Effects of Ozone and Water Stress on Plant Growth and Physiology

by

Sarah Rosemary Davidson

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Abstract

The aim of this project was to investigate the combined effects of ozone and water stress on the growth and physiology of *Vicia faba* and *Fagus sylvatica*.

In a series of experiments *Vicia faba* was exposed to ozone for one week, and to water stress for two weeks. Exposure to ozone either preceded, or coincided with, the first day of exposure to water stress. Exposure to ozone resulted in increased leaf conductance, and in some experiments, stimulated shoot growth, although there was no effect of ozone on root:shoot partitioning. Water stress either had no effect on plant responses to ozone, or reduced the positive impact of ozone. The timing of exposure to ozone and water stress influenced the degree of visible ozone injury and the occurrence of ozone/water stress interactions on gas exchange, but not growth.

From June to September 1988, *Fagus sylvatica* saplings were exposed to episodes of ozone and simultaneous water stress followed by 'recovery' periods. This study was designed to determine dose-response relationships over a range of ozone concentrations typical of different British summers. Ozone-induced increases in leaf conductance and photosynthesis occurred only in water stressed plants. Root weight measured in the subsequent spring was reduced by ozone in well watered plants, but increased by ozone in water stressed plants. Therefore ozone reduced the negative impact of water stress on root weight.

Ozone at concentrations typical of British summers can affect the growth and gas exchange of *Vicia faba* and *Fagus sylvatica*, and can affect plant sensitivity to water stress. In both species ozone consistently had a delayed impact on gas exchange. Plant responses to ozone and water stress appear to be dependant on the concentration of ozone, the severity of water stress, and the duration of the two stresses.

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Table of Contents

List of Figures

List of Tables

List of Plates

Chapter 1

Introduction

1 Ozone Pollution

In Europe, North America and other industrialised regions of the world, ozone pollution is widespread and causes extensive damage to vegetation (Fowler & Cape, 1982; Heck *et al.*, 1982). Ambient ozone has long been reported to have an adverse effect on vegetation in the United States. Damage to vegetation caused by photochemical smog was first recognised in the Los Angeles area in the 1940s, and in the 1960s ozone induced needle blight of eastern white pine was reported to be prevalent from Ohio to New York (Costonis & Sinclair, 1969). Today, ozone is considered to be the most important air pollutant affecting plant growth and productivity in the U.S. (Adams, 1987).

It has been suggested that ozone has a role in the widespread decline in forest health in Western Germany and other countries of central Europe over the past decade (Ashmore *et al.*, 1985). Mean ozone concentrations measured in some of the high elevation forests of South Germany, where the damage is most severe, are comparable to those recorded in areas of the USA where ozone damage is known to cause forest injury (UK PORG, 1987).

The potential for damage to vegetation by ozone in the U.K. was first demonstrated by the development of injury on the tobacco cultivar Bel-W3, exposed to ambient air at Ascot in the summer of 1972 (Bell & Cox, 1975). This cultivar of tobacco is exceptionally sensitive to ozone, showing visible symptoms when the concentration exceeds 40 parts per billion (ppb). Ambient ozone concentrations in the U.K. are now known to be sufficient to produce leaf injury and cause reductions in growth in a number of sensitive species (Ashmore, 1984).

1.1 The formation of ozone

Ozone is present in unpolluted air, primarily as a result of mixing from the stratospheric ozone layer. In unpolluted air over the U.K. and the European continent the ozone concentration varies at the present time between 20 and 50 ppb, depending somewhat on the time of year and weather conditions (UK PORG, 1987).

Ozone is not emitted directly into the atmosphere, but is a secondary pollutant, formed in certain conditions as a result of a complex series of reactions, some of which are photochemical. The set of conditions associated with the photochemical formation of ozone in the boundary layer are sunshine to drive the chemical reactions, and hydrocarbon and nitrogen oxide pollutants to feed the reactions. Both nitrogen oxides and hydrocarbons are emitted from motor vehicles and other sources of high temperature

combustion. Ozone will be formed in high concentrations only on days with high irradiances, high temperatures to promote certain of the reactions, and low wind speeds and a restrictive boundary layer, which inhibit atmospheric dispersion and allow the build up of pollutant concentrations. The simultaneous occurrence of all the above conditions is generally linked with anticyclonic conditions, and these have in fact been associated with most instances of elevated ozone levels in the UK (UK PORG, 1987).

1.2 Ozone concentrations in the United Kingdom

Because of the specific conditions necessary for its formation, ozone in highly episodic in its occurrence in the U.K., with high concentrations occurring mainly in the summer. In stable weather conditions ozone can persist for several days and may be transported long distances (UK PORG, 1987). Ozone is often found in higher concentrations in areas remote from urban and industrialised sources (Ashmore *et al.*, 1985).

Ozone concentrations have been monitored at about 30-40 rural sites in the U.K., but until recently monitoring has been intermittent and uncoordinated, and data do not extend over a sufficient length of time to establish trends (UK PORG, 1987). Mean U.K. concentrations of ozone are typically 25-35 ppb during the summer, and annual maximum concentrations are usually between 100 and 200 ppb (UK PORG, 1987). The highest ozone concentrations ever recorded in the U.K. were in 1976 when the concentrations of ozone recorded at several locations in south-east England exceeded 200 ppb (Apling *et al.*, 1977)

In the British Isles, a nationwide survey of phytotoxic ozone were carried out in the summer of 1977 using the Bel-W3 cultivar of tobacco as a biomonitor (Ashmore *et al.*, 1978). Characteristic symptoms of ozone injury were found on at least some occasions throughout the country, except in northern Scotland. The geographical distribution of damage was positively related to the number of hours of sunshine.

2 Ozone and plant water stress

High concentrations of ozone are associated with hot sunny weather and therefore will tend to coincide with periods of water stress in plants.

On a global scale water stress has long been recognised as a major environmental factor limiting plant productivity, and is considered to be the most important environmental factor determining the distribution, species composition, and growth of forests (Kozlowski, 1982). The numerous physiological responses of plants to water deficits

generally vary with the severity as well as the duration of the stress, and in temperate regions of the world, remarkably small reductions in plant water status trigger perturbations in plant function (Bradford & Hsiao, 1982).

It has been suggested that under certain conditions drought stress is an important trigger of forest decline (for example, Johnson, 1983), and drought years have been shown to have a marked impact on shoot growth of *Fagus sylvatica* in southern Britain (Lonsdale *et al.*, 1989). One of the major hypotheses proposed to explain forest decline suggests that ozone acts primarily to increase cellular permeability, so that key nutrients are more easily leached from the leaves by acidic mists and rain (Prinz *et al.*, 1987). Also ozone and acid deposition have been hypothesised to alter tree-water relations and drought resistance (McLaughlin, 1985).

Water stress is generally believed to "protect" plants from ozone pollution, by causing stomatal closure and therefore reduced pollutant uptake. However, relatively little is known of the physiological and growth responses of plants to combinations of these two stresses, or of how ozone may alter plant responses to water stress.

3 Plant responses to ozone

In predicting how ozone might alter plant responses to water stress we need to consider how the known effects of ozone on plant growth and physiology might disrupt plant water relations.
Altered plant-water relations can result from physiological changes within the plant, diminished capacity of the plant to take up water from the soil, or from a loss in capacity to control water loss to the atmosphere from foliage. There is considerable information concerning the impact of ozone on plant physiology, biomass partitioning between root and shoot, root function, stomatal function and cuticular integrity, and in the following sections this information is reviewed, with particular emphasis on data derived from long-term exposure to relatively low concentrations of ozone.

3.1 Ozone and plant gas exchange

It is well known that stomata respond to plant water deficits by closing if the leaf water content becomes too low or, in some species, to atmospheric vapour pressure deficits by closing as the atmosphere becomes drier (Willmer, 1983). Photosynthesis has long been known to be partially or completely suppressed by water stress if it is sufficiently severe (Bradford & Hsiao, 1982). Water deficits can inhibit photosynthesis either by a direct

effect on mesophyll tissues or by closing stomata and reducing carbon dioxide uptake. Stomatal and nonstomatal effects of water stress on photosynthesis are reviewed by Bradford and Hsiao (1982).

The sensitivity of ozone polluted plants to water stress will depend not only on the effects of ozone on plant gas exchange, but also on the effect of ozone in modifying these plant gas exchange responses to water stress.

3.1.1 Stomatal conductance

Exposure of plants to ozone at concentrations above 200 ppb generally results in reduced leaf conductance. This has been demonstrated with, for example, *Phaseolus vulgaris* (Rich and Turner, 1972), *Petunia hybrida* (Elkiey & Ormrod, 1979), and *Pisum sativum* (Olsyk & Tibbitts, 1981b). However, at concentrations below 200ppb, the effects of ozone on stomata are somewhat unpredictable, and a diversity of stomatal responses, including both stomatal opening and closure, has been reported (Darrall, 1989).

Numerous studies have shown that chronic exposure to a relatively low concentration of ozone results in reductions in leaf conductance. Daily exposure of soybean (*Glycine max*) to 50, 90 or 130 ppb ozone for eight weeks (Reich *et al.*, 1985), and cotton (*Gossypium hirsutum*) to 74, 125 or 150 ppb ozone for two months (Temple, Kupper *et al.*, 1988), resulted in ozone dose dependant reductions in leaf conductance in both species. Rowland-Bamford *et al.* (1989) reported reductions in leaf conductance in spring barley (*Hordeum vulgare*) during and after a twelve day exposure to 100ppb ozone; and Amundson *et al* (1987) observed stomatal closure in winter wheat (*Triticum aestivum*) following exposure to 96 ppb ozone for one day, and 54 ppb ozone for ten days. Reduced leaf conductance following exposure to ambient ozone has been demonstrated in filtration experiments, for example with *Capsicum annuum* in California on exposure to daily mean concentrations of 66 ppb ozone for four weeks (Takemoto *et al.*, 1988).

Ozone is also reported to cause stomatal opening. Olsyk & Tibbitts (1981a) reported stomatal opening in leaves of *Pisum sativum* following exposure to 130 ppb ozone for eight hours. This fumigation resulted in a 'trace' of ozone-induced necrosis. No change in conductance was observed following exposure to 130 ppb for two hours, and exposure to concentrations above 170 ppb resulted in stomatal closure. Keller & Hasler (1984) observed increased transpiration and stomatal sluggishness in response to light in *Picea abies* following daily exposure to 150 ppb ozone for thirty five days. Freer-Smith & Dobson (1989) observed increased stomatal conductance in both *Picea abies* and *P.*

sitchensis following exposure to 80 ppb ozone for just one hour, and Bucher *et al* (1988) reported increased stomatal conductance in *Abies alba* following continuous fumigation with 100 ppb ozone for between four and ten weeks.

The experiments reviewed here span a wide range of species, experimental conditions, and ozone concentrations, and demonstrate the unpredictability of effects of ozone on stomata, and the potential for ozone polluted plants to exhibit increased or decreased leaf conductance.

Many of the reports of stomatal closure have occurred after prolonged continuous ozone fumigations and are therefore not comparable with the short-lived ozone episodes typical of summer conditions in north west Europe. Many of the reports of increased conductance have been for conifers, which are generally considered to be less sensitive than agricultural crops or hardwoods to ozone (Reich, 1987). There is evidently a need to investigate further the effect of exposure to realistic doses of ozone before the stomatal responses of specific species to ozone can be predicted.

3.1.2 Photosynthesis

Photosynthesis is important in studies of ozone effects on plants since it is ultimately linked to plant yield. Mooney & Winner (1988) point out that the link between carbon gain and dry matter accumulation is complex, and although exposure of plants to pollutants may result in reduced growth, the relationship between photosynthetic reduction and growth reduction will not necessarily be linear because of changes in carbon allocation. If ozone and water stress affect carbon allocation between leaves and non-photosynthetic tissues, this may have important implications in terms of relationships between photosynthetic impairment and growth reduction.

Ozone has been associated with a reduction in photosynthesis in a wide variety of crop and tree species, with the degree of inhibition dependent upon the species, cultivar, age of plant, ozone dose (concentration and duration) and many environmental factors (see reviews by Darrall, 1989, and Pye, 1988). Stomata may respond to ozone-induced alteration of photosynthesis, since an effect of ozone on mesophyll activity may alter the intercellular CO_2 concentration, which may in turn result in stomatal opening or closure. Therefore an effect of ozone on photosynthesis might have important implications for plant water relations.

