlings, is to measure their transpiration rates during the day and night (Grace *et al.*,1975; Rees and Grace, 1981; Eamus *et al.*, 1989*b*).

5.2.1. Methods and results.

Day and night transpiration rates were measured on whole seedlings, which were fully watered prior to the experiment. The pots were sealed in polythene bags, which were tied around the base of the stem, below the lowest branch, to prevent evaporation from the soil. Ten plants from each of two replicate open-top chambers for the two acid mist treatment (pH = 5.0 and pH = 2.5) were then kept in an unheated greenhouse, five plants from each chamber had previously been treated in the wind tunnel at 16 m s^{-1} for 48 hours, while the other five plants from each chamber served as a 'no-wind' control. The pots with the plants were weighed (accurate to 0.1 g) at sunset on 30 October 1990, and at sunrise and sunset on the following two days. This allowed the calculation of the water loss during the next two nights and days. These values were then divided by the shoot dry weights determined in December 1990, as reported in Chapter 5.3, to standardise for differences in plant size. The average transpiration rates, in nmol H_2O per gram of dry weight of plant material per second, for the plants from each combination of acid mist and wind treatments are presented in TABLE 5.5, and the results from an analysis of variance performed on these data are shown in TABLE 5.6.

Table 5.5. Average whole plant transpiration, expressed in nmol H_2O g^{-1} dry weight s^{-1}, for the acid mist and wind treated plants, measured between 30 October and 1 November, 1990.

	pH = 5.0		pH = 2.5	
	Without wind	With wind	Without wind	With wind
Night 1	130	91	98	87
Day 1	593	540	527	530
Night 2	243	197	198	173
Day 2	657	614	602	561

Table 5.6. Results from the analysis of variance on the whole plant transpiration rates, of the acid mist and wind treated plants in 1990. The tabulated values represent the F-probabilities from the analysis of variance.

	Acid mist	Wind	Interaction
Night 1	0.521	0.078	0.321
Day 1	0.450	0.524	0.478
Night 2	0.370	0.048	0.568
Day 2	0.562	0.231	0.980

Both the acid mist and the wind treatments reduced the night and day transpiration rate (see TABLE 5.5). However, the only significant effect found was a reduction in the transpiration rate at night, caused by the wind treatment. During the first night the effect was almost statistically significant ($P = 0.078$), the second night the whole plant transpiration rate was significantly reduced ($P \leq 0.05$; see TABLE 5.6). There was no interaction between the two treatments.

In addition to the plants from the acid mist experiment, 40 plants from the ozone experiment were measured, in an identical experimental layout: ten plants from each of two replicate open-top chambers (supplied with filtered air at all times, or fumigated with ozone at 140 nmol mol^{-1} for about 200 hours during favourable meteorological conditions), with the same wind treatments as the plants from the acid mist experiment. The average transpiration rates for the plants from each combination of ozone and wind treatments are presented in TABLE 5.7, and the results from an analysis of variance performed on these data are shown in TABLE 5.8.

Table 5.7. Average whole plant transpiration, expressed in nmol H_2O g^{-1} dry weight s^{-1}, for the ozone and wind treated plants, measured between 30 October and 1 November, 1990.

	Filtered air		140 ppb ozone	
	Without wind	With wind	Without wind	With wind
Night 1	105	123	146	130
Day 1	631	515	532	492
Night 2	240	287	279	256
Day 2	750	668	704	561

Table 5.8. Results from the analysis of variance on the whole plant transpiration rates, of the ozone and wind treated plants in 1990. The tabulated values represent the F-probabilities from the analysis of variance.

	Ozone	Wind	Interaction
Night 1	0.529	0.923	0.114
Day 1	0.049	0.109	0.428
Night 2	0.880	0.588	0.122
Day 2	0.129	0.012	0.471

Two significant ($P \leq 0.05$) effects were observed (TABLE 5.8): the ozone treatment reduced the transpiration rate during the first day (see TABLE 5.7), while the wind treatment reduced the transpiration rate during the second day, and there were indications that ozone reduced the water loss the second day ($P = 0.129$) and the wind treatment the first day's transpiration rate ($P = 0.109$). The night transpiration rates were not affected by either treatment.

5.2.2. Discussion.

The plants were well-watered before the pots were sealed, and TABLES 5.5 and 5.7 show that the transpiration rates were higher during the second 24-hour period. This indicates the plants were not water-stressed over the 48 hours, which might have confounded any treatment effects.

Initially it was assumed that the shoot dry weight was a good estimate of the relative plant size. This means, however, that the plants have to be destructively harvested after the water loss measurement have been made. It would be advantageous if the measurements could be related to a non-destructive parameter. Therefore the data were re-analyzed using the sapwood area at the root collar instead of the shoot dry weight, as it has been shown that the sapwood area is closely correlated to the total leaf area for a number of species (Kendall-Snell & Brown, 1978; Kaufmann & Troendle, 1981; Waring *et al.*, 1982; Marchand, 1984). Although these four studies did not use Norway spruce, strong correlations between sapwood area and the total leaf area were found for 17 other species, among which were two spruce species (red spruce and Engelmann spruce (*Picea engelmannii* (Parry) Engelm.). It seemed therefore reasonable to assume a similar relationship for the seedlings from this study, and as the seedlings were only two years old, the sapwood area was calculated from the root collar diameter. For this the stem was assumed to be perfectly round and consisting of conducting tissue only. The results of this analysis of transpiration rates relative to the sapwood area, for the plants from the acid mist experiments are given in TABLE 5.9 and TABLE 5.10, while those for the plants from the ozone experiments are presented in TABLES 5.11 and 5.12.

It is clear from TABLE 5.9 that the transpiration rates, expressed relative to the sapwood area, are reduced by both the acid mist and the wind treatments. However, the analysis of variance (see TABLE 5.10) showed that for the acid mist treatment this reduction was almost entirely due to random variation ($P \gg 0.05$).

Table 5.9. Average whole plant transpiration, expressed in nmol H_2O mm^{-2} sapwood area s^{-1}, for the acid mist and wind treated plants, measured between 30 October and 1 November, 1990.

	pH = 5.0		pH = 2.5	
	Without wind	With wind	Without wind	With wind
Night 1	32	20	24	18
Day 1	147	119	121	106
Night 2	59	43	46	35
Day 2	159	138	137	110

Table 5.10. Results from the analysis of variance on the whole plant transpiration rates, of the acid mist and wind treated plants in 1990. The tabulated values represent the F-probabilities from the analysis of variance.

	Acid mist	Wind	Interaction
Night 1	0.472	0.021	0.444
Day 1	0.442	0.077	0.615
Night 2	0.333	0.011	0.687
Day 2	0.444	0.020	0.786

The effect by the wind treatment was significant ($P \leq 0.05$) for both nights and the second day, and almost significant ($P = 0.077$) the first day. There were no interactions between the acid mist and wind treatments.

For plants from the ozone experiment a similar change is found, when expressing the transpiration rate relative to the sapwood area instead of the shoot dry weight. Differences in water loss from the plants caused by the wind treatments, were explained better when expressed relative to the sapwood area, presumably because of the better correlation between the sapwood area and the total leaf area. Ozone reduced the day transpiration rates, expressed relative to the sapwood area, but the effect was not significant ($P > 0.05$). However, similar effects were also reported by Skärby *et al.* (1987), who found small reductions in daytime transpiration rates for Scots pine (*Pinus sylvestris* L.), indicating that ozone may influence stomata control of water loss. There were no significant interactions

Table 5.11. Average whole plant transpiration, expressed in nmol H_2O mm^{-2} sapwood area s^{-1}, for the ozone and wind treated plants, measured between 30 October and 1 November, 1990.

	Filtered air		140 ppb ozone	
	Without wind	With wind	Without wind	With wind
Night 1	26	24	31	26
Day 1	161	105	114	98
Night 2	60	59	59	50
Day 2	188	138	149	112

Table 5.12. Results from the analysis of variance on the whole plant transpiration rates, of the ozone and wind treated plants in 1990. The tabulated values represent the F-probabilities from the analysis of variance.

	Ozone	Wind	Interaction
Night 1	0.662	0.230	0.569
Day 1	0.136	0.017	0.169
Night 2	0.571	0.391	0.518
Day 2	0.227	0.001	0.605

between the ozone and wind treatments.

Eamus *et al.* (1989*b*) found no effects of a treatment with acid mist at pH = 2.5 on the day and night transpiration rates of red spruce (expressed in µmol tree^{-1} s^{-1}), and the same was found for Norway spruce in this study. In order to compare the results presented here quantitatively with those reported by Eamus *et al.* (1989*b*), the transpiration rates were also calculated in µmol tree^{-1} s^{-1}, and the night and day transpiration rates were found to be in the order of 5 and 18 µmol tree^{-1} s^{-1}, respectively. Eamus *et al.* (1989*b*) reported values of around 0.6 and 1.3 mmol tree^{-1} s^{-1}, i.e. there is a difference of two orders of magnitude. This can not be explained by the size of the trees, as the average height of their plants was virtually the same as that of the plants used here, nor by the timing of the measurements, as theirs were carried out in October 1987 (I.D. Leith, pers. comm.). However, 0.6 mmol H_2O tree^{-1} s^{-1} at night and 1.3 mmol H_2O tree^{-1} s^{-1}

during the day represents about 1.5 kg of water for the 24-hour period, which seems very unlikely for 3-year old seedlings, whereas the 18 g of water calculated for this experiment was correct when checked against the raw data.

McLaughlin (1985) suggested that changes in plant-water relations could be caused by either a diminished capacity to draw water from the soil, or loss of capacity to control water loss to the atmosphere. The second explanation, a reduced cuticular integrity, was also proposed by Dixon & Grace (1984), to explain an increase in night-time water loss of three broad-leaved tree species. Scots pine, however, did not respond to daily increases in wind speeds from 0.25 to 5.0 m s^{-1} (Dixon & Grace, 1984). It is clear that the Norway spruce seedlings used in this study did not suffer a reduced capacity to control evaporation, as the pollution treatments either reduced the whole-plant transpiration rates, or did not significantly affect them. The wind treatment significantly reduced the transpiration rates. It has to be concluded, therefore, that the plants lost some of their capacity to take up water. This also explains why the wind treatment exerted a stronger effect than the pollution treatments, which only resulted in small, non-significant reductions. Acid mist and ozone primarily affect plant foliage, and are not expected to directly influence the water uptake by the roots. A treatment in a wind tunnel, on the other hand, may well affect the water uptake by the roots, due to physical damage to the fine roots because of movement of the stem caused by the wind treatment (Harrington, 1986; Rizzo & Harrington, 1988). If this assumption is correct, then trees growing at higher altitudes, where they are frequently subjected to severe wind speeds, will be more vulnerable in subsequent times of drought. This susceptibility to drought would not have been found for the seedlings used in this study, as they were well-watered at all times.

5.3. Destructive Harvest.

In general, any growth limiting condition or resource will induce a change in the resource partitioning of the plant, which will result in a proportionally increased allocation in favour of that part of the plant which draws most upon the growth limiting part of the environment (Hunt & Nicholls, 1986; Johnson & Thornley, 1987; Smolders & Merckx, 1992). More particularly, defoliation or shoot removal can cause a reduction in root growth due to a reduction in the amount of assimilates available to the roots (Wilson, 1988). In this experiment the hypothesis was tested that similar effects are found when the total photosynthesising area is effectively reduced because of visible injury caused by air pollutants (see Section 5.1), or when damage to the photosynthetic apparatus occurs, resulting in reduced maximum assimilation rates (see Chapter 3).

5.3.1. Methods and results.

In December 1990, 10 plants, five treated in the wind tunnel in September, for 48 hours at 16 m s^{-1} at plant level, and five serving as a no-wind control, from each of two replicate chambers receiving either pH = 5.0 or pH = 2.5 acid mist from the third week in May onwards, were destructively harvested. The height of the shoot and the root collar diameter were measured to the nearest 0.01 mm, and the shoots and roots were oven-dried at 70 °C to constant weight, and weighed to the nearest milligram. Before any analysis was carried out, all the data were ln-transformed, as the height, diameter and shoot dry weight were highly skewed. The averages of the ln-transformed values were transformed back, and the results are presented in TABLE 5.13. The ln-transformed data were analyzed using an analysis of variance, and the resulting F-probabilities are shown in TABLE 5.14.

TABLE 5.14 shows there were no statistically significant ($P \leq 0.05$) effects on the size of the plants, by either the acid mist or the wind treatments, nor was there an interaction between the two. The wind treatment had very little effect, but the

Table 5.13. Results from the destructive harvest performed at the end of the 1990 growing season on the acid mist and wind treated plants. (Height and root collar diameter in mm, shoot and roots dry weights in g.)

	pH = 5.0		pH = 2.5	
	Without wind	With wind	Without wind	With wind
Height	461	429	394	402
Diameter	11.1	10.6	10.0	10.7
Shoot	37.0	30.6	29.2	28.3
Roots	24.9	20.5	16.7	19.1
S:R Ratio	1.50	1.54	1.87	1.54

Table 5.14. Results from the analysis of variance on the destructive harvest data of the acid mist and wind treated plants in 1990. The tabulated values represent the F-probabilities from the analysis of variance.