Several authors report reductions in both stomatal conductance and photosynthetic rate in response to ozone fumigation (Rowland-Bamford *et al.*, 1989; Amundson *et al.*, 1987). Generally it is not clear whether stomatal closure in response to ozone is the result or the cause of the depression in photosynthesis which is often observed at the same time (Mansfield & Freer-Smith, 1984). Takemoto *et al.* (1988) concluded that the reduction in photosynthetic rate of *Capsicum annuum* exposed to ambient ozone was due to stomatal closure. However, fumigation of *Populus deltoides* with 85 and 125 ppb ozone for fourteen days resulted in significant reductions in photosynthetic rate, which could not be attributed to reductions in stomatal conductance (Reich, 1983). Reich *et al.* (1985) observed linear declines in leaf conductance and water use efficiency in soybean (*Glycine max*) exposed to increasing concentrations of ozone. Photosynthesis was not measured, but the authors concluded that the reduced water use efficiency with increasing ozone concentrations suggests that decreased net photosynthesis due to ozone is inducing decreased conductance and not the reverse.

There are some indications that ozone at low concentrations can stimulate net photosynthesis. Freer-Smith & Dobson (1989) observed significant increases in the net photosynthetic rate of Norway and Sitka spruce needles (*Picea abies* and *P. sitchensis*) following exposure to 80 ppb ozone for one hour, and Sutinen *et al.* (1988) report significant increases in the photosynthetic rate of *Picea abies* exposed to ambient ozone (seasonal mean of 30 ppb) for one year compared to those in carbon filtered air.

Very little is known of the mechanisms of photosynthetic and stomatal responses to ozone, and a better understanding of these mechanisms is needed before predictions can be made concerning the impact of ozone and water stress on plant gas exchange.

3.2 The effect of ozone on plant cuticles

One of the symptoms associated with declining trees at high altitude is premature degrading of the epicuticular wax (Karhu & Huttunen, 1986). Epicuticular and intracuticular wax are believed to regulate water diffusion through the cuticle (Schonherr, 1976), and thus premature erosion of the wax might be expected to reduce the resistance of the cuticle to water loss, and to impair water conservation during drought. In evergreen plants, resistance to winter dessication is effected through stomatal closure, so that the cuticle provides the main barrier to winter water loss (Davison & Barnes, 1986).

The structural degradation of surface wax observed in declining high altitude forests has mainly been attributed to the known effects of acid mist rather than to a direct effect of ozone, because the wax component of the cuticle is generally not considered to react

readily with ozone (Skarby & Sellden, 1984). However, experimental evidence is conflicting. Skeffington and Roberts (1985) found no effect of ozone or acid mist on the needle surfaces of Scots pine saplings (*Pinus sylvestris*), but changes in wax chemistry have been reported following fumigation of *Pinus strobus* with ozone (Trimble *et al.*, 1982). Ashmore, Garretty *et al.* (1988) exposed Scots pine saplings to two levels of acid mist and three levels of ozone. The ozone concentrations used were typical of high elevation sites in central Europe, and of low elevation sites in central and northern Europe. There was a significant decrease in cuticular wax content with increasing ozone treatment, with no interaction between ozone and acid mist (Cape, 1988).

Barnes *et al.* (1988) found that ozone accelerated the structural degradation of epicuticular wax on *Picea abies* needles. They investigated the effect of ozone fumigation on the condition of the epicuticular wax that fills and surrounds the stomatal antechamber of the needles of this species. Fumigation of five clones with 120 ppb ozone, for 6 h day^{-1} for 70 days, consistently increased degradation of wax on the surface of both current and previous year's needles. In the previous year's needles of clones that showed the greatest degree of ozone accelerated degradation, more than 70 % of the stomata were completely occluded by an amorphous layer of structurally degraded wax (compared with 7-28 % in plants exposed to charcoal filtered air). Occlusion of the stomatal antechamber by an amorphous wax plug would be expected to reduce stomatal transpiration; however, accelerated structural degradation of epicuticular wax might be expected to reduce the resistance of the cuticle to water loss (Barnes *et al.*, 1988).

In this same study, drying curves were plotted for excised needles from fumigated and control trees and the rate of transpirational water loss was calculated (Barnes & Davison, 1988). Water loss was increased by ozone in only one out of ten cases (5 clones/2 needle ages) and some of this water loss could have been due to imperfectly closed stomata, but drying curves cannot reveal whether this was the case. Barnes *et al.* (1988) conclude that there is no simple relationship between wax degradation and cuticular resistance.

Other studies have specifically examined the effects of ozone on cuticles. Garrec (1988) fumigated isolated cuticles of *Ilex aquifolium* with 200 ppb ozone for one week. He found no effect of ozone on the water permeability of the cuticles. Kerstiens & Lendzian (1989) report that exposure to environmentally realistic ozone concentrations does not alter the water permeability of a wide variety of cuticles. They exposed isolated cuticles of various species, and non-isolated cuticles of astomatous leaf surfaces of *Fagus sylvatica* and ivy (*Hedera helix*) to ozone and acid fog in fumigation chambers. Ozone at a range of concentrations between 30 and 130 ppb was applied for two days at intervals of one to

three weeks. The specimens were exposed for 3 months (ivy plants), 5 months (beech plants), and 10 months (isolated cuticles), and thus beech leaves were exposed for almost all of their natural life span.

In summary, there is evidence that ozone alters the structure, morphology, amount and chemical composition of epicuticular wax. However, whether this results in changes in cuticular transpiration is unclear, and indeed several studies have shown that exposure to realistic concentrations of ozone has no effect on cuticular water permeability.

3.3 Ozone and plant growth

There is a vast body of evidence that exposure to ozone at realistic concentrations results in reductions in plant growth. However, there is now also abundant evidence that ozone affects allocation of biomass within the plant, and does so in a predictable manner. The sensitivity of ozone polluted plants to water stress will depend not only on these effects of ozone on plant growth, but also on the effect of ozone in modifying plant growth responses to water stress.

It is well established that changes in assimilate distribution occur in response to water stress. The first sign of water shortage in the field is usually a restriction in foliage growth (Bradford & Hsiao, 1982). This slowed canopy development restricts the transpirational surface area and conserves water in the soil. Increases in the root:shoot ratio in response to water shortage have been attributed mostly to these reductions in shoot growth. However water stress can result in preferential growth of roots relative to shoot, and an increase in absolute root biomass (e.g. Sharp & Davies, 1975) which will tend to increase water supply to the plant.

When plant growth is vegetative, relatively low levels of ozone (50-100 ppb) will generally divert assimilate to leaves rather than roots (Cooley & Manning, 1987). Numerous studies have documented that roots show proportionately greater growth reductions when exposed to ozone than do shoots (e.g. Bennett & Runneckles, 1977; Tingey *et al.*, 1971; Bennett & Oshima, 1976; Flagler & Younger, 1982).

Several authors have found ozone to affect root and shoot growth of tree species differentially. Kress and Skelly (1982) reported significant reductions in the root dry weight of *Pinus taeda*, *Pinus rigida*, *Liquidambar styraciflua*, and *Platanus occidentalis* following a 28 day exposure to between 50 and 150 ppb ozone. In all cases the percentage

reduction in root dry weight was greater than that of above-ground dry weight. Hogsett *et al.* (1985) observed greater reductions in root than above-ground dry weight in *Pinus elliottii* following chronic exposure to ozone.

These reductions in root growth reflect the reported reductions in root reserve carbohydrate for certain tree seedlings exposed to ozone (Tingey, Wilhour & Standley, 1976; Jensen, 1982). The longevity of woody species means that reductions in root growth may have important cumulative implications, in terms of the availability of reserve carbohydrates for new growth in the spring, or for recovery from other stresses, such as drought.

It is clear that ozone-polluted plants may be more vulnerable to drought stress since proportionately less root is available to supply water to transpiring leaves (Lechowicz, 1987). This vulnerability may be amplified if the diversion of assimilates to the shoots, at the expense of the roots, results in stimulations of shoot growth, and increases in leaf area. Stimulations of leaf length, dry weight or number resulting from ozone pollution were observed in some early studies (Bennett *et al.*, 1974; Bennett & Oshima, 1976; Bennett *et al.*, 1979), and several authors have reported increases in the leaf area ratio of ozone fumigated plants, for example in *Gossypium hirsutum* (Oshima *et al.*, 1979). This production of a greater transpiring leaf area per unit of biomass may further increase the sensitivity of ozone polluted plants to drought.

3.4 The effect of ozone on plant growth regulators

There is considerable evidence that endogenous hormones play an important part in the regulation of root:shoot partitioning (Mansfield, 1988). Evidence that ozone can alter plant processes such as assimilate partitioning, leaf senescence (Reich, 1983; Reich & Lassoie, 1985), leaf abscission (Ashmore *et al.*, 1988) and flowering (Amundson *et al.*, 1986) has led to speculation concerning the effect of ozone on plant growth regulators. Very little is known about the effects of pollutants on the biosynthesis, translocation or activity of plant growth hormones (Hughes & Laurence, 1984).

In an attempt to identify the cause of pollution induced changes in root:shoot partitioning, Mansfield (1988) speculated that receptor sites for hormones may be affected by pollution injury to the cell membranes, and this could then modify hormone-directed patterns of assimilate transport. Gaseous pollutants are known to increase synthesis of abscisic acid (ABA) and ethylene in leaves, and these hormones are both known to accelerate leaf

abscission (Fink, 1988), and to stimulate leaf senescence (Freer-Smith & Taylor, 1988). However, information concerning the effect of ozone on plant growth regulators is very scarce.

Increased ethylene production and ABA synthesis are also known to occur in response to water stress (Bradford & Hsiao, 1982). ABA is thought to have an important role as a hormonal signal from the root indicating soil water deficits (Davies *et al.*, 1982). ABA originating from the roots is transported to the shoots via the xylem stream, and the ABA concentration in the xylem sap has been shown to relate quantitatively to leaf conductance (Zhang & Davies, 1989).

Production of stress ethylene in response to ozone pollution is well documented (for example, Tingey, Standley & Field, 1976). Mehlhorn & Wellburn (1987) demonstrated that the formation of stress ethylene may also affect the sensitivity of plants to ozone. They exposed pea seedlings (*Pisum sativum*) to 50-150 ppb ozone for 7 h day^{-1} for their first three weeks of growth. After three weeks of fumigation the plants did not display any visible leaf injury, and rates of stress ethylene production were extremely low. By contrast, severe leaf necrosis developed when three week old seedlings (that had been grown in clean air) were fumigated with similar concentrations of ozone for only one 7 h period, and rates of stress ethylene evolution were much increased. The authors propose that the rate of ethylene production modifies the extent of visible leaf injury caused by ozone. Potentially, water stress could enhance ozone mediated leaf injury by increasing stress ethylene production.

Fuhrer & Grandjean (1988) reported significant increases in ethylene precursors in association with ozone induced yellowing of needles of *Abies alba*, and leaves of *Triticum aestivum*. They conclude that long term exposure of these plants to ozone does not cause an increase in ethylene production, but an accumulation of ethylene precursors. Ethylene is thought to be mobile in plants in the form of its precursors, and in this form ethylene is considered as a possible mechanism for hormonal communication of stress responses within the plant (Bradford & Hsiao, 1982).

4 Combined effects of ozone and water stress

Field and laboratory studies have established that plant water stress can strongly influence plant response to ozone. Water stressed plants generally show reduced responses to ozone. For example, in greenhouse studies plants that were water stressed just prior to ozone exposure showed little or no foliar injury compared to well watered plants (Harkov

& Brennan, 1980; Olszyk & Tibbitts, 1981b; Tingey *et al.*, 1982). However, this review is confined to experiments in which plants have been fumigated with ozone either before or during exposure to water stress, since the results from these experiments will aid our understanding of how exposure to ozone might modify plant sensitivity to water stress.

4.1 Ozone/water stress studies on crops: the NCLAN program

The National Crop Loss Assessment Network (NCLAN) was set up in the United States in 1980, in order to determine dose response relationships between seasonal mean ozone doses and yield of major crop species. In most agricultural regions of the United States drought periods occur during the growing season, and thus, NCLAN made a major effort to measure the effects of soil moisture deficit on yield responses to ozone (Heagle, Kress *et al.*, 1987). In a series of experiments, well watered and water stressed crops were exposed to a range of ozone concentrations in order to characterise the interaction of the two stresses on yield. Plants were exposed to ozone and water stress simultaneously, from the vegetative stage to maturity, in open top field chambers. These experiments are summarised in Table 1.1.