	Acid mist	Wind	Interaction
Height	0.237	0.669	0.457
Diameter	0.441	0.797	0.262
Shoot	0.117	0.259	0.415
Roots	0.072	0.812	0.176
S:R Ratio	0.411	0.397	0.303

acid mist treatment at pH = 2.5 appeared to reduce the size of the plants when compared with the pH = 5.0 control, exerting most effect on the root dry weight, although this was not significant (P = 0.072).

Treatment effects on the whole plant partitioning can be examined by analyzing the shoot : root ratios for the individual plants. TABLES 5.13 and 5.14 revealed that neither treatment had an effect in this experiment. However, using the allometric growth equation (Wilson, 1988) allows a more detailed analysis:

$$\ln(W_r) = \ln(b_a) + k_a \ln(W_s) \tag{5.1}$$

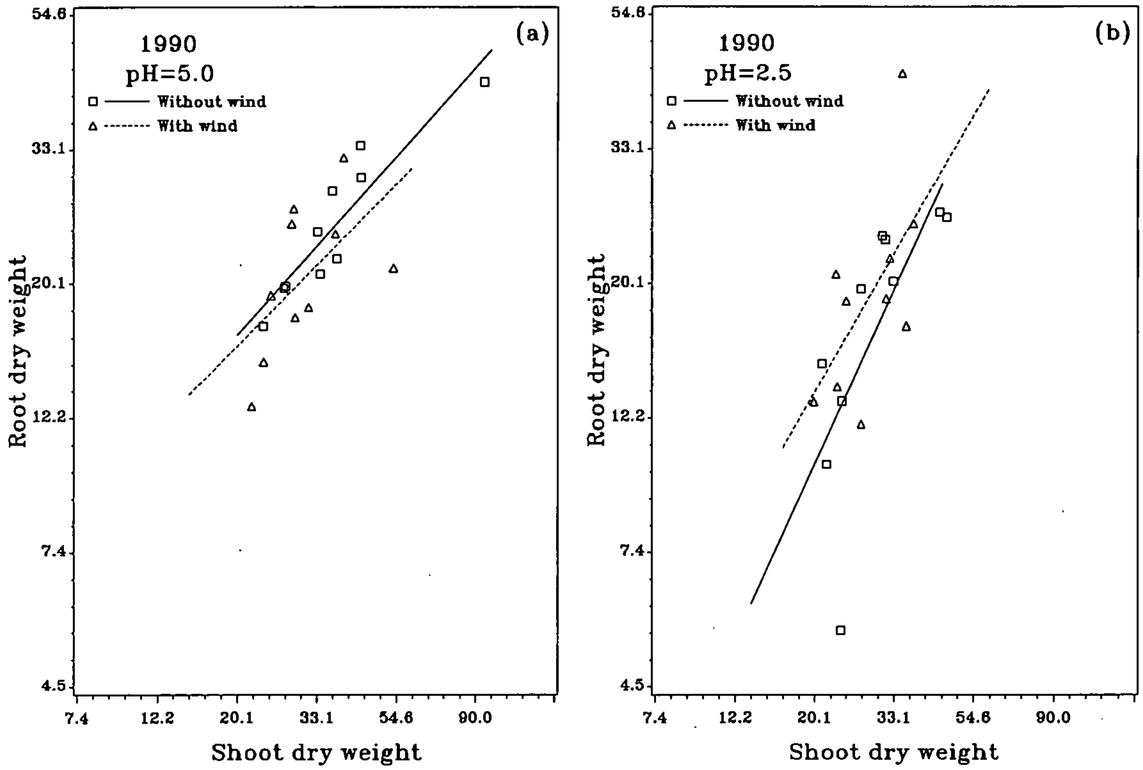


Figure 5.1. Relationship between the natural logarithms of shoot and roots dry weights (in g; ln-scale), together with straight lines fitted to the data sets, for the acid mist and wind treated plants, harvested in December of 1990.

Table 5.15. Average values for the allometric growth constant (k_a), with its standard error, calculated for the destructive harvest data for the acid mist and wind treated plants in 1990.

	pH = 5.0		pH = 2.5	
	Without wind	With wind	Without wind	With wind
k_a	0.67 ± 0.10	0.60 ± 0.30	1.30 ± 0.44	1.04 ± 0.49
k_a^*	1.29 ± 0.06	1.36 ± 0.08	1.27 ± 0.10	1.42 ± 0.09

[*] Lines forced through the initial harvest data point of shoot and root dry weights of 10.0 and 4.3 g, respectively.

where W_r is the dry weight of the roots, W_s is the dry weight of the shoot, and b_a and k_a are constants, which are known to be dependant on environmental parameters (Pearsall, 1927; Troughton, 1955).

In FIGURE 5.1 the ln-transformed data for the root dry weight is plotted against the shoot dry weight, together with straight lines fitted to the data points. The slopes, k_a, with their standard errors, of these fitted lines, are presented in TABLE 5.15, as any comparisons should be made by comparing the values of k_a (Troughton, 1955).

The wind treatment had very little effect on where the plants allocated their dry weight (FIGURE 5.1). The acid mist treatment had a somewhat larger effect, i.e. the plants allocated more assimilated in the shoot when they were sprayed with acid mist at pH = 2.5, which is indicated by the higher values calculated for k_a. However, both the scatter of the data points, and the standard error calculated for k_a, indicate a large amount of variation. This shows two points: 1. More than ten replicates are required per treatment to evaluate effects, and 2. Use of the allometric growth equation gives better results when more than one destructive harvest has been carried out, at appropriate intervals. A second destructive harvest can be simulated by forcing the fitted lines to go through the data point consisting of the average shoot and roots dry weights of the initial harvest, i.e. shoot and roots dry weights of 10.0 g and 4.3 g respectively. The values for k_a calculated this way are also presented in TABLE 5.15. The standard errors calculated for the slopes of these lines indicate the estimates were much more accurate, and combined curves analyses which were carried out on these lines, showed they were virtually identical. It can be concluded therefore, that the assimilate partitioning was not altered by the acid mist and wind treatments, as was found by the analysis of variance on the individual shoot : root ratios.

At the same time as the plants were harvested from the acid mist experiment, 40 plants from the ozone experiment were also destructively harvested. Twenty plants had been fumigated for a total of 200 hours between 13 June and 17 August 1990, with nominally 140 nmol mol^{-1} of ozone, and charcoal filtered air at other times, in two replicate open-top chambers. The other twenty plants had also been kept in two replicate chambers, and received charcoal filtered air at all times. The wind treatment was the same as for the acid mist treated plants, five were placed in the wind tunnel for 48 hours at 16 m s^{-1} in September, the other five were not treated with wind. Identical measurements to those made on the acid mist treated plants, height and root collar diameter to the nearest 0.01 mm, shoot and roots dry weights accurate to 1 mg, were taken. The data were analyzed after a ln-transformation, the back-transformed averages are presented in TABLE 5.16, and the results from an analysis of variance performed on the ln-transformed data, are presented in TABLE 5.17.

Table 5.16. Results from the destructive harvest performed at the end of the 1990 growing season on the ozone and wind treated plants. (Height and root collar diameter in mm, shoot and roots dry weights in g.)

	Filtered air		140 ppb ozone	
	Without wind	With wind	Without wind	With wind
Height	371	392	356	352
Diameter	9.06	9.70	9.35	8.97
Shoot	26.2	24.2	22.9	20.3
Roots	21.2	22.9	21.0	17.2
S:R Ratio	1.30	1.09	1.11	1.21

Table 5.17. Results from the analysis of variance on the destructive harvest data of the ozone and wind treated plants in 1990. The tabulated values represent the F-probabilities from the analysis of variance.

	Ozone	Wind	Interaction
Height	0.435	0.603	0.436
Diameter	0.644	0.739	0.206
Shoot	0.459	0.341	0.849
Roots	0.248	0.597	0.240
S:R Ratio	0.824	0.564	0.111

Neither the ozone treatment nor the wind treatment had a statistically significant effect on the height, root collar diameter, or shoot or roots dry weight, nor was there an interaction between the two treatments ($P > 0.05$). However, there appeared to be a slight antagonistic interaction on the shoot : root ratio, the effect of the combined treatment was less than that of either treatment alone, but this

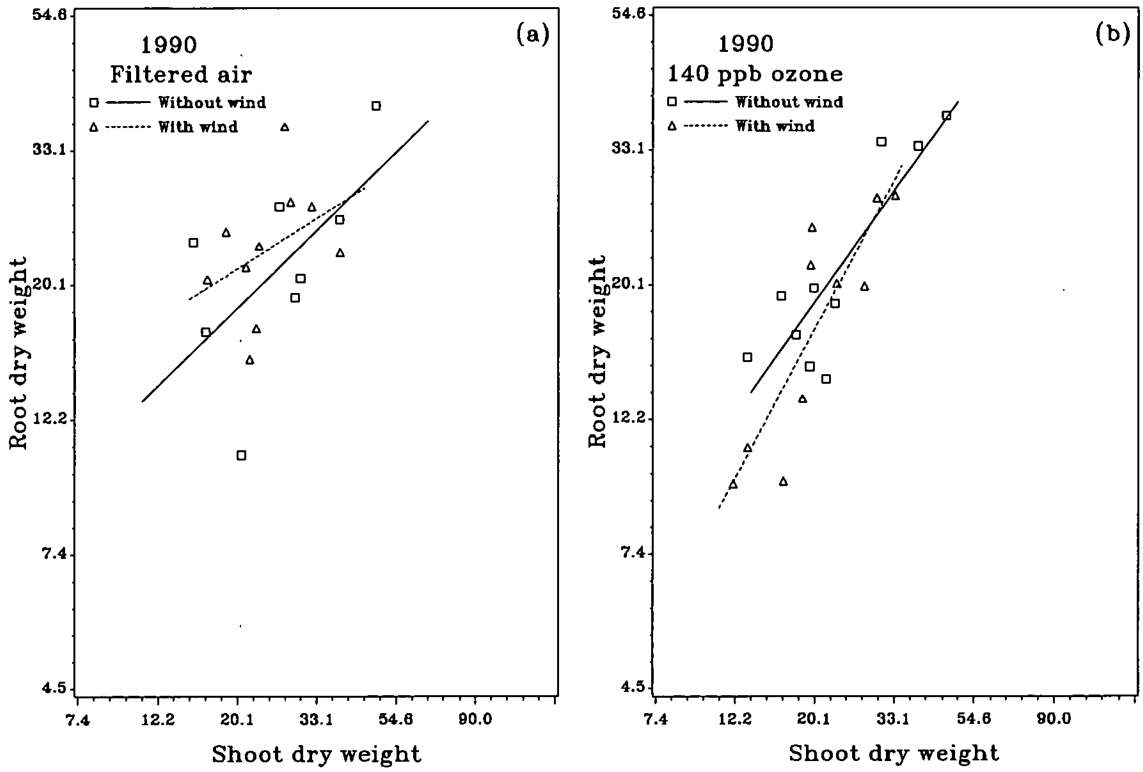


Figure 5.2. Relationship between the natural logarithms of shoot and roots dry weights (in g; ln-scale), together with straight lines fitted to the data sets, for the ozone and wind treated plants, harvested in December of 1990.

Table 5.18. Average values for the allometric growth constant (k_a), with its standard error, calculated for the destructive harvest data for the ozone and wind treated plants in 1990.

	Filtered air		140 ppb ozone	
	Without wind	With wind	Without wind	With wind
k_a	0.58 ± 0.31	0.37 ± 0.31	0.83 ± 0.17	1.10 ± 0.23
k_a^*	1.54 ± 0.17	1.79 ± 0.15	1.73 ± 0.15	1.81 ± 0.14

[*] Lines forced through the initial harvest data point of shoot and root dry weights of 10.0 and 4.3 g, respectively.

was not significant (P = 0.111).

Whole plant partitioning was again assessed using the allometric growth equation (EQUATION 5.1). The shoot and roots dry weights are presented in FIGURE 5.2, together with the fitted straight lines. The slopes, k_a, of these lines, with their standard errors, are shown in TABLE 5.18. Again the data scatter prevented any meaningful analysis of the data, and therefore the lines were forced through the initial dry shoot and roots dry weights. The resulting slopes for these lines are also presented in TABLE 5.18. The estimates for k_a were again more accurate (smaller standard errors), than when only the final harvest data were used, but, as with the results for the plants from the acid mist experiment, combined curves analyses showed the four fitted lines were essentially identical, indicating no treatment effects.