Ozone significantly reduced yield in all of these experiments except the one with *Hordeum vulgare* (Temple *et al.*, 1985a), in which there was no effect of ozone on yield at either moisture level.

In many of these studies soil water deficits were reported to reduce the impact of chronic ozone exposure on crop yield, although there was considerable year to year variation. For example, water stress reduced the impact of ozone on yield of *Gossypium hirsutum* in California during a normally hot and dry season, but not during a season when the weather was cool and cloudy (Temple *et al.*, 1985b). In experiments with *Glycine max* in Raleigh, North Carolina, water stress reduced the impact of ozone on yield in 1983 (Heagle, Flagler *et al.*, 1987), but not in 1984 (Heagle, Flagler *et al.*, 1987) or in 1986 (Miller *et al.*, 1989), and similarly water stress reduced the impact of ozone on yield of *Medicago sativa* in 1985, but not in 1984.

Table 1.1

The Effect of Soil Moisture Deficit on Ozone Dose-Yield Response.

Summary of Experiments Performed in the NCLAN Program.

- represents a significant ozone/water stress interaction in which water stress reduced the effect of ozone on yield, and + represents a significant interaction in which water stress increased the impact of ozone on yield. ns represents no significant interaction.

% yield supressions due to soil water stress (WS) are averaged across all ozone treatments.

Species	Year	% yield supression due to WS	Ozone/water stress interaction	Reference
Gossipium hirsutum	1981	16	-	Temple *et al.*, 1985b
	1982	2	ns	Temple *et al.*, 1985b
	1985	16	-	Heagle *et al.*, 1988
Glycine max	1982	13	+	Heggestad *et al.*, 1985
	1983	15	+	Heggestad *et al.*, 1985
	1983	50	-	Heagle, Flagler *et al.*, 1987
	1984	20	ns	Heagle, Flagler *et al.*, 1987
	1986	10	ns	Miller *et al.*, 1989
Medicago sativa	1984	10	ns	Temple, Benoit *et al.*, 1988
	1985	27	-	Temple, Benoit *et al.*, 1988
Festuca arundinacea with Trifolium repens	1984	12 (total forage)	ns	Heagle *et al.*, 1989
	1985	14 (total forage)	- (total forage)	Heagle *et al.*, 1989
Hordeum vulgare	1983	11	ns	Temple *et al.*, 1985a

In these examples there is some indication that water stress reduced the impact of ozone on yield only under relatively severe water stress conditions. When plants were moderately water stressed they tended to have similar yield responses to ozone as well watered plants.

As well as this effect of water stress severity, Moser *et al.* (1987) suggest that variations between seasons are partly the consequence of drought stress occurring at slightly different plant development stages. They reported that moderate water stress during the early reproductive stage reduced the effects of ozone on bush bean (*Phaseolus vulgaris*) growth, yield and dry matter partitioning more than drought stress during the late reproductive stage.

In experiments with *Glycine max*, Heggestad *et al.* (1985) observed a very different effect of water stress on yield responses to ozone. They observed more than additive reductions in yield (and leaf conductance) of three cultivars of soybean exposed to ambient ozone and soil moisture stress, compared to those exposed to either stress alone. However, in the same study, plants were exposed to non-filtered air with 30, 60 and 90 ppb ozone added, and at these elevated ozone concentrations water stress reduced the impact of ozone on yield.

This study demonstrates that the nature of ozone/water stress interactions on crop yield can depend on the level of ozone stress, as well as on the severity of water stress. This is an important point in considering how applicable the NCLAN results are to crops grown in north-west Europe. The mean seasonal ozone concentrations used in the NCLAN dose response studies are much higher than those experienced by field grown crops in the U.K. Also, in the NCLAN program, plants are exposed to elevated ozone concentrations daily throughout the growing season. This continuous ozone exposure is not representative of conditions in the U.K., where elevated ozone concentrations usually occur in episodes intermittently throughout the growing season. Similarly the continuous exposure to water stress, and in some studies (for example those in California) the severity of the water stress are not representative of field conditions in the U.K., where plants experience cycles of drought and rewetting in most summers.

Other ozone/water stress studies sponsored by NCLAN (and following NCLAN protocols) have investigated the impact of ozone and water stress on vegetative growth and gas exchange, rather than on yield. Amundson *et al.* (1986) report that ozone induced reductions in total plant dry weight of *Glycine max* were less for water stressed plants than for well watered plants. However, in the same experiment there were no ozone/water

stress interaction on leaf conductance; there was a linear decrease in conductance with increasing ozone dose in both well watered and water stressed plants (Reich *et al.*, 1985). Temple, Kupper *et al.* (1988) report ozone dose-dependant reductions in carbon fixation and vegetative growth of well watered and moderately water stressed *Gossypium hirsutum,* but severely water stressed cotton showed little response to ozone at ambient ozone concentrations. However, ozone-induced reductions in leaf conductance were comparable in all water stress treatments.

4.2 Ozone/water stress studies on trees

There are very few fumigation studies of ozone/water stress interactions on trees, and no studies on broadleaved species. There has been one filtration study of the effect of ambient air quality on well watered and water stressed *Fagus sylvatica* (Taylor *et al.*, 1989). This experiment was performed during the summer in southern Britain, and although ozone was a component of the ambient air pollution, the separate effects of individual gases could not be detected. Ambient air pollution had a significant effect on the gas exchange of well watered, but not of water stressed, plants.

Tseng *et al.* (1988) exposed three year old Fraser fir (*Abies fraseri*) seedlings to ozone (20, 50 or 100 ppb) and water stress simultaneously for ten weeks. Water stress significantly reduced total plant biomass, transpiration, needle conductance and net photosynthesis. The only significant effect of ozone was to reduce net photosynthesis measured after five weeks, and there were no significant interactions between ozone and water stress. However, in another experiment with fir (in this case Silver fir, *Abies alba*), Bucher *et al.* (1988) observed significant ozone/water stress interactions on xylem pressure, transpiration and needle conductance. Well watered and water stressed seedlings were exposed to 100 ppb ozone continuously for 4 weeks. Water stress significantly increased xylem pressure, and reduced transpiration and needle conductance. Exposure of water stressed plants to ozone enhanced these effects, whereas ozone significantly increased needle conductance and transpiration in well watered plants.

The timing of exposure to ozone and water stress may be important in determining the occurrence and nature of ozone/water stress interactions on plant growth and physiology. In the majority of ozone/water stress studies plants are exposed to ozone either prior to, or during, exposure to water stress. There is a great paucity of information regarding the effect of exposure to ozone on the subsequent response of plants to water stress.

5 The aims of this project

As this review of ozone/water stress studies illustrates, our understanding of the combined effect of ozone and water stress on plant growth and physiology is largely based on American studies on crop species, there is little information on the combined effects of ozone and water stress on forest trees (in particular broadleaved species) or on crops grown under typical British ozone and water stress conditions. The literature suggests that the severity of water stress and the ozone concentration can both be important in determining the nature of ozone/water stress interactions on plant growth and physiology, and so results from NCLAN studies are of limited value in predicting how ozone might affect plant sensitivity to water stress in the U.K.

Ozone is highly episodic in its occurrence in the U.K., and episodes of elevated ozone concentrations tend to coincide with periods of plant water stress. We know little of plant responses to episodic drought and ozone, or of the cumulative effects of intermittent exposure to ozone and water stress.

In the light of these observations this project was designed to investigate the combined effects of ozone and water stress on the growth and physiology of *Vicia faba* and *Fagus sylvatica*. The specific aims of the project were:

1. To expose plants to ozone concentrations typical of those monitored at rural sites in the United Kingdom, and for the duration of exposure to ozone and water stress to be realistic in relation to typical British summertime conditions.

2. To investigate how plant responses are influenced by the timing of exposure to ozone and water stress. Particular emphasis is placed on the effects of pretreatment with ozone on subsequent responses to water stress, and on the effects of simultaneous exposure to ozone and water stress.

3. To identify the major growth and gas exchange responses to ozone and water stress, and to elucidate how effects of ozone on plant growth and gas exchange might modify plant responses to water stress.

The first part of the project consisted of a series of four greenhouse experiments, in which *Vicia faba* was exposed to a variety of ozone and water stress regimes. The experiments were of two or three weeks duration and aimed to elucidate the main physiological and

morphological responses of this species to ozone and water stress.

The information gained from these greenhouse studies was used to develop a long term dose-response study with beech saplings in which plants were exposed to a range of ozone concentrations and to water stress intermittently over a four month period. Dose-response data were obtained for plant growth and gas exchange, and the effect of water stress on these dose response relationships was investigated. Plants were exposed to episodes of ozone and water stress followed by 'recovery' periods in order to determine any cumulative effects of successive stress exposures, and to determine the trees capacity to recover between these episodes.

The experiments with *V. faba* and *F. sylvatica* are presented in chapters 2 and 3 respectively. In chapter 4 the results from all the experimental work are brought together and discussed with reference to the published literature.

Chapter 2

Effects of Ozone and Water Stress on *Vicia faba*

1 Introduction

The combined effects of ozone and water stress on agricultural crops, at low ozone concentrations typical of North West Europe, have scarcely been investigated. The aim of these experiments was to determine the main growth and gas exchange responses of *Vicia faba* to low concentrations of ozone, and to investigate how these responses alter plant sensitivity to water stress.

Vicia faba was chosen for these experiments as it is amenable for use as experimental material in short term greenhouse studies, and it is considered to be relatively sensitive to both ozone and water stress.

Vicia faba has comparatively shallow roots and does not appear well adapted to drought (Day & Legg 1983). Legumes are considered to be relatively sensitive to ozone; ambient ozone in the U.K. has been demonstrated to inhibit growth and to cause visible leaf injury of various leguminous species (Ashmore 1984). Visible symptoms of ozone injury have been observed on *Pisum sativum* and *Trifolium repens* after episodes in which ambient ozone concentrations have exceeded 100 ppb (Ashmore *et al.*, 1980; Ashmore, 1984). Both these species are considered to be "very sensitive" to ozone, while *Vicia faba* is considered to be "moderately sensitive" to ozone (Ashmore, 1984).

In the U.K., episodes of high ozone concentrations are often brief, but they can persist for several days, or even for several weeks, under stable anticyclonic conditions (UK PORG 1987). In these experiments *Vicia faba* was exposed to ozone for approximately one week at concentrations between 50 and 60 ppb. Exposure to water stress was for approximately two weeks.

Examination of ozone and rainfall records for the U.K. reveal that ozone episodes commonly coincide with or precede periods of soil water stress. In these experiments emphasis was placed on differentiating between the effects of simultaneous and sequential exposure to ozone and water stress. A series of four experiments were performed in which exposure to ozone either preceded, or coincided with, the first day of exposure to water stress.

Ozone has been shown to alter assimilate partitioning and stomatal control of water loss in a wide variety of species. These are potential mechanisms by which exposure to ozone might disrupt plant-water relations. In an attempt to identify the important morphological and physiological responses of *Vicia faba* to ozone and water stress, detailed

measurements of plant growth and stomatal conductance were made in all experiments. Regular harvests and frequent gas exchange measurements were used to study dynamic responses of the plants to ozone and water stress.

2 Materials and methods

2.1 Description of experiments

In all experiments, plants were exposed to ozone for approximately one week, and to water stress for approximately two weeks. In experiment 1 (February 1987) exposure to ozone preceded exposure to water stress, while in experiments 2 and 3 (June 1987 and September 1987 respectively), exposure to ozone and water stress began simultaneously. In experiment 4 (March 1989), a third of the plants were exposed to ozone and water stress successively (Treatment a), a third were exposed to ozone and water stress simultaneously (Treatment b), and the remaining third were the control plants for both treatments a and b. For ease of description experiment 1, and treatment a of experiment 4 will be described as those in which exposure to ozone and water stress was "sequential", and experiments 2, 3 and treatment b of experiment 4 will be described as those in which exposure is "simultaneous".

Figure 2.1 summarises the timing and duration of exposure to ozone and water stress in each experiment.

2.1.1 Ozone and control treatments

The target ozone treatments for each experiment are summarised in Table 2.1. In experiments 1 and 3 ozone fumigation was continuous, and in experiment 4 plants were exposed to daily peaks of ozone. Experiment 2 was divided into two parts; experiment 2a in which the ozone fumigations were continuous; and experiment 2b in which the plants were exposed to daily peaks of ozone.

2.2 Greenhouse fumigation system

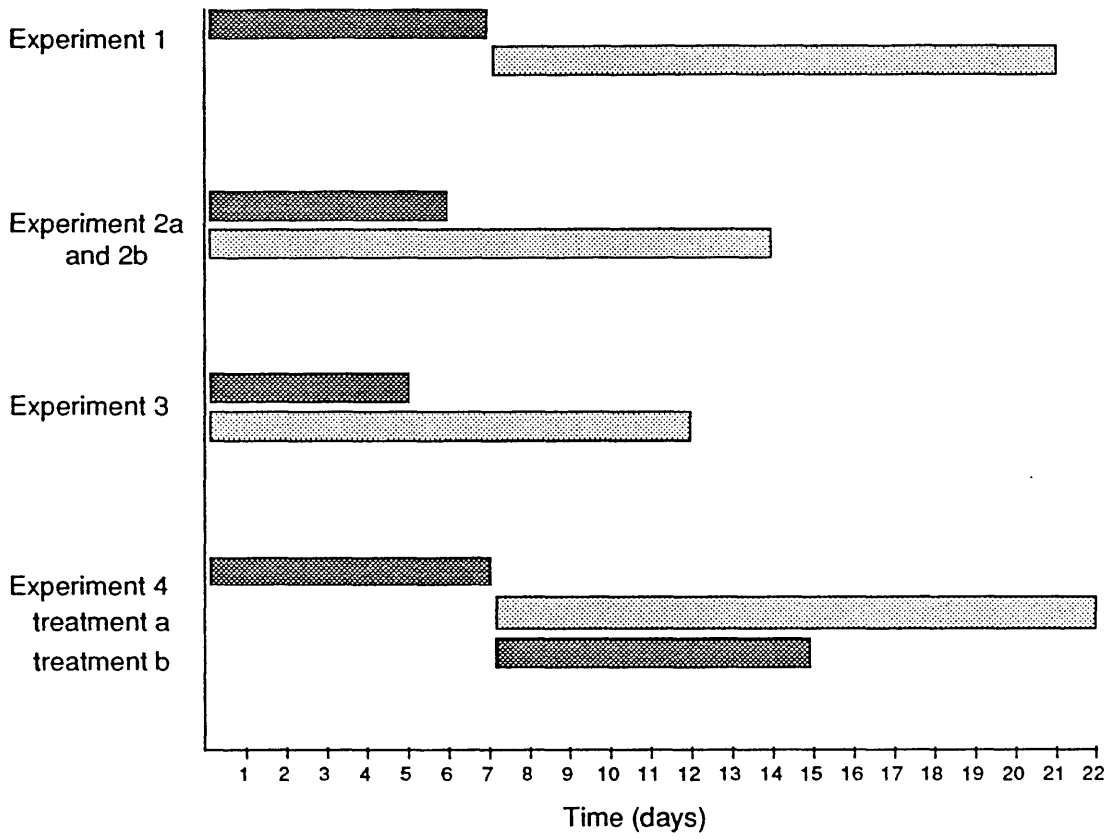
The plants were fumigated with ozone in a set of eight closed fumigation chambers, housed in a greenhouse. Each chamber was a Perspex cube (60cm x 60cm x 60cm),into which air was blown via an activated carbon filter. The air entered the chamber at the side, and exited via an exhaust at the back. The exhaust air was then blown out of the greenhouse. Air flow through the chambers averaged two air changes per minute.

The chambers had a removable front panel, and two port holes in the front panel, (which could be sealed), so that plants in the chambers could be manipulated without removing the front panel.

2.2.1 Ozone generation

In the first three experiments (experiments 1,2 and 3) ozone was generated from air by four UV generators. Each is a sealed metal box with six 4- watt ozone lamps (Philips type

Figure 2.1. The timing of exposure to ozone and water stress



Shaded bars represent the duration (in days) of ozone fumigation (▓▓) and the period over which water was withheld (▒▒).

Table 2.1
Target Ozone and Control Treatments

Experiment	Control Treatment	Ozone Treatment
1	Continuous exposure to filtered air	Continuous fumigation with 60 ppb ozone
2a	Continuous exposure to filtered air	Continuous fumigation with 50 ppb ozone
2b	Fumigation with daily peaks of 25 ppb ozone	Fumigation with daily peaks of 50 ppb ozone
3	Continuous exposure to filtered air	Continuous fumigation with 50 ppb ozone
4	Continuous fumigation with background ozone concentrations	Fumigation with daily peaks of 50 ppb

When daily peaks of ozone were administered, these were of 8 hours duration, and were usually from 0900 to 1700 hours. Plants were then exposed to filtered air at night.

OZ4SII) inside. Air is blown into the side of each box, and the air and ozone exits from the opposite side. In experiment 4, a commercial electric discharge ozone generator was used (type BA 0203012, Wallace and Tiernan, Tonbridge, England) and ozone was generated from oxygen. In experiment 4 the ozone was passed through water to remove any contaminant N_2O_5 before it entered the chambers.

From both generation systems the ozone was piped to each chamber, the ozone being introduced into the air flow before the chamber inlet. The ozone concentration in each chamber was controlled by a set of flowstats.

2.2.2 Ozone monitoring

The ozone concentration in each chamber was monitored with a Dasibi 1003-AH UV photometer, and recorded on a chart recorder. In experiments 2 and 4 a multichannel solenoid valve sampler was used, which sequentially sampled air from each chamber. Ozone loss through the sampling system was measured and a 10% correction was applied to all readings. Each chamber was sampled for approximately seven minutes each hour. In experiments 1 and 3, the chamber sample lines were changed manually.

2.3 Greenhouse conditions

The fumigation chambers were located in a greenhouse. In addition to natural daylight, supplementary lighting (averaging 408 umol m^{-2} s^{-1} PAR at plant height), was provided from 0600 to 2100 by three metal halide lamps. Fan heaters prevented the temperature from falling below about 10 °C, and extractor fans and shading prevented chamber temperatures from rising above approximately 35 °C on hot sunny days.

2.4 Plants

Seeds of *Vicia faba* cv. 'The Sutton' were surface sterilised with sodium hypochlorite (10% available chlorine) for 2 minutes, washed and then soaked in tap water overnight before planting.

The beans were planted in pots (8 cm^2, height 13cm) of pre-soaked compost, one seed per pot. In experiments 1, 2 and 3 the compost was a 1:1 mixture of peat and sharp sand, fertilised with a standard mix (UCD1 mix; Matkin and Chandler 1957). In experiment 4 resistance blocks were used for determination of soil water potential. These blocks did not respond well to changes in the water potential of the peat/sand compost, so in experiment 4 John Innes No.2 potting compost was used, with no additional fertiliser.

The plants were grown up in a heated greenhouse (day time temperature approximately 20 °C) with supplementary lighting from 0600 to 2100. Emergence began after 10 days; 3-4 weeks after planting (see Table 2.2 for details for each experiment) experimental plants were transferred to the fumigation chambers. Only plants with 3-5 leaves were selected, to improve initially uniformity of material.

2.5 Soil water stress

2.5.1 Control and measurement of soil water deficit

Soil water stress was monitored in all experiments by calculation of soil water deficits (SWD) for the water stressed treatments. The SWD is a measure of the decrease in the total weight of water in the soil compared to the total weight of water when the soil is at Field Capacity, see equation 1. A soil is at Field Capacity when it contains the maximum amount of water that it can hold against gravity (Fitter & Hay, 1983).

Soil water deficit is given by:

$$SWD_x = (1 - (WATER_x / WATER_{fc}))100$$

Where:

SWD_x is the soil water deficit at day x
$WATER_x$ is the weight of water in the soil at day x
$WATER_{fc}$ is the weight of water in the soil at Field Capacity.

The Field Capacities of the soils used were calculated and thus the weight of water in the soil at Field Capacity could be calculated for each pot. Before the onset of water stress the plants were watered to run off to ensure that they were at field capacity, and the plant, soil and pot were weighed for calculation of $WATER_x$ using the equation:

$$WATERx = WATERfc - (WTfc - WTx)$$

Where:

WTfc is the weight of the plant, soil and pot when the soil is at Field Capacity
WTx is the weight of the plant, soil and pot at day x.

Table 2.2
Details of Experiments

	Experiment			
	1	2a and 2b	3	4
Dates	17.2.87 to 9.3.87	10.6.87 to 24.6.87	18.9.87 to 30.9 87	1.3.89 to 22.3.89
Age of plants at start of experiment	4 weeks	3 weeks	3.5 weeks	4a: 3.5 weeks, 4b: 4.5 weeks
Age of plants at final harvest	7 weeks	5 weeks	5 weeks	4a and 4b: 6.5 weeks
No. of chambers	2 control & 2 ozone	2 control & 2 ozone	3 control & 3 ozone	4 control & 2 ozone
No. of plants per chamber	36	30	30	32
No. of plants per treatment	36	30	45	32

Thus, for all water stressed plants, the plant, soil and pot were weighed regularly throughout the water stress period in order to calculate the SWD for each pot.
In experiments 2, 3 and 4, the weight of the plant, soil and pot at a threshold SWD was calculated at the onset of water stress, and when the weight fell below this threshold the soil was watered back to the threshold weight. The threshold SWD used in experiments 2 and 3 was 70 %, and in experiment 4 was 55 %.

2.5.2 Measurement of soil water potential (Experiment 4).

In experiment 4, the soil water potential of six plants per treatment was measured every few days throughout the water stress period using gypsum Resistance Blocks and a Soil Moisture Meter (ELE International, Hemel Hempstead, England). The blocks have a diameter of 20mm, and were submerged in the soil 10 cm below the soil surface.

2.6 Plant water stress (Experiment 4)

2.6.1 Measurement of leaf water potential

A Pressure Chamber was used to measure leaf water potential in experiment 4. In this technique a leaf is cut and sealed in the chamber with the cut end of the petiole protruding through a rubber seal. The chamber pressure is increased by introducing compressed air from a cylinder until the xylem sap just appears at the cut end of the petiole. At this point the pressure inside the chamber equals the xylem pressure potential which closely approximates leaf water potential.

The pressure chamber apparatus used is manufactured by Chas W. Cook and Sons, Birmingham, England. Compressed air enters the chamber via a hand operated gas control valve, which can be pre set to allow a constant rate of pressure increase over a series of measurements.

The leaf petiole was threaded through a hole in a soft rubber seal and sealed into the lid of the chamber. The cut end of the petiole protruding through the top of the chamber was observed through a magnifying glass.

Measurement of leaf water potential of leaves of *Vicia faba*

Measurements were made in the middle of the day (1200 to 1400), at the end of each week of the experiment. Measurements were made on six plants per treatment, one leaf per plant.
A plant was removed from the fumigation chambers, brought to the pressure chamber

apparatus, where the youngest fully expanded leaf was selected, its position noted, and the petiole cut. The time between sampling and measurement was minimized; a leaf was sealed and a measurement made within 1 minute of cutting the petiole. When the end point was reached the pressure was noted, and then a repeat measurement was made on the same leaf. If the repeat measurements did not yield consistent values then the leaf was rejected and a new one was selected.

Damp filter paper was placed inside the pressure chamber, in order to reduce evaporative loss of water from the leaves during the period of measurement.

2.6.2 Measurement of relative leaf water content

An estimate of the relative water content of a leaf can be made by determination of the fresh and dry leaf weight:

Relative water content = (fresh weight - dry weight) / dry weight

When bean plants were removed from the fumigation chambers for determination of leaf water potential, a leaf was also sampled for measurement of relative water content. A fully expanded leaf was cut from the plant, weighed, oven dried (80 °C for at least 3 days), and weighed again.

The plants from which leaves had been sampled for water potential and water content determination were not returned to the chambers, but were destructively harvested. (See section 2.8 for details.)

2.7 Measurement of leaf conductance: porometry

Leaf conductance was measured using a Delta-T Automatic Diffusion Porometer (Mk. 1 model, Delta-T Devices, Burwell, Cambridge, England.)