5.3.2. Discussion.

Any adverse influence on plants will reduce their overall net growth (Honěk, 1991), but conditions which only effect part of a plant, will generally induce a change in the resource partitioning, reducing the effect on the whole plant by allocating an increased proportion of the resources to the affected part of the plant (Hunt & Nicholls, 1986). This allows analysis of where external conditions affect plants. TABLE 5.13 showed the plants' growth was reduced when they were sprayed with acid mist at pH = 2.5 as compared to the pH = 5.0 control, while the wind treatment had very little effect. Acid mist reduced the shoot dry weight at the end of the growing season by on average 15%, and by 21% for the roots. This suggests the acid mist treatment's primary effect was on the shoot, and the plants compensated for this by an increased carbon allocation to the shoot, thereby reducing the size of the roots more than that of the shoots. Deans *et al.* (1990) showed that especially the amount of course roots is reduced by an acid mist treatment. In the ozone experiment the overall plant size was also reduced by the pollution treatment, and less by the wind treatment, but both the shoot and roots dry weights were reduced by 14% on average, indicating an effect on the whole plant, rather than a specific part. Although acid mist primarily affected the shoot, and ozone the whole plant, the reverse would be expected as ozone can only be expected to reach above-ground parts of the plants, while acid mist can penetrate the soil to influence the roots directly. However, statistically the difference between the plants in the two ozone treatments has to be regarded as not significant, while although the effect of the acid mist treatment was not statistically significant either, there is a much stronger indication of an effect, especially on root growth (P = 0.072). This could be caused by a reduction in the effective leaf area, caused by needle necrosis and early senescence (see section 5.1), reducing the availability of assimilates to the root system (Wilson, 1988). Artificial defoliation of balsam fir (*Abies balsamea* (L.) Mill.) has also been found reduce the root dry weight more than the shoot dry weight (Piene & Little, 1990), which confirms the more detrimental effect of loss of foliage on the root system.

The calculated values for k_a, using the allometric growth equation (EQUATION 5.1), were on the high side when compared with the range for soil-grown plants, given by Pearsall (1927) and Troughton (1955), especially the plants from the ozone experiment. There are two possible explanations for this: 1. Pearsall reported values of k_a for annuals, and Troughton did his measurements on herbage grasses, and it is possible than conifers allocate relatively less assimilates to the roots, as has been found for herbaceous plants (Bourdôt *et al.*, 1984), which would increase k_a, or 2. Growing plants in water cultures can retard root growth, which would give higher values of k_a (Pearsall, 1927), and although the plants used in this study were grown in soil, they were well-watered, and the open-top chambers used for the ozone experiment did not exclude precipitation, and therefore the soil in which the plants were growing, especially the ozone treated plants, was possibly kept too moist for normal growth. It is unlikely that these relatively high values for k_a are caused by the treatments applied, as the values within the acid mist experiment, and within the ozone experiment are virtually identical.

5.4. Chlorophyll Analysis.

Decreased rates of biomass production in agricultural crops and trees resulting from exposure to elevated concentrations of ozone have been correlated with decreased rates of photosynthesis (Reich & Amundson, 1985). The decrease in photosynthesis has been attributed to a reduction in stomatal conductance (Reich, 1987), and oxidant damage to the biochemical processes of light harvesting and carbon fixation (Sasek & Richardson, 1989).

Oxidant damage can occur in chloroplasts, resulting in a reduced chlorophyll content of the needles. Ozone fumigation of plants has caused varying effects on chlorophyll concentrations. Sasek *et al.* (1991) reported a reduction of up to 65% in total chlorophyll content of loblolly pine (*Pinus taeda* L.), following ozone fumigation during one growing season, and Sasek & Richardson (1989) found a reduction after two years of treatment with ozone. Bermadinger *et al.* (1990) and Siefermann-Harms (1990) found no effect on the chlorophyll content of Norway spruce needles, of three age classes, after five years of ozone fumigation. Edwards *et al.* (1990) reported increases in foliar pigments after six months of ozone treatment of loblolly pine. In the current study the chlorophyll *a* and *b* concentrations were measured for the plants from the ozone experiment in 1991.

5.4.1. Methods and results.

Between 4 and 11 December 1991, needles were removed from the leaders of seedlings used for the 1991 gas exchange measurements on plants treated with ozone and/or wind. They were divided into two samples of about 0.2 g for each plant, and frozen in liquid nitrogen in two pre-weighed 1.5 ml polypropylene vials. After removal from the liquid nitrogen the vials with needles were weighed, to obtain the needle fresh weight, and then one samples per seedling was oven-dried at 70 °C, to constant weight, while the other sample was placed in 5 or 7 ml, depending on needle sample size, of N,N-dimethylformamide (DMF) for

96 hours in the dark at room temperature, to extract the chlorophyll (Moran & Porath, 1980; Moran, 1982). The absorbtion of the resulting chlorophyll solution was measured at 647, 664 and 750 nm (A_{647}, A_{664} and A_{750}, respectively), using a Cecil spectrophotometer (Model CE303, Series II). The chlorophyll concentrations were then calculated in µg ml^{-1} from the following equations (Porra *et al.*, 1989):

$$Chl\ a=12.00(A_{664}-A_{750})-3.11(A_{647}-A_{750}) \tag{5.2}$$

$$Chl\ b=20.78(A_{647}-A_{750})-4.88(A_{664}-A_{750}) \tag{5.3}$$

Using the fresh weight : dry weight ratios obtained from the other sample of each seedling, the calculated concentrations in µg ml^{-1} of chlorophylls *a* and *b*, were converted to µg chlorophyll per gram dry weight of needle material. The average chlorophyll *a* and *b* contents and their ratio are presented in TABLE 5.19, and the results from an analysis of variance performed on the data are shown in TABLE 5.20.

Neither the ozone nor the wind treatment significantly affected the chlorophyll *a* or *b* or their ratio, and there was no interaction between the ozone and wind treatments (TABLE 5.20). However, the ozone treated plants had an almost significant ($P = 0.065$) higher average chlorophyll *a* content than the plants from the charcoal-filtered air chambers (see TABLE 5.19), and there was a similar wind effect on the chlorophyll *a* : *b* ratio ($P = 0.061$), the plants treated in the wind tunnel during the previous dormant period showing a lower ratio than the no-wind control plants, and the plants treated for five days had a relatively high ratio.

5.4.2. Discussion.

The average chlorophyll content reported here was about 0.6 mg per gram dry weight, which is considerably less than values reported for Norway spruce by

Table 5.19. Average chlorophyll *a* and *b* content, in μg g^{-1} dry weight, and their ratio, measured for the ozone and wind treated plants between 8 and 15 December 1991.

	Filtered air				
	no wind	winter	budburst	autumn	120 hrs
Chl *a*	397	372	354	387	297
Chl *b*	225	249	171	239	125
Chl *a* : *b*	1.87	1.76	2.20	1.83	2.37
	140 ppb ozone				
	no wind	winter	budburst	autumn	120 hrs
Chl *a*	429	379	393	438	401
Chl *b*	205	247	189	262	201
Chl *a* : *b*	2.11	1.65	2.15	1.87	2.10

Table 5.20. Results from the analysis of variance on the chlorophyll contents, measured for the ozone and wind treated plants in 1991. The tabulated values represent the F-probabilities from the analysis of variance.

	ozone	wind	interaction
Chl *a*	0.065	0.558	0.882
Chl *b*	0.523	0.285	0.896
Chl *a* : *b*	0.916	0.061	0.671

Siefermann-Harms (1990; about 2 mg g^{-1} chlorophyll *a*) and Bermadinger *et al.* (1990; about 4 mg g^{-1} total chlorophyll), and by Sasek & Richardson (1989; 2 to 4 mg g^{-1} total chlorophyll) for loblolly pine. It is possible this difference is caused by a difference in extraction technique, but no chlorophyll standards for absolute measurements were included in the experiment reported here. However, as comparisons are mainly made between treatments, this will not significantly influence the results.

Sasek & Richardson (1989) showed a significant reduction in chlorophyll content after two years of ozone fumigation, but not after one year. Also, the reported reduction was higher when expressed per unit area; when expressed per unit dry weight, effects were small. The chlorophyll content expressed in mg g^{-1} increased slightly after one year treatment. Edwards *et al.* (1990) reported that concentrations of chlorophylls *a* and *b* in the second flush needles of loblolly pine, harvested in October after fumigation during the growing season, were significantly higher in twice-ambient ozone treated seedlings compared with those of needles exposed to ambient and subambient concentrations of ozone. This compares well with the results presented here, which would tend to indicate that ozone, when only applied for relatively short periods, i.e. one growing season, at chronic rather than acute concentrations, does not seriously affect chlorophyll concentrations. Higher concentrations, however, do appear to cause damage to the photosynthetic apparatus. Sasek *et al.* (1991) found the total chlorophyll content declined by up to 65% at concentrations of three times ambient, compared to charcoal-filtered air, but these authors applied ozone for 12 hours each day from June to October 1987. As their highest hourly concentration was 347 nmol mol^{-1}, the results can not be compared with those from this study, where the plants were protected from concentrations over 160 nmol mol^{-1}.

Chapter 6.

Summarizing Discussion and General Conclusions.

In this concluding chapter the results presented in Chapters 3, 4 and 5, and appropriate work from the literature, are summarized, and discussed in relation to each other where appropriate in Section 6.1. The concept, presented diagrammatically in FIGURE 1.1, describing the potential environmental stresses involved in forest decline, has been adapted in Section 6.2., to incorporate the findings of this study, and general conclusions have been drawn. The main conclusions of this study are presented in the thesis summary in Section 6.3.

6.1. Results Presented in this Thesis.

This section is based on the topics outlined in the literature review in Chapter 1. The effects of the acid mist, ozone and wind treatments are first discussed separately in Subsections 6.1.1., 6.1.2. and 6.1.3. respectively. In Subsection 6.1.4. the interactions between the pollutant and wind treatments are provided. Each subsection starts with a brief summary of the current knowledge from the literature, after which the results from this study are presented in summarizing tables, followed by an overall discussion.

6.1.1. Acid mist.

Large inputs of the major ions in acidic deposition, SO_4^{2-}, NO_3^-, NH_4^+ and H^+, have generally resulted in a reduced tree vigour, both in the field and under controlled conditions. 'Tree vigour' in this respect, encompasses a wide variety of physiological and growth responses, such as radial and height growth, photosynthesis, visible injury (discoloration, lesions, etc.), mycorrhizal associations, and cold tolerance. Not all of these parameters are influenced directly, i.e. some of these effects are secondary. Reductions in growth are a consequence of a reduced availability of assimilates or nutrients, due to effects on photosynthesis or mycorrhizal associations. The process of photosynthesis has been shown to be directly affected by acid mist or rain, but the assimilating area of a tree is also reduced through needle necrosis and premature senescence, resulting in a lower overall potential for carbon assimilation. Similarly, mycorrhizal associations may be directly affected through acidification of the soil, but their effectiveness could also be lowered through a reduced availability of assimilates. Frost hardiness is influenced by the carbohydrate status of the needles (Aronsson *et al.*, 1976), and a reduction in frost hardiness due to acid mist treatments, as found in several studies (Fowler *et al.*, 1989; Cape *et al.*, 1990; Jacobson *et al.*, 1992) could be an indirect result of a reduced photosynthetic activity prior to or during the hardening period.

The effects of acidic mists have been found to be modified by other conditions encountered by trees growing at higher altitudes. Well-fertilized plants are much less susceptible than plants growing in natural soils of low base status (McLaughlin & Tjoelker, 1992). The ionic composition of the mists also exerts a strong influence on the effects found (Jacobson *et al.*, 1990). Generally, mists containing sulphate as the dominant anion, are more damaging than mists dominated by nitrate. This was reported for foliar necrosis (L'Hirondelle *et al.*, 1992), alteration of crystalline wax structures (Rinallo *et al.*, 1986; Adams *et al.*, 1990), and reductions in frost hardiness (Cape *et al.*, 1991; Jacobson *et al.*, 1992).

In this study acid mist, containing equimolar concentrations of sulphuric acid and ammonium nitrate at pH = 2.5, was found to influence some gas exchange parameters of Norway spruce (*Picea abies* (L.) Karst.) seedlings, but only in 1990, and not in 1991 (see TABLE 6.1). The absence of effects in 1991 may have been caused by differences in the growth regime between the two years. In 1990 the plants were potted up immediately prior to being placed in the open-top chambers. The plants used in 1991 were potted up in the preceding autumn, and were left on the standing out area during the winter, providing the seedlings with

Table 6.1. Overview of the effects of the acid mist treatments in 1990 and 1991 on the parameters presented and discussed in Chapters 3, 4 and 5.

	Chapter	Acid mist treatments
Maximum assimilation rate	3	Reduced in August 1990, no effect in September 1990 and August 1991.
Light compensation point	3	No effect in August 1990 and August 1991, reduced in September 1990.
Apparent quantum yield	3	Not altered significantly.
Dark respiration	3	Reduced in August and September 1990, no effect in August 1991
Stomatal conductance	3	Not altered significantly.
Stomatal response time	3	Not altered significantly.
Initial conductivity	4	Highly significantly increased.
Electrolyte leakage rate	4	Significantly increased.
Overall frost hardiness	4	Highly significantly reduced.
Visible injury	5.1	Significantly increased.
Whole plant transpiration rate	5.2	Not altered significantly.
Height	5.3	Not altered significantly.
Root collar diameter	5.3	Not altered significantly.
Shoot dry weight	5.3	Reduced, although not significantly.
Root dry weight	5.3	Reduced, although not significantly.
Shoot : root ratio	5.3	Not altered significantly.

six months to recover from planting shock. However, this difference did not affect the time of flushing, which was late April and early May in both years, and it seems unlikely the timing of potting up caused the differences in effects for the gas exchange parameters. Another difference between the two years was the frequency of spraying. In 1990 the seedlings were received 2 mm precipitation equivalent twice a week, while in 1991 the seedlings were sprayed with 1 mm for four times each week. It has been shown in other studies, however, that an increasing the spraying frequency exacerbates damage (Jacobson *et al.*, 1990; Sheppard *et al.*, 1993*b*), while this study revealed no significant changes in the carbon assimilation processes after application of acid mist at pH = 2.5.

Possibly the most obvious explanation for the different effects on photosynthesis in the two years, is the difference in fertilizer. In 1991 Osmocote Mini (containing 18-6-11 N:P:K) was used instead of Vitax Q4 (composition 5.3-7.5-10 N:P:K, plus trace elements), as it was found that N:K ratios in plants fertilized with Vitax Q4 were unusually low, with N-content lower, and K-content higher than usual. In addition, sulphur levels in these plants were very high (L.J. Sheppard, pers. comm.), but the manufacturer would not supply information on the sulphur content of Vitax Q4. Similar results were obtained by McLaughlin & Tjoelker (1992), who found that the maximum photosynthetic rate was reduced by the application of acid rain and mist when plants were grown in natural soil, but increased in fertilized soil. L'Hirondelle *et al.* (1992) also reported red spruce (*Picea rubens* Sarg.) seedlings of low nutrient status to be more sensitive to acidic mist than well-fertilized ones.

In general, the data collected in this study support the hypothesis as suggested by McLaughlin & Tjoelker (1992), that the effects of the acid mist treatment can be mitigated by a well-fertilized soil, although not all parameters were measured in 1991. There were two exceptions (see TABLE 6.1). The maximum assimilation rate (A_{max}) was reduced by the acid mist treatment in August 1990, as would be expected, but three weeks later no effect was found, and in 1991 serious foliar injury was found, even though the seedlings were well-fertilized.

The foliar injury observed on the seedlings treated with acid mist at pH = 2.5 in 1991 was probably a result of the increased frequency of mist application. It has been shown by Sheppard *et al.* (1993*b*) that at a constant dose applied, a higher frequency had a larger effect on red spruce seedlings. There are two possible explanations for this, although these are linked to some extent. When mist droplets remain on plant foliage after an event, liquid water will evaporate, increasing the concentration in the remaining droplets, and potentially increasing the toxicity to foliage (Unsworth, 1984; Milne *et al.*, 1988), and this effect was doubled with the frequency used in the 1991 experiment. Secondly, it is feasible that the total leaf area of the seedlings could not hold 2 mm precipitation equivalent, in which case some of the applied mist would drip off the foliage, and the effective dose would be less than assumed. This reduced effective dose will also reduce the quantity of pollutants remaining on the foliage after evaporation of the water, limiting its toxicity.

The second apparent anomaly, no effect on maximum assimilation rates in September 1990, may have been caused by the onset of the frost hardening process in the acid mist control plants. At the end of August there was a clear difference between the A_{max} of plants treated with acid mist at pH = 5.0 and pH = 2.5, while three weeks later, after the wind treatment, no effect was found. An analysis of variance showed that the wind treatment had no effect, nor was there an interaction between the two treatments. Under normal circumstances, trees start to harden against freezing temperatures, in early autumn, in response to a combination of day length and cold temperatures, resulting in a reduced physiological activity, and therefore lower maximum assimilation rates. Eamus & Murray (1991) found higher light-saturated assimilation rates in winter for Norway spruce treated with ozone and acid mist, which would suggest that these seedlings were more physiologically active than their controls, as well as less frost hardy. If this effect occurred in the present study, this might have resulted in a time during the hardening period when the seedlings from the two acid mist treatments exhibited similar maximum assimilation rates, as was found in September 1990.

If the acid mist treatment at pH = 2.5 caused the seedlings to delay or reduce the frost hardening, relative to the control seedlings sprayed with pH = 5.0 acid mist, the maximum assimilation rates might be expected to remain constant, or reduced less for the acid mist treated plants, compared to the control plants. They were, however, some 15% higher in September 1990 than in August 1990. This can be explained by the ambient temperatures before the measurement periods. Eamus & Fowler (1990) acclimated their red spruce seedlings for seven days prior to gas analysis measurements, in a temperature controlled greenhouse, to remove variations caused by differences in cultivation temperature. Neilson *et al.* (1972) showed that a difference in cultivation temperature of 6 °C for three months, resulted in a reduction in the light saturated assimilation rate of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) of 40%. The average ambient temperature during the fortnight before the first round of measurements in this study in 1990 was 13.1 °C, and before the second series this was 13.8 °C. This relatively small difference in temperature seems unlikely to account for a 15% increase in maximum assimilation rates, but it probably contributes to the difference in A_{max}.

The dark respiration and light compensation point were reduced in the 1990 acid mist experiment, in contrast to the expectation. As this effect was also found in response to the wind treatments in the 1990 and 1991 acid mist experiments, together with a (non-significant) synergistic interaction, this will be discussed in Section 6.1.4.

6.1.2. Ozone.

Detrimental effects of ozone have been observed on a wide variety of physiological processes at all levels of plant function (see Section 1.3.2). The maximum assimilation rates are generally reduced, the extent of the effect depending on the total dose of ozone absorbed by the foliage, relative to its useful lifespan, as was shown in FIGURE 1.4, which was reproduced from Reich (1987). Varying effects on the dark respiration have been reported (Wolfenden & Mansfield, 1991), fumigation with ozone has resulted in increased, decreased or unchanged rates of dark respiration. The stomatal conductance is usually unaffected by ozone concentration below 200 nmol mol^{-1}, except for a decline paralleling that in photosynthesis, with stomatal closure caused by increased internal CO_2 concentrations (Reich *et al.*, 1985; Temple, 1986). A few workers found slower stomatal responses to changes in light conditions (Keller & Häsler, 1984, 1987) or increasing water deficit in needles (Barnes *et al.*, 1990*c*). Growth may be reduced, with the roots frequently earlier and more affected by ozone fumigation than the above-ground plant parts (Darrall, 1989). Results obtained by a number of researchers have shown that fumigation with ozone accelerates the processes of plant senescence, resulting in visible symptoms of leaf damage (Smith *et al.*, 1990), and early senescence and abscision of foliage (Coyne & Bingham, 1982; Reich *et al.*, 1984). This early senescence may also limit the resources available for winter hardening, resulting in increased susceptibility to frost (Barnes & Davison, 1988; Cape *et al.*, 1990).

Environmental conditions modify plant responses to ozone. Drought-stressed plants generally show reduced responses to ozone fumigation, presumably due to a decreased stomatal conductance, lower rates of gas exchange (Tingey & Hogsett, 1985), and therefore smaller fluxes of ozone into the plant for the same ambient concentrations.

In this study fumigation with ozone for one growing season, exerted only limited effects on Norway spruce seedlings, see TABLE 6.2., even though the ozone exposure was as large as those in areas of Europe where ozone concentrations are the highest. The maximum assimilation rates in the two series of measurements in 1990 were reduced, but no effects were found in 1991. The stomatal conductance was reduced in early September 1990, but no effect was found some four weeks later in early October 1990. However, in early November 1990 the whole plant transpiration rates were reduced during the day, for the ozone fumigated plants compared with the charcoal-filtered control plants. This suggests a variable stomatal behaviour in response to ozone fumigation. A reduced stomatal conductance, concurrent with a reduced maximum assimilation rate, is consistent with effects reported in the literature, i.e. stomatal closure being a secondary effect (Reich *et al.*, 1985; Temple, 1986; Sasek *et al.*, 1991). The reduction in stomatal response time, i.e. the time needed by the stomata to respond to changes in the photon flux from about 600 to 1200 μmol m^{-2} s^{-1}, was the opposite to that reported by Keller & Häsler (1984, 1987), and the cause of this response

Table 6.2. Overview of the effects of the ozone treatments in 1990 and 1991 on the parameters presented and discussed in Chapters 3, 4 and 5.

	Chapter	Ozone treatments
Maximum assimilation rate	3	Reduced in September and October 1990, no effect in November/December 1991.
Light compensation point	3	Not altered significantly.
Apparent quantum yield	3	Not altered significantly.
Dark respiration	3	Not altered significantly.
Stomatal conductance	3	Reduced in September 1990, no effect in October 1990 and November/December 1991.
Stomatal response time	3	Stomata responded faster to changes in photon flux after treatment with 140 nmol mol^{-1} ozone.
Initial conductivity	4	No effect in 1990, unexplained increase at two freezing temperatures in 1991.
Electrolyte leakage rate	4	Not altered significantly.
Overall frost hardiness	4	Not altered significantly.
Visible injury	5.1	No visible injury observed in ozone experiments.
Whole plant transpiration rate	5.2	Reduced during the day.
Height	5.3	Not altered significantly.
Root collar diameter	5.3	Not altered significantly.
Shoot dry weight	5.3	Not altered significantly.
Root dry weight	5.3	Not altered significantly.
Shoot : root ratio	5.3	Not altered significantly.
Chlorophyll *a*	5.4	Increased, although not significantly.
Chlorophyll *b*	5.4	Not altered significantly.
Chl *a* : *b* ratio	5.4	Not altered significantly.

remains unclear.

The chlorophyll *a* content of the needles from the ozone fumigated plants in 1991 was larger than that of the charcoal-filtered control plants. This may explain why no effects on the maximum assimilation rates were found.

Under natural conditions the development of frost hardiness in trees involves a series of metabolic and physiological processes, the timing of which depends on seasonal temperature fluctuations and day length (Wolfenden & Mansfield, 1991). Early senescence often brought on by ozone, together with increased rates of dark respiration, may impose a limit on the resources needed for normal winter hardening. In this study neither early senescence, nor increases in dark respiration were found, which may explain the absence of an effect by the ozone treatment on frost hardiness. Eamus & Murray (1991) reported an increased susceptibility of ozone-fumigated plants to frost, together with increased light-saturated assimilation rates, due to a higher physiological activity compared to the charcoal-filtered control plants. Several other studies have linked increased frost susceptibility to lower concentrations of raffinose in the foliage (Rehfuess, 1987; Alscher *et al.*, 1989), and higher concentrations of starch (Rehfuess, 1987). This also suggests that the ozone-fumigated plants did not attain the degree of dormancy (and frost hardiness) found in the control plants. The ozone-fumigated seedlings from the present study, however, did not show reductions in frost hardiness, or maximum assimilation rates in November/December 1991, although it is likely that the seedlings used in 1991 were less susceptible to ozone, possibly due to the nutrient status of the soil. In early October 1990 the maximum assimilation rates were reduced, but the frost hardiness was not affected some six weeks later. Using realistic exposure regimes, conifers appear to be relatively insensitive to ozone after just one growing season of exposure. Effects are often only found after several successive year of treatment (Sutinen *et al.*, 1990; Wallin *et al.*, 1992*b*).

The limited effect of one season of ozone fumigation on trees, and especially on conifers, is also shown in FIGURE 6.1, which is reproduced from FIGURE 1.4, encompassing data points from the current study. The ozone dose was calculated from the average ozone concentration during the fumigation periods, multiplied by the total number of hours of fumigation. In 1990 this was 24.6 ppm-h, and in 1991 34.2 ppm-h (see Section 2.4). This assumes that the dose for the charcoal-filtered control plants was nil, as was the dose for the fumigated