The Delta-T porometer measures conductance of abaxial leaf surfaces by measuring the rate of humidification of a cup clamped to the leaf surface. This rate is timed electronically over a fixed interval of relative humidity and is expressed as a count. The count is compared with those for known diffusion conductances obtained using a calibration plate, and thus counts obtained from leaf measurements can be converted into diffusion conductance values. Relative humidity in the cup is measured with a thin film capacitative sensor, and the leaf and cup temperatures are measured by two thermistors in the cup.

Measurement of the conductance of leaves of *Vicia faba*

Leaf conductance was measured with the porometer in experiments 1,2 and 3. Table 2.3 outlines the details of these measurements for each experiment.

The porometer was calibrated at the beginning and end of each set of leaf conductance measurements. The count is highly sensitive to temperature changes, and to temperature differences between the leaf and the cup, so care was taken to perform the calibration at the temperature at which the measurements were made, and to check cup and leaf temperatures regularly throughout the set of measurements.

Measurements were made only on fully expanded leaves, on one leaf per plant, and the same leaf was measured throughout each experiment.
The porometer sensor head was passed through the chamber porthole and clamped carefully onto a leaf (Plate 2.1). In this way measurements were made with minimum disturbance to chamber conditions. Counts were recorded as soon as they had stabilised (usually after approximately 20 seconds), but the leaf was never left in the cup for more than one minute before the count was recorded.

2.8 Gas exchange measurements

In experiment 4, carbon dioxide assimilation and transpiration of single leaves was measured with a portable infra red gas analyser (IRGA) system, the LCA-2 Leaf Chamber Apparatus (Analytical Development Company Ltd., Hoddesdon, Herts.). This system consists of an IRGA, a leaf cuvette, an apparatus to supply air to the cuvette (the Air Supply Unit), and a data processor and logger.

The principle of operation of the LCA-2 is different to that of the porometer. The porometer is a closed system; the leaf is sealed into a cuvette, and conductance is derived from the rate of change of water vapour concentration within the cuvette. The LCA-2 is an open system; the leaf is placed in a cuvette into which there is a measured flow of air of known water vapour and carbon dioxide content. Gas exchange rates are determined from flow rate and the concentration differences between inlet and exhaust air.

A 6.25 cm^2 square of leaf is sealed into the cuvette, into which there is a measured flow of dry air from the Air Supply Unit. The cuvette contains an air temperature sensor, a PAR sensor, and a humidity sensor positioned in the air exhausting from the cuvette. A fan is fitted inside the cuvette, which vigorously mixes the dry air entering the cuvette with many times its volume of moist air, and as a result the leaf experiences (with very small

error) the humidity of the exhaust air. This fan also ensures a small boundary layer resistance and rapid response. The transpiration rate is the product of the flow rate per unit leaf area and the exhaust humidity.

Air emerging from the cuvette (the 'analysis' gas stream), and a sample of air emerging directly from the Air Supply Unit (the 'reference' gas stream), are passed to the IRGA. The IRGA measures the difference in carbon dioxide concentration of these two air streams, and the product of this and the flow per unit leaf area gives the assimilation rate. The gas flow through the measurement cell of the IRGA alternates between 'analysis' and 'reference' air every two seconds, enabling a continuous correction of measurements for changes in source and detector characteristics (e.g. due to temperature).

The data logger is micro-processor controlled, and both processes and stores data from the IRGA and cuvette. The logger stores the basic sensor data ('analysis' and 'reference' carbon dioxide concentrations, the flow rate from the Air Supply Unit, the time, air temperature, exhaust humidity, and PAR), and calculates and stores the leaf conductance, the photosynthetic rate, leaf temperature, and the sub-stomatal cavity carbon dioxide concentration. The calculated variables are derived from inputs from the IRGA and cuvette, and from constants entered into the data logger by the operator (leaf area, boundary layer resistance to water vapour, and atmospheric pressure). The data were downloaded from the logger to a printer at the end of each session of measurements.

Table 2.3
Gas exchange Measurements
Experiments 1-4

	Experiment			
	1	**2a and 2b**	**3**	**4**
Method of measurement	porometry	porometry	porometry	LCA-2 portable IRGA
Frequency of measurements	Every 3 days (approx.)	Every 3 days (approx.)	Measured on two days only, at six day intervals.	Every 2-3 days.
Timing of measurements	1100 to 1200	1300 to 1400	At 0700-0800, 1000-1100, 1400-1500 and 1800-1900.	1200 to 1400
Number of plants per treatment measured.	14 (1 leaf per plant)	10 (1 leaf per plant)	15 (1 leaf per plant)	8 to 12 (1 or 2 leaves per plant)

Plate 2.1
Measurement of Plant Gas Exchange *(Vicia faba)*

Automatic Diffusion Porometer

LCA-2 Portable IRGA

Gas Exchange Measurements In *Vicia faba.*

Gas exchange measurements were made with the LCA-2 in experiment 4. The details of these measurements are given in Table 2.3. The measurements were made with the front panels of the fumigation chambers removed, i.e. under greenhouse conditions (Plate 2.1). Leaves were sealed into the cuvette and a measurement recorded within one minute. The inlet tube to the Air Supply Unit was suspended in the greenhouse roof, and the flow rate from the Air Supply Unit to the cuvette was maintained at 300 ml min^{-1}. Problems in obtaining a steady reading of leaf conductance were encountered, which were thought to be due to humidification of the cuvette during measurements. In an attempt to clarify this the last set of measurements (20.3.89) were made at the normal flow rate of 300 ml min^{-1}, and then repeated at a higher flow rate (500 ml min^{-1}).

2.9 Destructive harvests

Plant growth and partitioning of assimilates were assessed in each experiment by destructive harvests. At each harvest, plants were divided into their constituent parts, main and side stem leaves, stem, roots (and, where appropriate, flowers and dead leaves); the leaves were counted; the leaf areas of all, or a sample of plants per treatment were determined (using an Optomax Image Analyser); and then the plant parts were dried (80 °C for three days) and weighed.

The details of the harvests in each experiment are given in Table 2.4. There was a final harvest at the end of each experiment, and intermediate harvests were made at the end of each week in all experiments except experiment 3. In each experiment an initial harvest was made before the treatments began in order to assess initial plant size.

2.10 Non destructive growth measurements and visible injury assessments

In experiments 2, 3 and 4, the number of main and side stem leaves on each plant was counted initially (before the treatments began), and at the end of each week. The number of dead leaves, if any, on each plant was also counted.

In experiments 1 and 2 the number of plants with visible ozone injury was counted at the end of the fumigation, while in experiments 3 and 4, the number of injured leaves on each plant was counted. In experiment 3, this assessment was made once, at the final harvest, and in experiment 4, the plants were assessed for ozone injury at the end of each week of the experiment.

Table 2.4
Sample size for Destructive Harvests
Experiments 1-4

	Experiment			
	1	**2a and 2b**	**3**	**4**
initial harvest	9	15	15	16
intermediate harvest(s)	14	15	--	8
final harvest	14	15	15	20

Tabulated values are numbers of plants harvested per treatment. There were no intermediate harvests in experiment 3.

3 Data analysis

(See Appendix 1)

The majority of the data were analysed by two-way analysis of variance using the Genstat 4 package. Any exceptions to this rule are outlined below. When the F ratio was significant (at $p<0.05$) the differences between individual treatments were tested using the least significant difference (calculated from the pooled standard error) at the 95% significance level.

Before performing the analysis the data were examined to ensure that they were normally distributed. There was no evidence of non-normal distribution in gas exchange and water stress data. However, some of the growth and leaf injury data were subjected to logarithmic transformations to ensure that they were normally distributed.

When appropriate, covariates were used in the analysis of variance to explain part of the residual variation. The details of the covariates used in the analysis of each data set are included in the following sections. Only covariates which were not significantly affected by ozone or water stress were used. Whenever covariates were used all values presented are adjusted means.

3.1 Ozone concentration: variation between chambers

In experiments 1 and 3 the sequential ozone sampler was not available, so concentrations were monitored from just one chamber, with spot checks of the concentration in other chambers being made regularly. Therefore, in these experiments it was not possible to analyse statistically any differences in ozone concentration between chambers of the same treatment. However, in experiments 2 and 4, the sequential ozone sampler sampled the air from each chamber for approximately seven minutes each hour, so data were available of the ozone concentration in each chamber throughout the experiment.

These data were used to calculate a mean 24 hour ozone concentration in each chamber for each day of the fumigation in experiment 2a, (in which ozone fumigation was continuous) and a day time 8 hour mean for each chamber for each day of the fumigation in experiments 2b and 4 (in which fumigation was daily). The overall difference between the means for each pair of chambers was analysed using a paired t test.

3.2 Soil water stress

3.2.1 Soil water deficit

The effect of ozone on the SWD of water stressed treatments was analysed in each experiment. Attempts were made to use various harvest data as covariates, (total plant dry weight, leaf area, leaf dry weight), but none of these were significant covariates (at $p<0.05$) and were not included in the final analysis.

3.2.2 Soil water potential

In experiment 4 the mean soil water potential of each treatment was calculated for each day that it was measured. Attempts were made to use various harvest data as covariates in the analysis of variance (total plant dry weight, leaf area, leaf dry weight), but none of these were significant (at $p<0.05$) and were not included in the final analysis.

3.3 Plant water stress

Measurements of plant water stress were made in experiment 4. The leaf water potential of water stressed plants only was measured, and the effect of ozone on the leaf water potential was analysed using the age of the leaf selected for measurements as a covariate in the analysis of variance.

The leaf water content of fully watered and water stressed plants was measured. The effect of ozone and water stress on the leaf water content was analysed using a two way analysis of variance, using leaf age as a covariate.

3.4 Growth data

After each harvest the dry weight data obtained (stem, root, live and dead leaf dry weight) were used to calculate total plant dry weight, total above ground dry weight and root:shoot ratio, RSR (root dry weight/above ground dry weight). The measurements of leaf area were used to calculate Leaf Area Ratio, LAR (total leaf area/total plant dry weight), and Specific Leaf Area Ratio, SLAR (total live leaf area/live leaf dry weight).

In experiments 2, 3 and 4, initial leaf number (counted one day before the treatments began) was used as a covariate in the analysis of variance. No covariate was used in the analysis for experiment 1.

3.5 Gas exchange

3.5.1 Leaf conductance

In experiments 1, 2a and 2b the initial measurements of leaf conductance, made before the treatments began, were used as a covariate. No initial measurements were made, and no covariates were used in the analysis of the leaf conductance data from experiment 3. In experiment 4, the age of the leaf selected for measurement was used as a covariate, as in this experiment, unlike the others, leaf age varied within one set of measurements.

3.5.2 Photosynthesis

Net photosynthesis and intercellular carbon dioxide concentration were measured with the LCA-2 in experiment 4. The covariates used in the analysis of these data were leaf age and PAR measured with the LCA-2 for each gas exchange measurement made.

3.6 Leaf injury

In experiments 1 and 2 accurate assessments of ozone damage were not made. The number of plants with ozone damage at the end of the fumigation was counted, but no attempt was made to assess the effect of water stress on the extent and development of damage.

In experiments 3 and 4 the number of injured leaves on each fumigated plant was counted. In experiment 3 counts were made only at the final harvest, while in experiment 4 the number of injured leaves was counted at the end of each week of the experiment. The effect of water stress on the number of injured leaves was assessed in experiment 3 using a t-test, and in experiment 4 using analysis of variance. In experiment 4, analysis of variance was also used to test the difference between the mean number of injured leaves on plants in treatment a, compared to plants in treatment b, at the end of the fumigation, and one week after the end of the fumigation. No covariates were used in this analysis.

4 Results

4.1 Greenhouse conditions

The greenhouse conditions for each experiment are summarised in Table 2.5. The conditions varied between experiments; mean temperatures were highest in experiment 2, and lowest in experiment 1.

4.2 Ozone exposures

The ozone and control treatments for each experiment are summarised in Table 2.6. The timing of exposure to ozone and water stress is illustrated in Figure 2.1.

In experiments 1, 2a and 3, the plants were continuously fumigated with ozone, day and night. The actual ozone concentrations administered in each of these experiments are illustrated in Figure 2.2. In experiment 1, the 24 hour means for each day of the fumigation varied between 49 and 61 ppb; in experiment 2a, they varied between 35 and 59 ppb; and in experiment 3, they varied between 36 and 65 ppb.