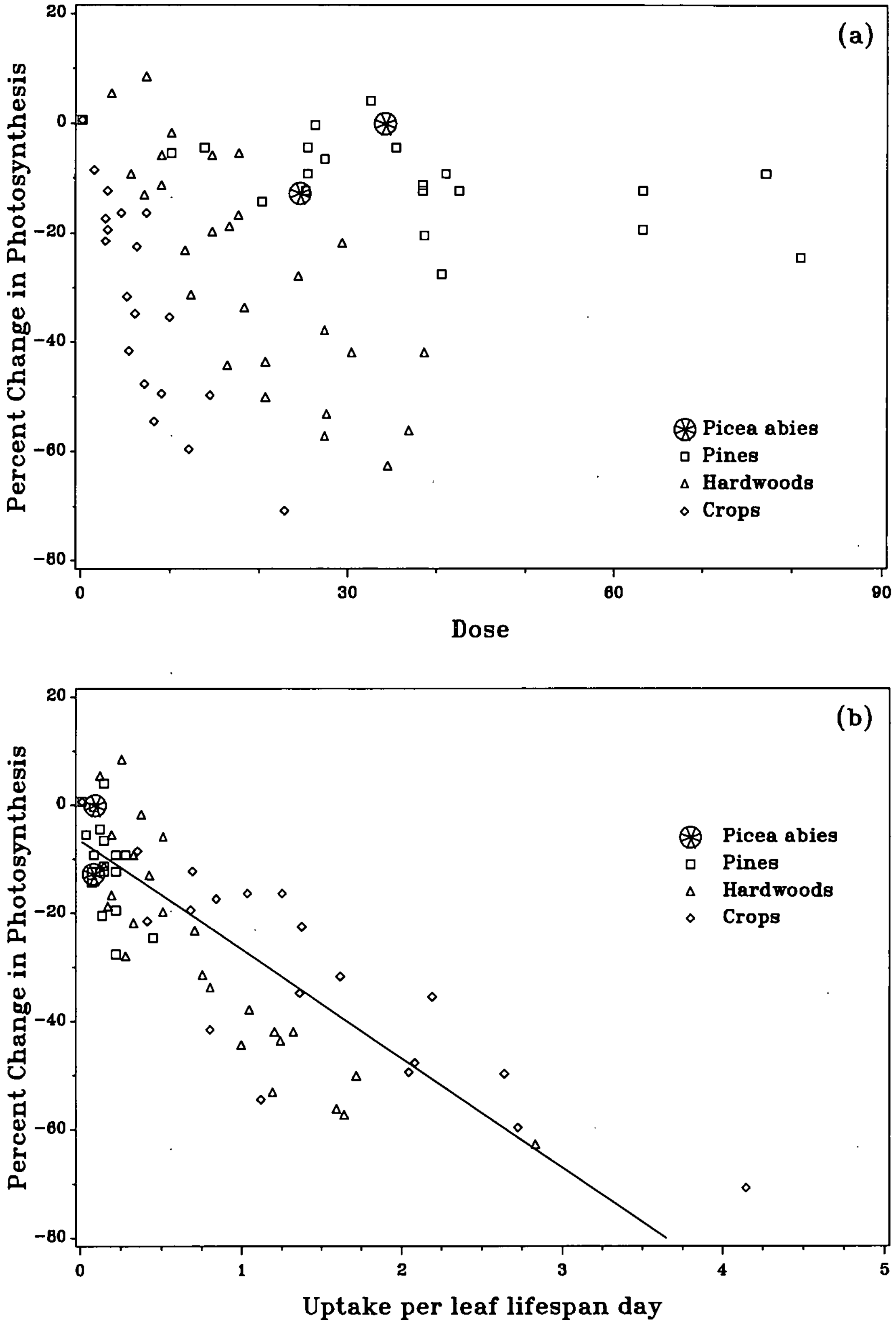


Figure 6.1. Percent reduction in the net photosynthesis of conifers, hardwoods and agricultural crops in relation to (a) the total ozone dose (in ppm-h), and (b) the ozone uptake divided by days in the leaf lifespan (in μg cm^{-2} day^{-1}). (After Reich (1987), incorporating data points from the present study.)

seedlings in between episodes. The small error which this introduced, was assumed to be negligible. The reduction in photosynthesis was averaged across wind treatments, as was the stomatal conductance (g_s). The stomatal conductance to ozone ($g_{s,o}$) was calculated from (after Leuning *et al.*, 1979):

$$g_{s,o}=\left(\frac{D_o}{D_w}\right)g_s \approx 0.61 g_s \qquad (6.1)$$

where D_o and D_w are the molecular diffusivities of ozone and water vapour (in $m^2\ s^{-1}$).

Ozone uptake was estimated as the product of the dose and the stomatal conductance to ozone, and divided by an arbitrary leaf life span of 438 days (Reich, 1987). Recalculating ozone uptake from the dose data presented by Reich (1987), assuming an average stomatal conductance (g_s) of 120 mmol $m^{-2}\ s^{-1}$ (Körner *et al.*, 1979; Cowan, 1977), resulted in values of about 1 µg cm^{-2} day^{-1}, i.e. two orders of magnitude smaller than the uptake calculated by Reich (1987). Therefore the units in FIGURE 6.1b were altered with respect to FIGURE 1.4b. FIGURE 6.1 shows that the reduction in maximum assimilation rates found in this study fit in well with other data presented for conifers, mostly pine species, found in the literature. FIGURE 6.1 also shows the extremely high doses applied in some studies reported in the literature. Where reductions in maximum assimilation rates have been found for conifer species, these are usually a result of unrealistic exposure regimes, and have to be regarded as of relatively little importance under normal conditions.

The straight line in FIGURE 6.1b, relating the ozone uptake per leaf life span day to the reduction in photosynthesis, fitted by Reich (1987), is hardly affected by the addition of the two data points from this study. However, both from a theoretical point of view, and from the data points in FIGURE 6.1b, it would seem that a rectangular hyperbola would provide a better fit to the data points, with an asymptote at 100% reduction in photosynthesis. The fitted straight line indicates some damage without any ozone uptake, and it would also theoretically be possible to obtain a reduction in photosynthesis of more than 100%, by applying a very high dose of ozone. A reduction in maximum assimilation rates of more than 100% indicates respiration rates higher than gross assimilation rates, a condition which would very quickly result in the death of the plant.

This study clearly indicated that ozone fumigation of Norway spruce at 140 nmol mol^{-1} for one growing season has only limited effect. Where negative responses of conifer species after ozone fumigation have been reported, these were frequently the result of unrealistic ozone concentrations (e.g. Wiselogel *et al.*, 1991), or were only found after several successive years of fumigation (e.g. Sasek *et al.*, 1991; Wallin *et al.*, 1992*b*).

6.1.3. Wind.

Although acid deposition or some other anthropogenic stress has been proposed as the primary cause of spruce-fir decline, wind appears to be an overlooked but significant natural stress factor that might contribute to the growth decline and mortality of spruce and fir at high elevations (Harrington, 1986).

Exposure to high wind speeds can induce a range of biological responses (van Gardingen & Grace, 1991; see also Section 1.4.2.). Plant water relations can be altered, physical damage can occur on foliage and roots, and consequently other parameters may be affected, such as photosynthesis, dark respiration and growth and assimilate partitioning. Photosynthetic rates have been found to be reduced, although this is probably a secondary effect of exposure to wind, resulting from changes in foliage orientation (Caldwell, 1970) or decreases in carboxylation rates due to a reduced water content (Grace & Thompson, 1973). Where the dark respiration has been measured following exposure high wind speeds, increased rates were usually found.

Plant water relations may be modified in several ways by exposure to high wind speeds, and both increases and decreases in transpiration rates have been reported. The influence of wind on transpiration rates appeared to be determined by the radiation levels: at low light levels the evaporation was generally increased at higher wind speeds, while at high radiation levels reductions in evaporation have frequently been found in response to exposure to high wind speeds (Dixon & Grace, 1984; MacKerron & Waister, 1985). This reduction in evaporation at higher wind speeds has been shown to occur because of the effect of wind on the surface temperature, and thus on the vapour pressure deficit (Bunce, 1985).

Wind speeds can physically damage plant material. Foliage is affected at relatively low wind speeds, but ultimately virtually all plant parts become susceptible to extremely high wind speeds. The actual wind speeds at which damage occurs depends on the species, conifer needles, and especially the short needles on species such as Norway spruce, are much less easily damaged than broad leaves. For whole trees the susceptibility increases with age (Foster, 1988*a*), but for foliage the reports are less clear. In grasses damage is more pronounced on older leaves (Russell & Grace, 1979), while Wilson (1980) demonstrated that young expanding sycamore (*Acer pseudoplatanus* L.) leaves were far more susceptible than mature leaves. Effects of high winds on root systems have also been reported.

The effects of the wind treatments in this study are summarized in TABLE 6.3. Several parameters which were found to be affected in other studies, showed no response in the work presented here. Neither the maximum assimilation rate nor the stomatal conductance were altered in response to the wind treatments. However, the gas exchange measurements were not made in the wind tunnel, and therefore the needle orientation will not have been altered compared to the no-wind control plants. This supports the findings of Caldwell (1970), who showed reductions in photosynthesis of Arolla pine (*Pinus cembra* L.) were

Table 6.3. Overview of the effects of the wind treatments in the 1990 and 1991 acid mist and ozone experiments, on the parameters presented and discussed in Chapters 3, 4 and 5.

	Chapter	Wind treatments
Maximum assimilation rate	3	Not altered significantly.
Light compensation point	3	Reduced in the acid mist experiments in 1990 and 1991, no effect in the ozone experiments.
Apparent quantum yield	3	Not altered significantly.
Dark respiration	3	Reduced in the acid mist experiments in 1990 and 1991, no effect in the ozone experiments.
Stomatal conductance	3	Not altered significantly.
Stomatal response time	3	Increased by 120 hours wind treatment (ozone experiment 1991), no significant effect otherwise.
Initial conductivity	4	Reduced on plants sprayed with acid mist at pH = 2.5 only, unexplained increases at two freezing temperatures in the 1991 ozone experiment.
Electrolyte leakage rate	4	Differences between the five wind treatments in the 1991 ozone experiment, especially on ozone fumigated plants at colder temperatures.
Overall frost hardiness	4	Increased on plants sprayed with acid mist at pH = 5.0 only, increased by the winter wind treatment in the 1991 ozone experiment.
Visible injury	5.1	Not altered significantly.
Whole plant transpiration rate	5.2	Reduced at all times when combined with acid mist treatments, reduced during the day only when combined with ozone treatments.
Height	5.3	Not altered significantly.
Root collar diameter	5.3	Not altered significantly.
Shoot dry weight	5.3	Not altered significantly.
Root dry weight	5.3	Not altered significantly.
Shoot : root ratio	5.3	Not altered significantly.
Chlorophyll *a*	5.4	Not altered significantly.
Chlorophyll *b*	5.4	Not altered significantly.
Chl *a* : *b* ratio	5.4	Small, non-significant differences.

primarily due to a substantial change in needle orientation. Similarly, stomata of Sitka spruce seedlings have been shown to partially close in response to high wind speeds (Grace *et al.*, 1975). In this study the stomatal conductance was measured after the wind treatment, under the same conditions for all seedlings, and no effects were found. This suggests that effects of high winds on stomata are short-lived, as the measurements in 1990 were made within 8 hours of the seedlings being taken out off the wind tunnel, or that the Norway spruce seedlings used in this study did not respond at all to the wind treatments.

The dark respiration and light compensation point were reduced in the 1990 and 1991 acid mist experiments, contrary to what was expected, while no effect was found in the ozone experiments. As this effect was also found in response to the acid mist treatments, together with a (non-significant) synergistic interaction, this will be discussed in Section 6.1.4.

This study simulated newly planted seedlings at higher elevations, where abrasion caused by physical contact between seedlings does not occur due to the spacing generally used. Therefore, physical contact was avoided during the treatment in the wind tunnel, and no visible injury, such as might arise from branches rubbing against each other, was found directly attributable to the wind treatments. Similarly, no effect were found on the growth parameters, although it was inferred from the whole plant transpiration measurements, that the root systems must have been damaged by the wind treatments. Whole plant transpiration rates were reduced at most times, which indicates a reduced water uptake by the roots. Physical damage to the fine roots because of movement by the stem caused by high winds, has been shown before (Harrington, 1986; Rizzo & Harrington, 1988), and reduces the capacity of the root system to draw water from the soil. The seedlings used in this study were well-watered at all times, and no adverse effects were found. However, as there was a potential for an increased susceptibility to drought, this study confirms the need for testing seedlings under all possible environmental conditions (Barnes *et al.*, 1988). On the other hand, trees growing at higher altitudes rarely encounter prolonged drought conditions, and water stress has been shown to be unlikely as a primary cause for forest decline at higher altitudes (Andersen & McLaughlin, 1991).

Although whole plant transpiration rates were significantly reduced during the day time, in both the 1990 acid mist and ozone experiments, no significant effects were found on the stomatal conductance at saturating photon fluxes. The stomatal response time (τ_{1200}) was increased by the extremely severe 120 hours wind treatment, but the 48 hours treatments did not significantly alter τ_{1200}. This supports the conclusion that the changes in whole plant transpiration are not due to changes in the stomatal behaviour, and suggests that the reduced whole plant transpiration rates were caused by a reduced water uptake from the soil, due to damage to the root systems.

6.1.4. Combinations of acid mist, ozone and wind.

No investigations into the combined effects of pollutants and high wind speeds have been reported in the literature, although it has been recognised that cloud water deposition is much more efficient at higher wind speeds (Fowler *et al.*, 1991). In addition, if some aspect of cloud water deposition causes canopy deterioration in subalpine forests, a positive feedback is possible between the canopy thinning and the cloud water deposition, due to the negative correlation between the canopy density and the average wind speed in the canopy (Lovett & Reiners, 1986).

The main objectives of this study were to investigate the effects of acid mist, ozone and wind on the physiology of Norway spruce, and especially the interactions between the pollutant and wind treatments. The working hypothesis was that treatment with wind altered uptake pathways into the foliage, rendering the seedlings more susceptible to pollution treatments. This hypothesis would have been proved correct if significant interactions had been found between the

Table 6.4. Overview of the interactions between the pollution (acid mist or ozone) and wind treatments in 1990 and 1991, on the parameters presented and discussed in Chapters 3, 4 and 5.

	Chapter	Interactions
Maximum assimilation rate	3	No significant interactions.
Light compensation point	3	Slight, non-significant synergistic interaction between the acid mist and wind treatments in 1990.
Apparent quantum yield	3	No significant interactions.
Dark respiration	3	Slight, non-significant synergistic interaction between the acid mist and wind treatments in 1990.
Stomatal conductance	3	No significant interactions.
Stomatal response time	3	Almost significant (P = 0.058) interaction between the ozone and wind treatments in 1991.
Initial conductivity	4	Antagonistic interaction between the acid mist and wind treatments in 1990.
Electrolyte leakage rate	4	No significant interactions.
Visible injury	5.1	Wind treatment in winter synergistic with acid mist treatment, wind treatment in summer appeared antagonistic.
Whole plant transpiration rate	5.2	No significant interactions.
Height	5.3	No significant interactions.
Root collar diameter	5.3	No significant interactions.
Shoot dry weight	5.3	No significant interactions.
Root dry weight	5.3	No significant interactions.
Shoot : root ratio	5.3	No significant interactions.
Chlorophyll *a*	5.4	No significant interactions.
Chlorophyll *b*	5.4	No significant interactions.
Chl *a* : *b* ratio	5.4	No significant interactions.

treatments with acid mist or ozone on the one hand and wind on the other. It was expected that the wind treatments would cause physical damage to the foliage, increasing the opportunity for the pollution treatments to affect the seedlings, which would have resulted in synergistic interactions. The interactions between the pollutant and wind treatments are presented in TABLE 6.4.

TABLE 6.4 shows that synergistic interaction occurred only very occasionally among the measured variables, and the interactions for the light compensation point ($Q_{A=0}$) and the dark respiration (R_d) in the 1990 acid mist experiment were not quite significant ($P > 0.05$). This reduction in $Q_{A=0}$ and R_d was opposite to that expected, and generally reported in the literature. At first, a reduction in R_d would seem beneficial to trees, but it may well indicate a reduced capacity of the maintenance respiration, rendering the trees vulnerable to stress in the long term (Wolfenden & Mansfield, 1991). Increased respiration rates are generally considered to be a result of detoxification and repair mechanisms, allowing the plant to prevent toxic concentrations of pollutants to build up (Black, 1984). If this respiration is impaired, plants are more susceptible to further doses of pollutants, a situation which would be even more damaging in non-photosynthetic parts of the plant, such as roots (Hofstra *et al.*, 1981).

The only significant interactions found were between the acid mist and wind treatments. The initial conductivity in the frost hardiness measurements, representing the solutes readily washed from the needle surfaces, was increased by the acid mist treatment as was expected (highly significant: $P \leq 0.001$). This increase was significantly ($P \leq 0.05$) counteracted by the wind treatment ten weeks before the frost hardiness measurements, which suggests that at least part of the deposited ions were removed from the needles by the wind treatment. A similar antagonistic interaction was found on the visible injury, between the acid mist treatment and a simulated storm in late summer, although this was not a statistically significant result in 1990, and was assumed to be coincidental in 1991, considering the short time between the wind treatment and the visible injury assessments. It is possible, however, that trees growing in areas with frequent cloud immersion, would benefit from a strong gale at the end of the growing season, causing the removal of deposited ions from the foliage. When remaining on the foliage, these deposited ions would redissolve in cloud droplets subsequently captured by the foliage, thereby increasing the actual concentration of ions in the water attached to the needles, and a partial removal of ions from the foliage by high winds will reduce this effect. This might explain why the wind treatment appeared to increase the frost hardiness of the seedlings from the acid mist experiment in 1990, especially the reduction in the number of shoots killed after relatively warm freezing temperatures, i.e. temperatures of -19 °C and -15 °C, and the non-frozen control.

The other significant interaction between the acid mist and wind treatments was found in the visible injury assessments in 1991, between the spring storm and the mist treatment. It is clear that the timing of the wind treatment is crucial in determining subsequent effects. When the wind treatment was applied at budburst, it did not directly affect the seedlings' new needles, but did render them more vulnerable to the acid mist treatments during the following summer.

The ozone and wind treatments did not interact significantly to produce effects on any of the parameters, measured in this study. The only indication of a significant interaction (P = 0.058) between the ozone and wind treatments was on the stomatal response time after changes in the photon flux, but this was entirely caused by the very different response of the charcoal-filtered control plants, treated for 120 hours in the wind tunnel. This wind treatment, however, is so extremely severe, that similar conditions are not expected to occur in the field.

6.2. Conclusions.

There is a general consensus that forest health has declined during the last few decades across large areas of central Europe (Skeffington & Roberts, 1985; Rehfuess, 1987) and eastern North America (McLaughlin, 1985; Johnson, 1987). There are, however, a few reports indicating no adverse effects on forest trees in the UK (Innes, 1992), and forests in Germany appear to be recovering (Blank *et al.*, 1990*a*; Schulze & Freer-Smith, 1991). The limited reduction in forest health in the

UK, as indicated by a recent Forestry Commission survey (Innes, 1992), has been attributed to the maritime climate with generally lower pollutant inputs (Schulze & Freer-Smith, 1991). However, this is only correct at lower altitudes. The total input of pollutants at higher elevations in Britain is at least as high as in central Europe, due to the much higher wet deposition (RGAR, 1990).

It is clear from the study presented here, and from others reported in the literature recently, that the concept presented diagrammatically in FIGURE 1.1 requires revision, to include current understanding of effects of, and interactions between environmental stresses on trees (see FIGURE 6.2).

The most obvious difference between FIGURES 1.1 and 6.2 is the possibility that the nutritional status of the seedling affects the influence of air pollution on tree

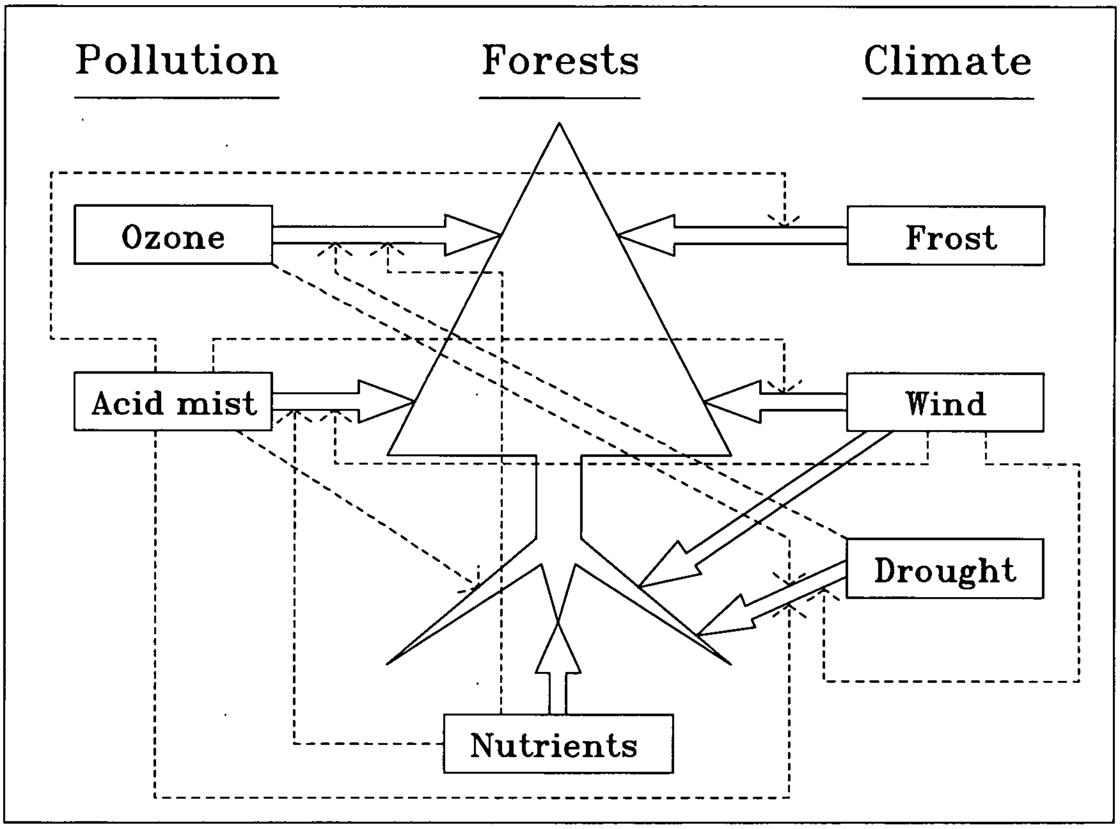


Figure 6.2. Revised schematic representation of the potential effects of, and interactions between, those environmental factors which have been discussed in this thesis, on forests. (See text for a further explanation.)

growth. Soil nutrients directly affect trees (large arrow, ⇨), but more important in this context is the fact that well-fertilized trees appear to be less sensitive to air pollutants, as was suggested as an explanation for the difference in effects between the two experimental years in this study. This is indicated by the dotted lines (representing interactions) between the nutrients box and the two large arrows, which show the direct effects of acid mist and ozone on the above-ground parts of trees. This indirect effect of a reduced, or even reversed, impact of pollutants on foliage of well-fertilized trees was also found by Keller & Matyssek (1990) and McLauglin & Tjoelker (1992).

Another addition to the diagram is the potential interaction between the wind and drought treatments. This interaction has been clearly demonstrated in grasses (Grace & Russell, 1982), but not yet in trees. Based on the results from the whole plant transpiration experiments in Section 5.2, it was concluded that the wind-treated seedlings had a reduced ability to take up water from the soil, which would render them more susceptible in subsequent periods of drought, although this was not tested in the current study.

The ozone treatments in 1990 and 1991 did not affect the frost hardiness of the Norway spruce seedlings during the following hardening period, measured at different stages of the hardening process, and it was concluded that there was unlikely to be a delay in hardening, as had been suggested by Lucas *et al.* (1988) for Sitka spruce. The dotted line shown in FIGURE 1.1, indicating an interaction between the ozone treatment and the effects of frost on trees was therefore removed. Similarly, no interactions were found between the wind and ozone treatments, and the dotted lines indicating these indirect effects were also removed.

Interactions between the acid mist and wind treatments were found, especially on the visible injury measurements, and the initial conductivity in the frost hardiness assessments. In both cases the wind treatments modified the effects of the acid mist. The reverse, the acid mist treatment altering the effects of high wind speeds, was only shown in the non-significant synergistic interactions on the dark respiration and the light compensation point. However, foliar injury caused by acid mist, will reduce the density of the foliage, which in turn will result in an increased average wind speed through the foliage. This can be expected to increase the damage caused by high wind speeds in the long term, and the dotted line in FIGURE 1.1, indicating this interaction was reproduced in FIGURE 6.2.

In this study the seedlings were kept well-watered, and drought conditions were avoided. Therefore, the direct effect of drought on the root system, and the interactions involving a drought treatment, were not studied, and can only be speculated on. In FIGURE 6.2 these effects and interactions were therefore copied from FIGURE 1.1, except for the potential indirect effect of high wind speeds mentioned previously.

As has been shown in other studies (Fowler *et al.*, 1989; Cape *et al.*, 1990), treatment with acid mist highly significantly ($P \leq 0.001$) reduced the frost hardiness of the seedlings in autumn, which clearly demonstrated the vulnerability of trees growing at higher altitudes, to winter injury. This increased susceptibility to cold temperatures has been proposed by many studies as one of the main factors involved in forest decline (Friedland *et al.*, 1984; DeHayes, 1992).

This study suggested that the nutritional status of trees can modify the effects of air pollution. The capacity of trees to absorb and neutralize pollutants may be higher when all elements are available in sufficient amounts, compared to deficient growing conditions, and foliage might benefit from additional nitrogen inputs from acid mist or rain, as was shown by McLaughlin & Tjoelker (1992). When damage occurred, the parameter most clearly affected was frost hardiness. Frost hardiness is normally achieved by trees by maintaining high levels of soluble sugars in the vacuole, which results in the water in the cell walls freezing before that in the cells, thus avoiding cell rupture. The reduced frost hardiness as a result of treatment with air pollution, was not solely an indirect result of a reduced photosynthetic activity prior to or during the hardening period, but was at least partly caused by a reduced level of dormancy, as indicated by the relatively high maximum assimilation rates during the second series of gas exchange measurements in 1990.

The concept of critical loads, i.e. "a quantitative estimate of an exposure to one or more pollutants below which significant harmful effects do not occur on specified sensitive elements of the environment, according to present knowledge" (Last & Watling, 1991), has been applied to the problem of forest decline, and is especially relevant with acid mist and rain (Brydges & Wilson, 1991). Problems with sulphur and nitrogen in acid mist and rain are not caused by some inherent toxic feature, as they are essential elements, necessary for plant growth. Damage is dependent on the amount and chemical form of the supplied sulphur and nitrogen, and it is clear from several studies that the critical load for nitrogen, especially when it is deposited as nitrate, is (much) higher than for sulphur, and possibly ammonium. Several other factors do have to be taken into account, however. The concentrations of the major ions in deposited cloud water are likely to be more important than the total amounts for studies of effects on foliage (Fowler *et al.*, 1991), especially when liquid water evaporates subsequent to cloud water deposition, further increasing concentrations (Unsworth, 1984; Milne *et al.*, 1988). The frequency of cloud water deposition has also been shown to be important (Jacobson *et al.*, 1990; Sheppard *et al.*, 1993*b*), and the present study indicated that episodes of high wind speeds may reduce the effects in tree foliage, but this depends on the timing of the high winds. If trees encounter high wind speeds during budburst, damage later during the growing season will be enhanced. Finally, the effects of air pollution might be modified by the nutritional status of the trees.