In experiment 2b, all plants were exposed to daily peaks of ozone (Figure 2.3). The control plants received 8 hour daily peaks with mean concentration of between 16 and 25 ppb, while the ozone fumigated plants received daily peaks of between 45 and 53 ppb (8 hour mean).

In experiment 2a, the ozone concentrations tended to dip during the day and rise at night. This was because the ozone generators failed to produce enough ozone to supply day time ozone peaks to plants in experiment 2b, and at the same time to maintain the continuous supply of ozone to plants in experiment 2a. The lack of capacity of the ozone generation system also accounts for the relatively low ozone levels in the early part of experiment 3. The gaps in the ozone record for experiments 2a and 2b are due to technical problems with the monitoring equipment.

In experiment 4, the control plants were continuously fumigated with 'background' ozone concentrations (13 ± 3 ppb), while the ozone fumigated plants received daily peaks of ozone (Figure 2.3). The mean 8 hour concentration of these peaks varied between 48 and 60 ppb in treatment a, and between 48 and 57 ppb in treatment b.

Table 2.5

Experimental Greenhouse Conditions

	Experiment			
	1	2a and b	3	4
Month	February	June	September	March
Mean mid day temperature (°C)	17 ± 3	27 ± 5	21 ± 8	25 ± 5
Mean mid day relative humidity (%)	49 ± 7	25 ± 8	43 ± 7	34 ± 9
Temperature minimum-maximum (°C)	6 - 27	13 - 37	13 - 36	12 - 33
Relative humidity minimum-maximum (%)	34 - 68	14 - 43	34 - 53	24 - 52

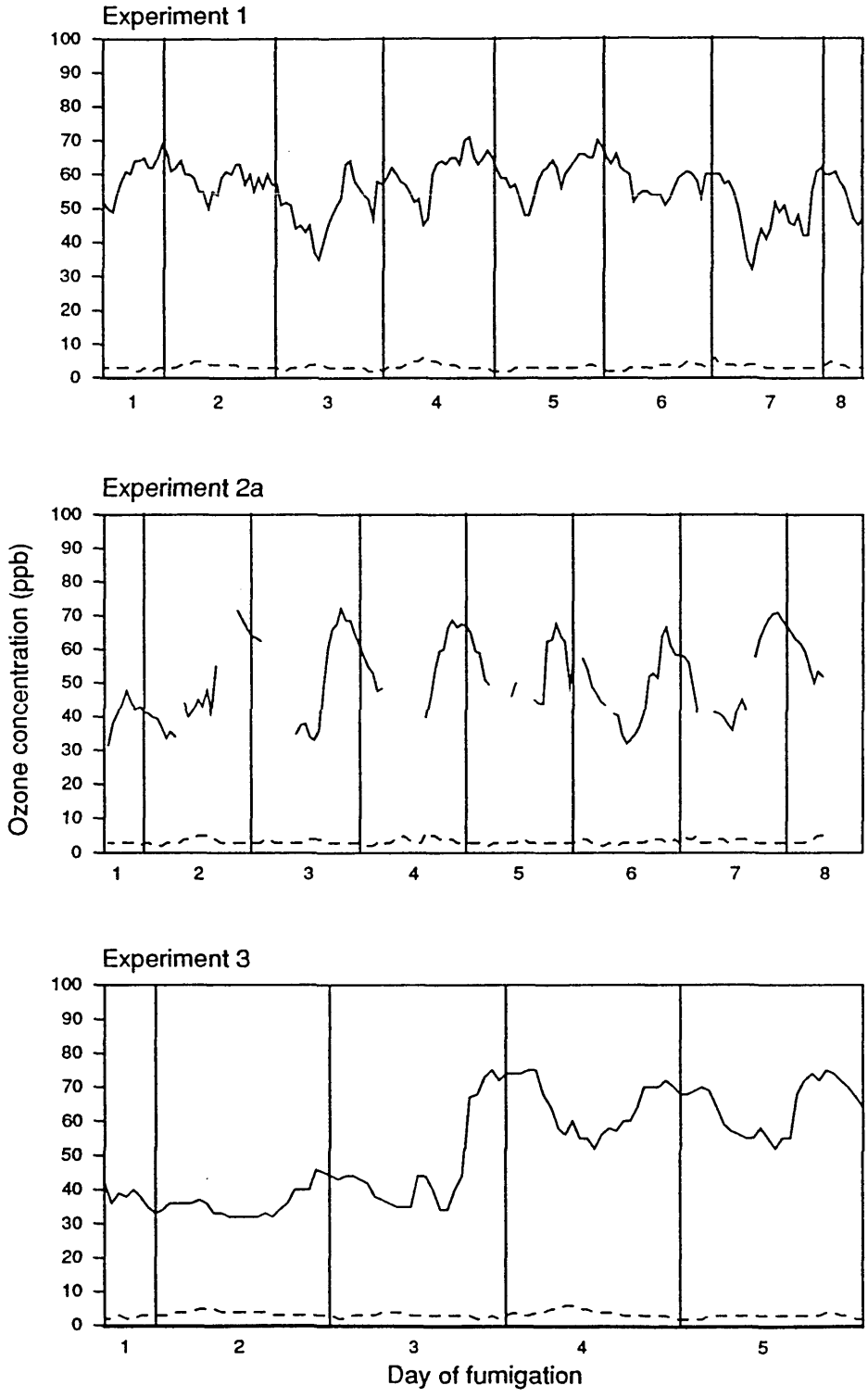
Mid day data were recorded at 1200 and at 1400 on each day of the experiment. Figures presented are overall means for the whole experiment ± standard deviation of this mean. The minimum and maximum data are for the whole experiment, recorded over 24 hours.

Table 2.6
Details of Control and Ozone Treatments

Exp.	Duration	Control	Ozone Fumigation
1	7 days	3 ± 2	56 ± 8
2a	6 days	3 ± 2	52 ± 12
2b	6 days	22 ± 7 (day) 3 ± 2 (night)	47 ± 7 (day) 3 ± 2 (night)
3	5 days	3 ± 2	52 ± 15
4 a	7 days	13 ± 3	53 ± 12 (day) 13 ± 3 (night)
b	8 days	13 ± 3	51 ± 9 (day) 13 ± 3 (night)

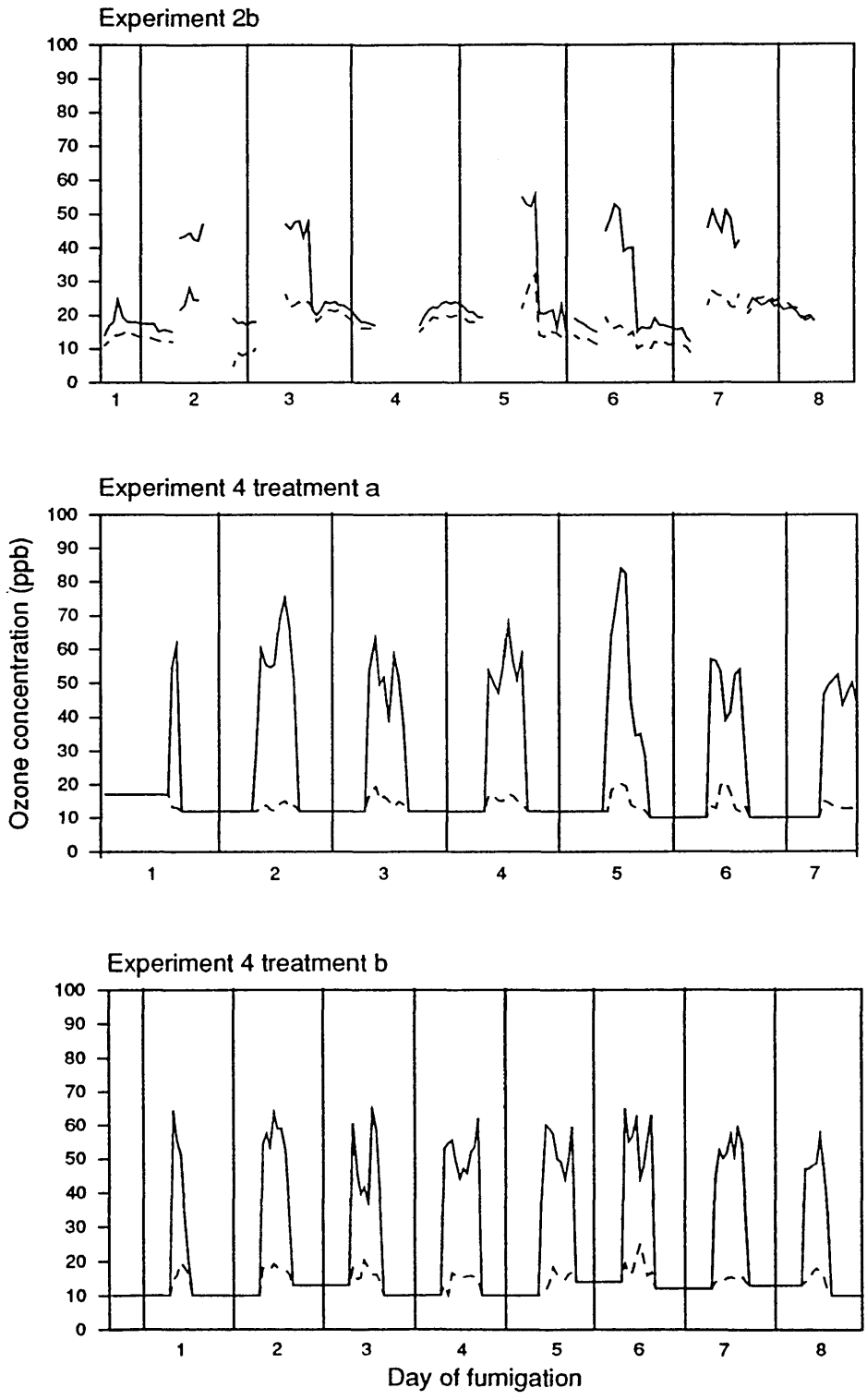
Tabulated values are overall mean ozone concentrations (in ppb) for the whole fumigation ± standard deviation of the hourly means.
In experiments 2b, 4a and 4b daily peaks were of 8 hour duration; 0900 to 1700

Figure 2.2. Mean hourly ozone concentrations in experiments 1, 2a and 3 (continuous fumigation).



Ozone concentration is plotted against day of fumigation. Solid lines represent ozone fumigated chambers, broken lines represent control chambers.

Figure 2.3. Mean hourly ozone concentration in experiments 2b and 4 (daily peaks of ozone).



Ozone concentration is plotted against day of fumigation. Solid lines represent ozone fumigated chambers, broken lines represent control chambers.

Variation Between Chambers

In experiments 2 and 4, two chambers were used for each of the ozone fumigations, and the plants to be fumigated were split evenly between these chambers. In experiment 2a, in which the fumigation was continuous, the difference between the 24 hour mean ozone concentration in each chamber was analysed, and in the other three experiments, the 8 hour daily mean was used in the analysis. In all these experiments, there was no significant difference in the overall mean ozone concentration supplied to each chamber in a treatment pair.

4.3 Soil water stress

4.3.1 Soil water deficit

The development of SWD in each experiment is illustrated in figure 2.4. Plants in experiments 2a and 2b were exposed to the same water stress regime, as were plants in treatments a and b of experiment 4. Exposure to ozone before or during the period of water stress had no significant effect on the development of SWD in any experiment.

4.3.2 Soil water potential

In experiment 4, the soil water potential of water stressed and fully watered treatments was monitored throughout the water stress period. The effect of ozone and of water stress on soil water potential is summarised in Table 2.7.