This reiterates the conclusion that forest decline cannot be attributed to one single factor, but is a consequence of complex interactions (Last & Watling, 1991). These involve both biotic and abiotic agents, including habitat variables such as high wind speeds, droughts and unseasonal periods of cold, sub-zero weather, which influence tree responses to air pollutants (McLaughlin, 1985; Rehfuess, 1987; Blank *et al.*, 1990*b*; Schulze & Freer-Smith, 1991). In turn, except where their concentrations are very large, pollutants are now regarded as agents of stress that debilitate, rather than kill, plants, unless their effects are exacerbated by interplay with other stress factors. Therefore, the concept of the determination of critical loads, which was developed to unify air pollution research, will need to be modified, to allow for several other factors, specific to a particular environment for which the critical load is to be determined.

6.3. Thesis Summary.

This project was set up to test the hypothesis that treatment with wind will alter pathways into the needles of Norway spruce, and will render them more susceptible to air pollutants. To test this hypothesis two year old seedlings were treated with either acid mist or ozone in open-top chambers, and these pollution treatments were combined with treatments in a controlled environment wind tunnel at various stages in the growing season. The actual treatments applied were chosen to simulate the climatological conditions found at higher altitudes, and were compared with conditions more typical of a clean atmosphere at lower altitudes. Acid mist was applied at pH = 2.5, which represents the acidity of the most polluted cloud water found in upland Britain, and this was compared with a pH = 5.0 control. The ozone applications were designed to fumigate the plants with air containing on average 140 nmol mol^{-1} of ozone, close to the upper limits of ozone concentrations measured regularly in the UK. The ozone treatment was paired with a charcoal-filtered control. The wind treatments were applied in a wind tunnel, at various times during the year, usually for 48 hours, at an average wind speed of 16 m s^{-1}, a velocity found regularly at higher altitudes. The results of the wind treatments were compared with a 'no-wind' control.

The main series of measurements made on the Norway spruce seedlings in this project were gas exchange and frost hardiness measurements. The gas exchange measurements provided information on maximum assimilation rate, light compensation point, apparent quantum yield, dark respiration, stomatal conductance, and the stomatal response time. In 1990 two series of measurements were made, shortly before and straight after the wind treatments, during August and September. In 1991 the plants from the acid mist experiment were measured during August, while the plants from the ozone experiment were measured in November, both after the wind treatments. The frost hardiness was assessed for plants from both pollution experiments in November 1990, while in 1991 only the frost hardiness of the seedlings from the ozone experiment was determined, in early October, straight after the last wind treatment. A few smaller experiments, on visible injury, whole plant transpiration rates, chlorophyll concentrations and a destructive harvest, were also carried out, to support the findings of the main experiments.

Treatment with acid mist reduced the maximum assimilation rate, but only in 1990, when the fertilizer used contained an unbalanced mixture of the major elements. In 1991, using a better balanced fertilizer, no effects were found. The dark respiration, and consequently the light compensation point, was reduced by the acid mist treatment, contrary to expectation, which may indicate damage to repair mechanisms, rendering the plants more susceptible to further pollution treatments. The other gas exchange parameters were not significantly altered. The frost hardiness of the seedlings treated with acid mist was seriously affected. The temperature killing 50% of the shoots, *LT50*, was reduced by 7 °C in November 1990. Visible injury to current-year foliage, also recorded in November 1990, was about 20% for the seedlings treated with acid mist at pH = 2.5, while no damage was found on the pH = 5.0 control plants. In 1991, when a different spraying regime was employed, visible damage was so severe that no frost hardiness measurements were carried out. The whole plant transpiration measurements and the final harvest showed no acid mist effect.

The ozone fumigation copied the concentrations regularly found in the UK, but after treatment for one growing season only limited effects were found, which suggests that Norway spruce planted in upland Britain is unlikely to be seriously affected by ozone episodes. Assimilation rates were found to be reduced, but only when comparing the response at all photon fluxes, using a combined curves analysis. There was also a reduction in stomatal conductance in September 1990, but this effect had disappeared three weeks later. The stomata of seedlings treated with ozone responded faster to changes in the photon flux, contrary to results reported in the literature, and the cause of this response remains unclear.

Like the ozone treatment, the wind treatments had only limited effects on the Norway spruce seedlings. Treatment in the wind tunnel reduced the dark respiration rate and light compensation point of seedlings from the acid mist experiment, but in the ozone experiments no effects were found. Again, this probably indicates impaired repair mechanisms. A significant result of this study was the reduction of the whole plant transpiration rate by the wind treatment in 1990. This was attributed to damage to the root systems, caused by movement of the stem, resulting in a reduced capacity of water uptake from the soil, rendering the seedlings more susceptible to drought.

The main hypothesis of this study, of wind treatments altering pathways into the foliage, would have been proved correct if significant synergistic interactions had been found between the pollutant and wind treatments. This study on Norway spruce showed that these interactions were only found for a few of the variables measured, probably a consequence of the tough cuticle on this species, and most of these were not statistically significant, i.e. dark respiration and light compensation point in the acid mist experiment. A significant antagonistic interaction was found between the acid mist and wind treatments on the initial conductivity in the frost hardiness measurements, representing the solutes readily washed from the needle surfaces. It was concluded that a wind treatment during late summer removes part of the deposited ions from the foliage, reducing their harmful effect. A similar result, although not statistically significant, was found in the visible injury scores. The ozone and wind treatments did not interact significantly to produce effects on any of the parameters measured in this study.

This study suggested that the nutritional status of trees can modify the effects of air pollution. The capacity of trees to absorb and neutralize pollutants may be higher when all elements are available in sufficient amounts, compared to deficient growing conditions, and foliage might benefit from additional nitrogen inputs from acid mist or rain. This study also demonstrated that high wind speeds may reduce the effects of pollutants on tree foliage, but this depends on the timing of the high winds.

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Appendix 1.

Data Logger Program in the Ozone Fumigation System.

Program Table 1:

```
01: P10, 28
02: P91, 11, 30
03: P91, 12, 0
04: P95
05: P20, 1, 6
06: P02, 1, 5, 8, 1, 0.05, 0
07: P02, 1, 2, 4, 2, 91.7, 0
08: P07, 1, 3, 3, 1, 4300, 6, 100.5, 0
09: P16, 1, 6, 3, 1, 0
10: P02, 1, 5, 5, 4, 1.0785, -23.207
11: P02, 1, 5, 6, 5, 1.1072, -31.368
12: P92, 0, 30, 3
13: P92, 2, 30, 16
14: P92, 6, 30, 4
15: P92, 8, 30, 16
16: P92, 12, 30, 5
17: P92, 14, 30, 16
18: P92, 18, 30, 6
19: P92, 20, 30, 16
20: P92, 24, 30, 7
21: P92, 26, 30, 16
22: P91, 17, 30
23: P86, 27
24: P91, 28, 30
25: P20, 1, 5
26: P31, 18, 8
27: P31, 19, 9
28: P86, 18
29: P94
30: P31, 8, 18
31: P31, 9, 19
32: P30, 0, 8
33: P30, 0, 9
34: P21, 1, 10
35: P21, 2, 10
36: P86, 28
37: P20, 0, 5
38: P95
39: P86, 10
40: P77, 110
41: P70, 1, 8
42: P86, 20
43: P95
44: P91, 16, 30
45: P91, 11, 1
46: P91, 12, 1
47: P91, 13, 1
48: P91, 14, 1
49: P91, 15, 1
50: P95
51: P77, 110
52: P70, 5, 21
```

```
53: P71, 4, 2
54: P20, 11, 1
55: P20, 12, 2
56: P20, 13, 3
57: P20, 14, 4
58: P21, 1, 8
59: P21, 2, 9
60: P00
```

Program Table 3:

```
01: P85, 1
02: P31, 11, 12
03: P33, 12, 1, 11
04: P95
05: P85, 2
06: P30, 20, 13
07: P38, 11, 13, 14
08: P30, 0, 11
09: P86, 26
10: P95
11: P85, 3
12: P86, 25
13: P86, 11
14: P86, 2
15: P31, 14, 25
16: P86, 10
17: P95
18: P85, 4
19: P20, 0, 1
20: P86, 21
21: P86, 12
22: P86, 2
23: P31, 14, 21
24: P91, 18, 30
25: P89, 21, 3, 160, 8
26: P95
27: P95
28: P85, 5
29: P20, 0, 2
30: P86, 22
31: P86, 13
32: P86, 2
33: P31, 14, 22
34: P91, 18, 30
35: P89, 22, 3, 160, 8
36: P95
37: P95
38: P85, 6
39: P20, 0, 3
40: P86, 23
41: P86, 14
42: P86, 2
43: P31, 14, 23
44: P95
45: P85, 7
46: P20, 0, 4
47: P86, 24
48: P86, 15
```

```
49: P86, 2
50: P31, 14, 24
51: P95
52: P85, 8
53: P20, 0, 5
54: P20, 0, 6
55: P30, 0, 10
56: P21, 1, 10
57: P21, 2, 10
58: P86, 28
59: P86, 11
60: P86, 12
61: P86, 13
62: P86, 14
63: P86, 10
64: P77, 110
65: P70, 5, 21
66: P70, 4, 2
67: P86, 20
68: P95
69: P00
```

The equipment used in the ozone fumigation system is described in Chapter 2.4.1. The first line in Program Table 1 monitered the battery voltage (P10). Lines 2 to 4 prevented program execution in the event of a automatic system shut-down. Line 5 controlled the mains power to the solemoid valves. Lines 6 to 11 monitored the ozone analyser, solar radiation (both P02), ambient temperature (P07 and P16) and the two flow controllers (P02, individually calibrated). Lines 12 to 21 controlled the sampling sequence (P92), six minutes for each chamber and ambient air, with the first two used for flushing the tubing and ozone analyser. Lines 22 to 43 were used to switch the system on and of, using the User Control Flags 7 and 8 (P91). Lines 54 to 59 were used to set the Control Ports 1 to 4 according to User Control Flags 1 to 4 (P20), and the Analogue Output Voltage to the flow controllers (P21). The rest of Program Table 1, together with the subroutines (P85) in Program Table 3, was necessary for the averaging and storing of the data. Subroutines 4 and 5 also checked whether the ozone concentrations in the high-ozone open-top chambers were above 160 nmol mol^{-1}, in which case subroutine 8 was executed, which shut down the fumigation system automatically.

Appendix 2.

Data Logger Programs in the Gas Exchange System.

Logger designated to data acquisition.

Program Table 1:

```
01: P10, 28
02: P89, 28, 4, 11.9, 0
03: P91, 11, 30
04: P86, 10
05: P86, 21
06: P86, 1
07: P95
08: P91, 12, 30
09: P86, 10
10: P86, 22
11: P86, 1
12: P95
13: P91, 13, 30
14: P01, 1, 3, 1, 1, 9.9925, -29.239
15: P94
16: P01, 1, 3, 1, 1, 1.1806, -37.479
17: P95
18: P01, 1, 3, 2, 25, 2.5894, -76.871
19: P56, 25, 2
20: P01, 1, 5, 3, 3, 1.0858, 102.02
21: P01, 1, 5, 4, 4, 1.0849, -3.0637
22: P17, 16
23: P13, 1, 1, 6, 1, 16, 20, 1, 0
24: P56, 20, 5
25: P07, 1, 3, 7, 1, 4300, 17, 99.582, 0
26: P16, 1, 17, 21, 1, 0
27: P56, 21, 6
28: P07, 1, 3, 11, 3, 4300, 18, 99.582, 0
29: P16, 1, 18, 22, 1, 0
30: P56, 22, 7
31: P01, 2, 5, 15, 8, 1, 0
32: P30, 20, 26
33: P56, 26, 27
34: P36, 8, 6, 8
35: P38, 8, 27, 8
36: P37, 8, 2.4795, 8
37: P34, 8, -41.764, 8
38: P36, 9, 7, 9
39: P38, 9, 27, 9
40: P37, 9, 2.5107, 9
41: P34, 9, -59.807, 9
42: P01, 1, 3, 5, 10, 166.93, 0
43: P32, 12
44: P89, 12, 1, 30, 30
45: P86, 10
46: P30, 0, 12
47: P26, 11
48: P37, 11, 0.1, 11
```

```
49: P45, 11, 11
50: P77, 10
51: P70, 1, 11
52: P78, 1
53: P95
54: P71, 10, 1
55: P00
```

Program Table 3:

```
01: P85, 1
02: P26, 0
03: P80
04: P77, 110
05: P78, 1
06: P70, 2, 23
07: P86, 20
08: P30, -1, 12
09: P95
10: P00
```

The equipment used in the gas exchange system is described in Chapter 3.1. The first two lines in Program Table 1 monitored the battery voltage (P10), and switched the data logger off in case of a power failure (P89), to protect the lead acid batteries. The next ten lines detected whether the photon flux density had been altered, or whether a different leaf chamber was being sampled, indicated by User Control Flags 1 and 2 (P91), and executed the subroutine in Program Table 3 in either case (P86), which reset the timer, and produced a line in the output to indicate this occurrence. Lines 13 to 17 in program table 1 monitored the Infra-Red Gas Analyser (P01), its mode being indicated by the state of User Control Flag 3. Lines 18 to 42 monitored the dew-point meter (P01), the two flow meters (P01), the two platinum resistance temperature sensors (P07, P16), the water vapour pressure in the chambers (P01) and the photon flux density (P01) respectively. The dew-point and air temperature measurements were converted into saturated water vapour pressures (P56), and the water vapour pressure measurements were corrected for air temperature in the leaf chambers (P36, P38). The last twelve lines incremented a counter (P32), initiated averaging at the end of each thirty seconds, and stored the data in the logger's memory.