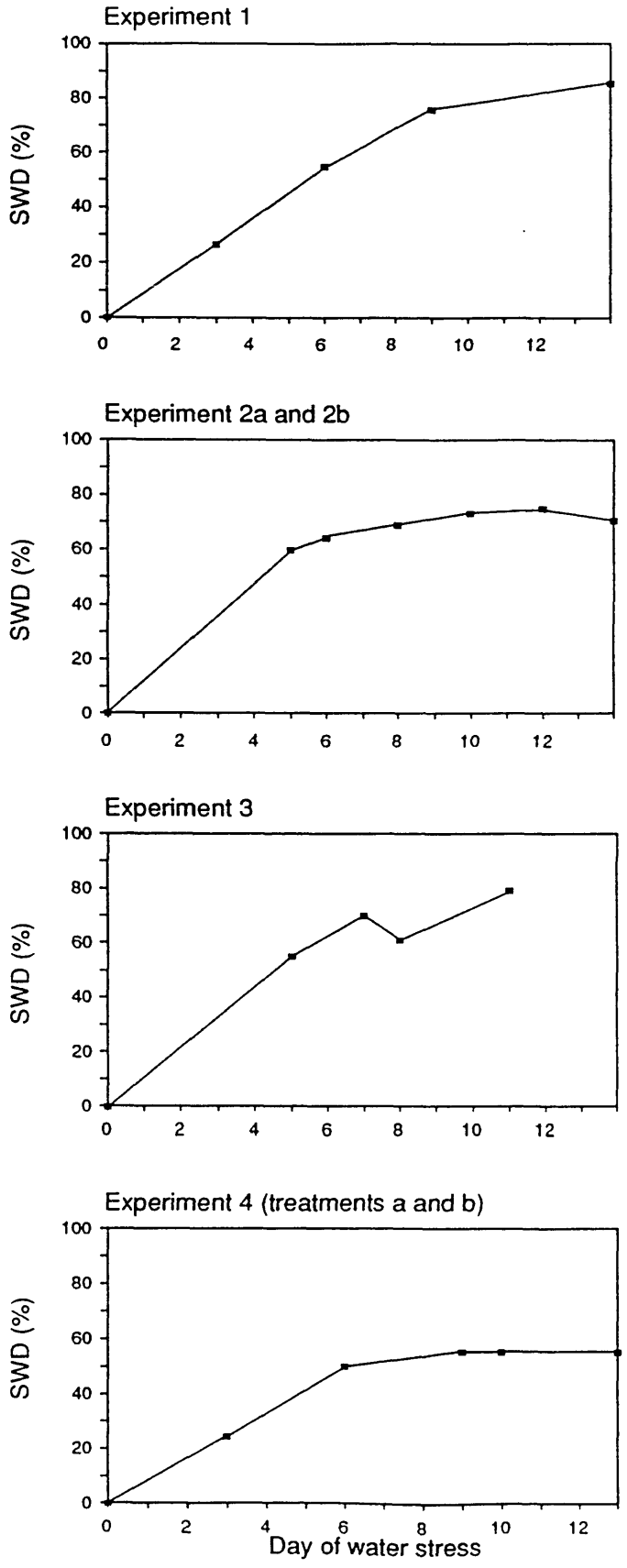
The soil water potential of water stressed treatments was significantly less than that of fully watered treatments from the sixth day of the water stress episode onwards.

Ozone had no effect on the soil water potential of fully watered treatments throughout the experiment. In water stressed treatments, there was evidence of a decrease in the soil water potential of treatments that had been fumigated with ozone (treatments a and b) compared to the controls (Figure 2.5), but this effect of ozone was not significant at $p<0.05$.

4.4 Plant water stress

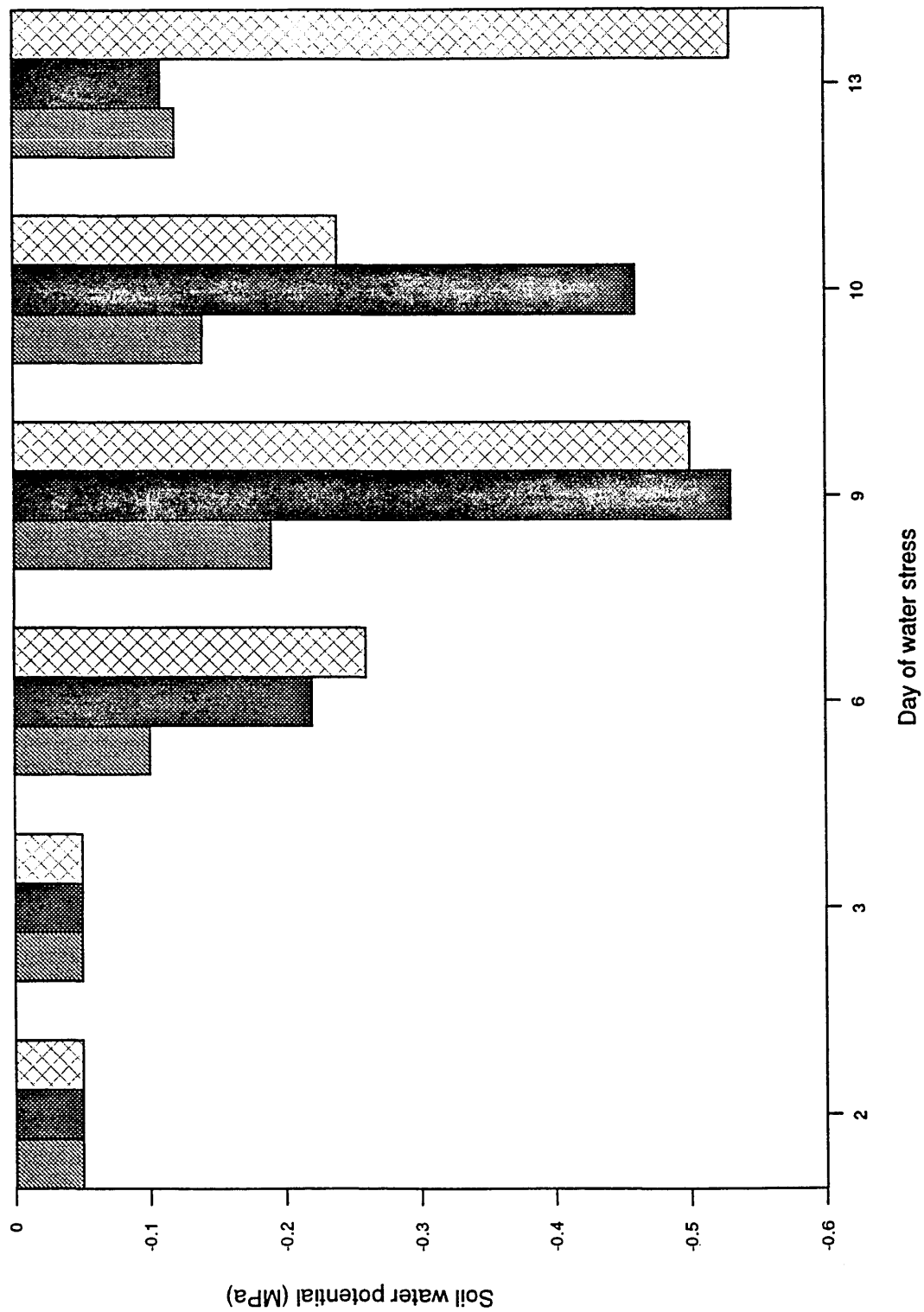
The mid day leaf water potential and leaf water content of fully watered and water stressed plants was measured in experiment 4. The effect of ozone and water stress on leaf water stress is summarised in Table 2.8.

Figure 2.4. The development of soil water deficit in water stressed treatments



Soil water deficit (SWD) is plotted against day of water stress. Plotted values represent the mean of ozone fumigated and control treatments.

Figure 2.5. The effect of ozone on the soil water potential of water stressed treatments (experiment 4).



The soil water potential of control (▨), treatment a (▩) and treatment b (▧) is plotted against day of water stress.

Table 2.7

The effect of Ozone and Water stress on Soil Water Potential (Experiment 4)

Summary of Analysis of Variance

Day of water stress	O_3	WS	0_3*WS
2	0.07	0.05	1.20
3	0.59	0.49	0.43
6	0.68	**5.65** -	0.57
9	3.16	**38.91** -	2.63
10	0.57	**5.80** -	0.32
13	2.37	**8.86** -	1.98

Tabulated values are variance (F) ratios. F ratios in bold type are significant at $p<0.05$. - indicates a significant decrease in ozone (O_3) or water stress (WS). The covariate used in this analysis was leaf age.

Table 2.8
Plant Water stress (Experiment 4)
Summary of Analysis of Variance

	Leaf Water Potential			Leaf Water Content		
Day of WS	O_3	WS	O_3*WS	O_3	WS	O_3*WS
Pre WS	0.52	n/a	n/a	1.32	n/a	n/a
7	0.09	0.02	0.31	0.57	1.08	2.43
14	0.99	2.69	2.15	0.55	**36.61** -	0.48

Tabulated values are variance (F) ratios. F ratios in bold type are significant at $p<0.05$. - indicates a significant decrease in ozone (O_3) or water stress (WS). *Pre* water stress the effect of WS is not applicable (n/a). The covariate used in this analysis was leaf age.

4.4.1 The effect of water stress

The effect of water stress on mid day leaf water potential and leaf water content is illustrated in figure 2.6.

Leaf Water Potential

Water stress had no significant effect on leaf water potential; the mean mid day leaf water potential of water stressed plants was no lower than that of the well watered plants.

A possible explanation for this somewhat surprising result is that well watered plants experienced plant water stress at mid day, brought about by relatively high mid day temperatures. During this experiment the weather was generally sunny and settled, and mid day temperatures in the chambers frequently exceeded 30 °C.

Relative Leaf Water Content

The relative leaf water content of water stressed plants was reduced by 6% after one week of water stress, and by 13 % after two weeks of water stress. Only the 13 % reduction was significant.

4.4.2 The effect of ozone on leaf water status

Ozone had no significant effect on the leaf water potential or leaf water content of fumigated plants in either treatment a or b.

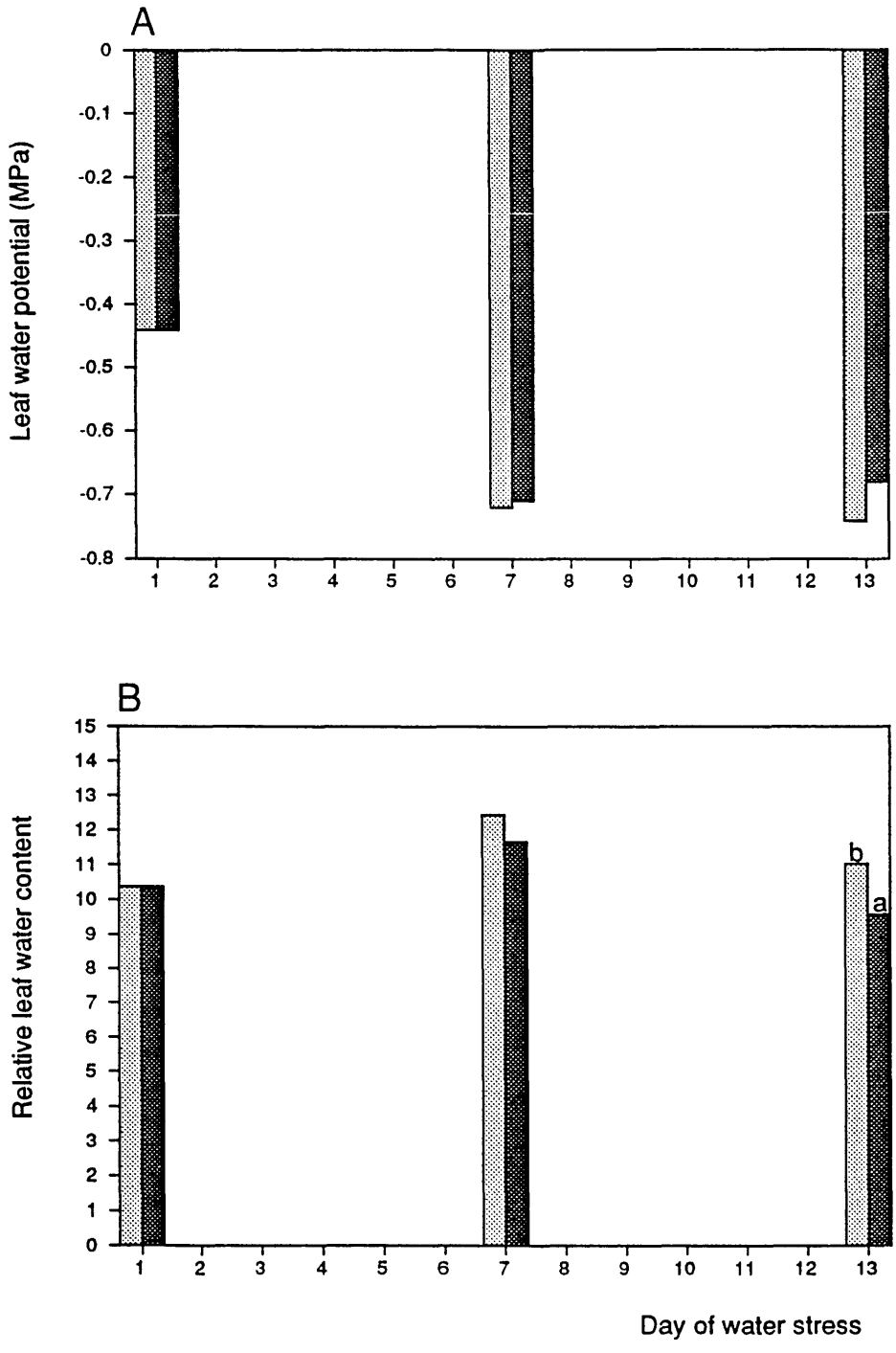
4.5 Visible ozone injury

Ozone fumigation resulted in visible ozone damage in all experiments. In experiments 1 and 2 accurate assessments of ozone damage were not made, while in experiments 3 and 4 the number of injured leaves on each fumigated plant was counted.