Logger designated to leaf chamber temperature control.

Program table 1:

```
01: P10, 10
02: P89, 10, 4, 11.9, 0
03: P17, 1
04: P14, 2, 1, 4, 1, 1, 2, 1, 0
05: P92, 0, 5, 10
06: P77, 110
07: P71, 1, 2
08: P73, 1, 0, 2
09: P74, 1, 0, 2
10: P71, 1, 3
11: P73, 1, 0, 3
12: P74, 1, 0, 3
13: P91, 17, 30
14: P89, 2, 3, 20.2, 30
15: P20, 1, 2
16: P95
17: P89, 2, 4, 20, 30
18: P20, 0, 2
19: P95
20: P89, 2, 4, 19.8, 30
21: P20, 0, 1
22: P86, 27
23: P95
24: P95
25: P91, 27, 30
26: P89, 2, 4, 19.8, 30
27: P20, 1, 2
28: P95
29: P89, 2, 3, 20, 30
30: P20, 0, 2
31: P95
32: P89, 2, 3, 20.2, 30
33: P20, 1, 1
34: P86, 17
35: P95
36: P95
37: P91, 18, 30
38: P89, 3, 3, 20.2, 30
39: P20, 1, 4
40: P95
41: P89, 3, 4, 20, 30
42: P20, 0, 4
43: P95
44: P89, 3, 4, 19.8,30
45: P20, 0, 3
46: P86, 28
47: P95
48: P95
49: P91, 28, 30
50: P89, 3, 4, 19.8, 30
51: P20, 1, 4
52: P95
```

```
53: P89, 3, 3, 20, 30
54: P20, 0, 4
55: P95
56: P89, 3, 3, 20.2, 30
57: P20, 1, 3
58: P86, 18
59: P95
60: P95
61: P00
```

The first two lines once again monitored the battery voltage, and switched the logger off if necessary. Lines 3 and 4 measured the air temperature in the two chambers (P14), and lines 5 to 12 caused the logger to store the average, minimum and maximum temperatures in Final Storage. Lines 13 to 36 were concerned with the control of the temperature in the first leaf chamber, and lines 37 to 60 controlled that in chamber 2. These instructions took the measurements from line 4, and depending on the actual temperatures (P89) set control ports (P20) which activated the electrical circuitry described in FIGURE 3.2 and the associated text.

Appendix 3.
Statistical Analyses.

In 1990 two rounds of gas exchange measurements were carried out, the first before the wind treatments had been applied, and the analysis of the data determined only effects by the pollution treatments. These effects were calculated on chamber averages, as indicated in TABLE A3.1, since the individual seedlings within each chamber were not statistically independent.

Table A3.1. Example of an analysis of variance, testing the effects of pollutants only (i.e. after the first round of gas exchange measurements in 1990).

Source of variation	d.f.	s.s.	m.s.	v.r.	F-prob.
Chamber stratum					
Acid mist	1	94.556	94.556	23.29	0.040
Residual	2	8.121	4.061	0.44	
Chamber*units stratum	36	333.926	9.276		
Total	39	436.603			

Most analyses of variance in this thesis were carried out to determine effects of both pollutant and wind treatments, together with their interaction, and an example of this is presented in TABLE A3.2. This table shows that the effects of the pollution treatment were still determined on the basis of chamber averages, while the effects of the wind treatments, and the interaction between the wind and pollution treatments were analysed using the results for individual seedlings.

After the second round of gas exchange measurements in 1990, the data were also analysed taking earlier measurements on the same seedlings into account, using an analysis of covariance. An example of this type of analysis is presented in TABLE A3.3. The partitioning of the variance was the same as in the analysis of

Table A3.2. Example of an analysis of variance, testing the effects of pollution and wind treatments.

Source of variation	d.f.	s.s.	m.s.	v.r.	F-prob.
Chamber stratum					
Acid mist	1	3.618	3.618	3.17	0.217
Residual	2	2.281	1.141	0.17	
Chamber*units stratum					
Wind	1	0.024	0.024	0.00	0.953
Acid mist * wind	1	9.305	9.305	1.39	0.251
Residual	24	161.151	6.715		
Total	29	172.966			

variance, but the previous measurements were used as a covariate.

In 1991 more wind treatments were applied, and the data were first analysed with an analysis of variance, but this only tests for wind effects across all treatments. Results of individual wind treatments were compared with the no-wind controls

Table A3.3. Example of an analysis of covariance, testing the effects of pollution and wind treatments, taking earlier measurements into account (i.e. after the second round of gas exchange measurements in 1991).

Source of variation	d.f.	s.s.	m.s.	v.r.	cov.ef.	F-prob.
Chamber stratum						
Acid mist	1	0.920	0.920	0.41	0.17	0.638
Covariate	1	0.035	0.035	0.02		0.920
Residual	1	2.246	2.246	0.49	0.51	
Chamber*units stratum						
Wind	1	3.023	3.023	0.66	0.95	0.425
Acid mist * wind	1	1.731	1.731	0.38	0.95	0.545
Covariate	1	55.573	55.573	12.11		0.002
Residual	23	105.578	4.590		1.46	
Total	29	172.966				

within the pollution treatments, using the standard errors of differences of means as provided by GENSTAT, and effects were tested using a *t*-test (A. Scott, pers. comm.).

Appendix 4.
Curve Fitting and Combined Curve Analyses.

Curve fitting.

In the analyses of effects of wind and pollutants on the gas exchange and frost hardiness, several types of curves were fitted to the data sets. This was done with purpose written software, using "C" as the programming language, and was based on the least squares method (Box *et al.*, 1978). Initial estimates were assigned to each parameter of a function, these functions drawn from the available literature. The initial estimates either depended on the data set, or were set values used for all data sets, based on initial tests. Depending on the number of parameters to be estimated, one or two stepsizes were added to or substracted from these initial estimates, and error sums of squares were calculated for each combination of these three or five estimates (initial estimates, and ± one or two stepsizes). With three or more unknown parameters only one stepsize was added or deducted, resulting in at least 27 combinations (3^3); with two parameters to be estimated, one and two stepsizes were added and deducted, giving 25 combinations (5^2).

Error sums of squares were calculated from:

$$\sum (y_{meas} - y_{calc})^2 \tag{A4.1}$$

where y_{meas} is the measured value at each x-value, and y_{calc} is the value calculated using a particular set of estimates for all parameters of a specified curve.

The set of parameter estimates was chosen that gave the smallest error sum of squares, and the initial estimates were adjusted. In case of three values for each parameter, the initial estimate was altered in case either of the two extremes gave the smallest error sum of squares, or, if the initial estimate was the best, the stepsize was reduced by 50 %. In case of five values for each parameter, the same basic principle was applied, but when either of the two extremes was found to give the best error sum of squares, the initial estimate was adjusted, when either of the two intermediate values was needed, the stepsize was also reduced to 50 %, and when the initial value was retained, the stepsize was reduced to 25 %. This process was repeated until the stepsizes for all parameters were smaller than or equal to a preset value, generally a quarter to a tenth of the order of presentation (e.g. A_{max} in Chapter 3 is given in 0.01 µmol m^{-2} s^{-1}, minimum stepsize 0.001 µmol m^{-2} s^{-1}). The stepsizes at the start were set to about 45 % of the initial estimate. If at some stage one or more of the stepsizes were adjusted to less than the minimum, this minimum stepsize was subsequently used.

Combined curve analysis.

The combined curve analysis is described by Ross (1981), and basically tests whether combining the data sets adds to the total variation not accounted for by the model. Curves were fitted to each individual data set, and to the combined data set. Once each parameter of these curves has been estimated, it is possible to calculate the total variation, which can be calculated from the individual data points, together with the variation accounted for by the fitted curve, and the residual variation is the difference between the two. Then an analysis of variance is carried out, to test for a significant increase in the error terms. Theoretically the

Table A4.1. Theoretical partitioning of the variance in a combined curve analysis.

	d.f.	s.s.
Combined curve	p-1	SSRC
Separate curves	(j-1)*p	SSEC-SSE1-SSE2-..
Residual	n-j*p	SSE1+SSE2+..
Total	n-1	SSTC

where p is the number of unknown parameters in the fitted curve, j is the number of individual curves, n is the number of points in the combined data set, SSRC is the variation in the combined data set accounted for by the fitted curve, SSTC is the total variation in the combined data set, SSEC is the residual variation in the combined data set, and SSE1, SSE2 etc are the residual terms for the individual curves.

variance is partitioned as in TABLE A4.1.

As an example the combined curve analysis for FIGURE 3.6 is presented in TABLE A4.2.

Table A4.2. Combined curve analysis performed on the lines fitted for the shoots from the acid mist experiment, measured between 22 August and 1 September 1990.

	d.f.	s.s	m.s.	v.r.
Combined curve	2	4704.24	2352.12	1569
Separate curves	3	62.40	20.80	13.87
Residual	234	350.86	1.50	
Total	239	5117.50		

In this particular analysis *p*, the number of parameters in EQUATION 3.5, is 3, *j*, the number of curves is 2, and *n*, the total number of data points, is 240. The analysis on the combined curve in TABLE A4.2 effectively test whether the model used to describe the data is adequate, and this is shown to be the case, as it was in all combined curve analyses throughout this thesis (very high variance ratios). The analysis on the separate curves in TABLE A4.2 test whether the individual curves are statistically different, which in this case was also highly significant.

Appendix 5.
Symbols Used.

Table A5.1. Symbols used in this thesis.

Symbol	Description
A	Assimilation rate (μmol m^{-2} s^{-1})
A_{max}	Maximum assimilation rate (μmol m^{-2} s^{-1})
a_l	Constant
b_a	Constant
b_l	Constant
C_0	Initial conductivity (μS)
C_{auto}	Conductivity after autoclaving (μS)
C_t	Conductivity at time t (μS)
Δc	Difference in CO_2 concentration between air entering and leaving the leaf chamber (μmol mol^{-1})
c_a	CO_2 concentration in ambient air (μmol mol^{-1})
D_o	Molecular diffusivity of ozone (m^2 s^{-1})
D_w	Molecular diffusivity of water vapour (m^2 s^{-1})
E	Transpiration rate (mol m^{-2} s^{-1})
f	Molar flow rate (mol s^{-1})
f_v	Volumetric flow rate (ml min^{-1})
g_b	Boundary layer conductance (mmol m^{-2} s^{-1})
g_c	Cuticular conductance (mmol m^{-2} s^{-1})
g_s	Stomatal conductance (mmol m^{-2} s^{-1})
$g_{s,0}$	Stomatal conductance when changing the photon flux (mmol m^{-2} s^{-1})
$g_{s,1200}$	Stomatal conductance at the highest photon flux (mmol m^{-2} s^{-1})
$g_{s,n}$	Stomatal conductance 25 min after changing the photon flux (mmol m^{-2} s^{-1})
$g_{s,o}$	Stomatal conductance to ozone (mmol m^{-2} s^{-1})
k	Photon flux at which the assimilation rate is half of A_{max} (μmol m^{-2} s^{-1})
k_a	Constant
k_e	Electrolyte leakage rate (% day^{-1})

Table A5.1. Continued.

Symbol	Description
k_l	Constant
LT50	Freezing temperature killing 50% of the shoots (°C)
Q	Photon flux (μmol m^{-2} s^{-1})
$Q_{A=0}$	Light compensation point (μmol m^{-2} s^{-1})
P	Atmospheric pressure (kPa)
ΔP	Pitot static tube reading (Pa)
P_{STP}	Standard pressure (101.3 kPa)
$R_{control}$	Number of shoots killed without a freezing treatment
R_d	Dark respiration (μmol m^{-2} s^{-1})
R_{dead}	Number of shoots killed after a freezing treatment
s	Leaf area (m^2)
T	Temperature (K)
T_{STP}	Standard temperature (273.15 K)
T_{corr}	Constant
t	Time (s or h)
u	Wind speed (m s^{-1})
v_e	Vapour pressure of air entering the leaf chamber (kPa)
v_o	Vapour pressure of air leaving the leaf chamber (kPa)
v_{sat}	Saturated vapour pressure (kPa)
W_r	Root dry weight (g)
W_s	Shoot dry weight (g)
α	Slope of straight line
ρ	Air density (kg m^{-3})
ρ_{STP}	Air density at standard temperature and pressure (1.292 kg m^{-3})
τ	Time constant (unit time)
τ_{1200}	Response time after changing the photon flux to its highest level (s)
ϕ	Quantum yield (mol mol^{-1})