In all experiments ozone injury was visible from approximately seven days after the beginning of the ozone fumigation. Thus, in experiments 1, 2 and 4, visible injury was first observed on the last day of the fumigation, and in experiment 3, was first observed two days after the end of the five day fumigation.

In experiments 1, 2a and 2b, approximately 8% of the plants exposed to ozone developed symptoms of ozone damage. This percentage increased to approximately 40% in treatment b of experiment 4, and to approximately 80% in experiment 3 and treatment a of experiment 4.

Figure 2.6. The effect of water stress on the leaf water potential and relative leaf water content of plants in experiment 4.



The leaf water potential (A) and relative leaf water content (B) of well watered (▭) and water stressed (▩) plants is plotted against day of water stress. Plotted values represent the mean of ozone fumigated and control treatments. Within each day entries not headed by the same letter are significantly different at $p<0.05$.

4.5.1 The effect of water stress on ozone injury

The effect of water stress on the extent and development of ozone injury was analysed in experiments 3 and 4.

In experiment 4, plants in treatment a were exposed to water stress after the end of the ozone fumigation. Water stress had no significant effect on the number of injured leaves counted after one and after two weeks of water stress. However, in experiment 3 and treatment b of experiment 4, plants were water stressed during the ozone fumigation. In both these cases, one week after the end of the fumigation water stressed plants had significantly (at $p<0.05$) less ozone injured leaves than fully watered plants (experiment 3: $t=2.79$; treatment b of experiment 4: $F=5.313$). See Figure 2.7. In experiment 4 (treatment b) the number of injured leaves was also counted at the end of the fumigation, and at this time there was no significant difference in the number of injured leaves on fully watered and water stressed plants.

4.5.2 Leaf injury in experiment 4: comparison of treatments a and b.

Plants in treatment a of experiment 4 had considerably more ozone injury than those in treatment b. This difference was seen in the percentage of plants with injury at the final harvest, (80 % in treatment a, and 40 % in treatment b), and also in the mean number of leaves injured (Figure 2.7). One week after the end of the ozone fumigation, the mean number of injured leaves on plants in treatment a was significantly (at $p<0.05$) greater than the number on plants in treatment b. This difference between the experiments was seen in both fully watered ($F=7.53$) and water stressed ($F=49.12$) plants.

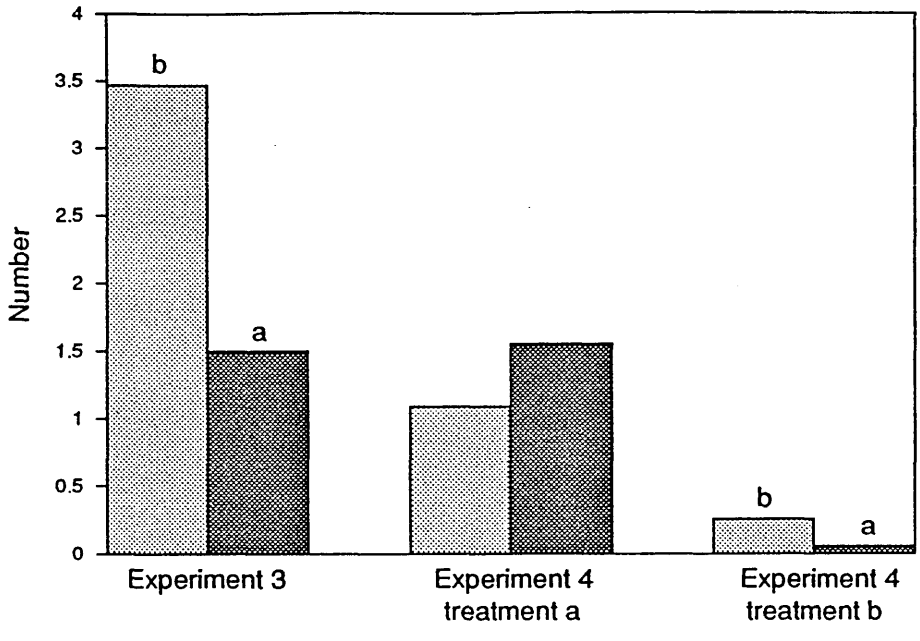
The mean daily ozone concentrations during the fumigation in treatments a and b were very similar; between 48 and 60 ppb in treatment a, and between 48 and 57 ppb in treatment b. However, in treatment a plants were accidentally fumigated with > 70 ppb on the fifth day of the fumigation (Figure 2.3). In treatment b, ozone concentrations did not exceed 70 ppb. The accidental fumigation of plants in treatment a with > 70 ppb ozone may explain the increased ozone injury observed in this treatment.

4.6 Plant growth

4.6.1 Intermediate harvests

There was no intermediate harvest in experiment 3. In experiments 2a, 2b and 4b there was one intermediate harvest at the end of the ozone fumigation (and therefore at the end of the first week of water stress).

Figure 2.7. The effect of water stress on ozone injury (experiments 3 and 4).



Plotted values represent the mean number of injured leaves on well watered (▭) and water stressed (▩) plants, assessed one week after ozone fumigation. Within each experiment entries not headed by the same letter are significantly different at $p<0.05$.

In experiments 1 and 4a there were two intermediate harvests. The first was made at the end of the ozone fumigation, before the water stress treatments began. There was no effect of ozone on plant growth at this stage in either experiment. The second intermediate harvest was made half way through the water stress episode, after one week of water stress, and is equivalent (in terms of length of time since beginning of experiment) to the final harvest in experiments 2, 3 and 4b.
In general any significant effects of ozone or water stress seen at these intermediate harvests were then seen at the final harvest. All exceptions to this rule are discussed below.

4.6.2 Final harvests

The effects of ozone and water stress on plant growth are summarised in Tables 2.9, 2.10 and 2.11.

4.6.2.1 The effect of water stress

The effects of water stress on plant growth in all experiments are summarised in Figures 2.8 and 2.9.

Water stress reduced total plant dry weight by 14 % in experiment 1; by 25 % in experiment 2a; by 27 % in experiment 2b; and by 14% in experiment 4. Water stress had no effect on total plant dry weight in experiment 3.

The effect of water stress on total plant dry weight was largely the result of a decrease in shoot growth in water stressed plants. Water stress significantly reduced the total number of leaves, live leaf dry weight and area in experiments 1, 2a, 2b, and 4, but had no effect on live leaf growth in experiment 3. The reduction in leaf dry weight in water stressed plants was accompanied by an even greater reduction in leaf area, and thus a reduction in the leaf area ratio and specific leaf area ratio of water stressed plants (Figure 2.9).

Root dry weight was not affected by water stress in experiments 1, 2b, 3 and 4, and was increased by water stress in experiment 2a. The increase in root dry weight and/or reduction in above ground growth in water stressed plants in experiments 1, 2b and 2a resulted in these plants having a significantly greater root:shoot ratio than the fully watered plants (Figure 2.8).

In experiment 4, unlike the other experiments, the effect of one week of water stress on plant growth was very different to the effect of two weeks. At the intermediate harvest

Tables 2.9, 2.10 and 2.11
The effect of ozone and water stress on plant growth at the final harvest.
Summary of Analysis of Variance

Table 2.9. Experiments 1 and 3.

Table 2.10. Experiments 2a and 2b.

Table 2.11. Experiment 4.

Tabulated values are variance (F) ratios. F ratios in bold type are significant at $p<0.05$. + indicates a significant increase in ozone (O_3) or water stress (WS), and - indicates a significant decrease. * represents a significant ozone/water stress interaction (O_3*WS).

In experiments 2a, 2b, 3 and 4, initial leaf number (counted one day before the treatments began) was used as a covariate in the analysis. No covariates were used in the analysis of experiment 1.

Dead leaf dry weight was not measured in experiment 1.

Table 2.9

Parameter	Experiment 1			Experiment 3		
	O_3	WS	O_3*WS	O_3	WS	O_3*WS
Total dry weight	0.13	4.10 -	1.17	0.01	0.16	0.28
Above ground dry weight	0.25	10.32 -	1.21	0.04	0.37	0.04
Root dry weight	0.21	0.01	1.22	0.11	0.07	1.22
Root:shoot ratio	0.70	4.80 +	0.21	0.27	0.65	0.87
Live leaf dry weight	0.01	13.56 -	1.67	1.28	0.90	1.28
Dead leaf dry weight				24.76 +	8.13 +	20.87 *
Total leaf number	0.91	15.23 -	0.05	1.76	0.30	1.24
Leaf area	0.01	106.7 -	2.75	1.03	0.51	5.97 *
Leaf area ratio	0.15	143.6 -	0.76	1.69	0.43	3.58
Specific leaf area ratio	0.01	135.1 -	0.29	1.17	1.10	3.12
Stem dry weight	0.91	5.31 -	0.32	0.05	0.65	0.01

Table 2.10

Parameter	Experiment 2a			Experiment 2b		
	O_3	WS	O_3*WS	O_3	WS	O_3*WS
Total dry weight	0.24	40.14 -	0.01	0.06	44.12 -	3.08
Above ground dry weight	0.25	93.29 -	0.01	0.06	101.9 -	2.86
Root dry weight	0.01	6.05 +	0.07	0.99	1.05	2.67
Root: shoot ratio	0.31	123.4 +	0.08	2.39	203.4 +	0.01
Live leaf dry weight	0.09	77.99 -	0.17	1.16	86.65 -	2.25
Dead leaf dry weight	0.35	13.16 +	1.35	0.08	17.75 +	0.85
Total leaf number	0.16	129.0 -	0.23	1.19	108.7 -	0.19
Leaf area	0.03	36.39 -	0.10	4.44 +	111.5 -	1.92
Leaf area ratio	0.03	21.25 -	0.21	8.69 +	74.54 -	0.01
Specific leaf area ratio	0.96	6.56 -	1.30	1.01	33.60 -	4.15
Stem dry weight	0.57	57.74 -	1.10	0.04	70.49 -	2.89

Table 2.11

Experiment 4

Parameter	O_3	WS	O_3*WS
Total dry weight	**6.24** +	**7.24** -	**3.59** *
Above ground dry weight	**9.27** +	**9.55** -	**5.52** *
Root dry weight	1.22	1.77	0.50
Root: shoot ratio	0.56	0.06	0.49
Live leaf dry weight	**4.10** +	**15.41** -	**3.64** *
Dead leaf dry weight	0.05	0.99	0.05
Total leaf number	1.23	**10.49** -	0.91
Leaf area	1.10	**39.83** -	2.51
Leaf area ratio	0.91	**17.99** -	0.02
Specific leaf area ratio	4.04	**13.15** -	1.52
Stem dry weight	**3.40** +	**4.67** -	1.18

Figure 2.8. The effect of water stress on total plant dry weight and root:shoot ratio.

Plotted values represent the mean total plant dry weight (A) and root:shoot ratio (B) of well watered (▭) and water stressed plants (▩), averaged across ozone fumigated and control treatments. Within each experiment entries not headed by the same letter are significantly different at $p<0.05$.

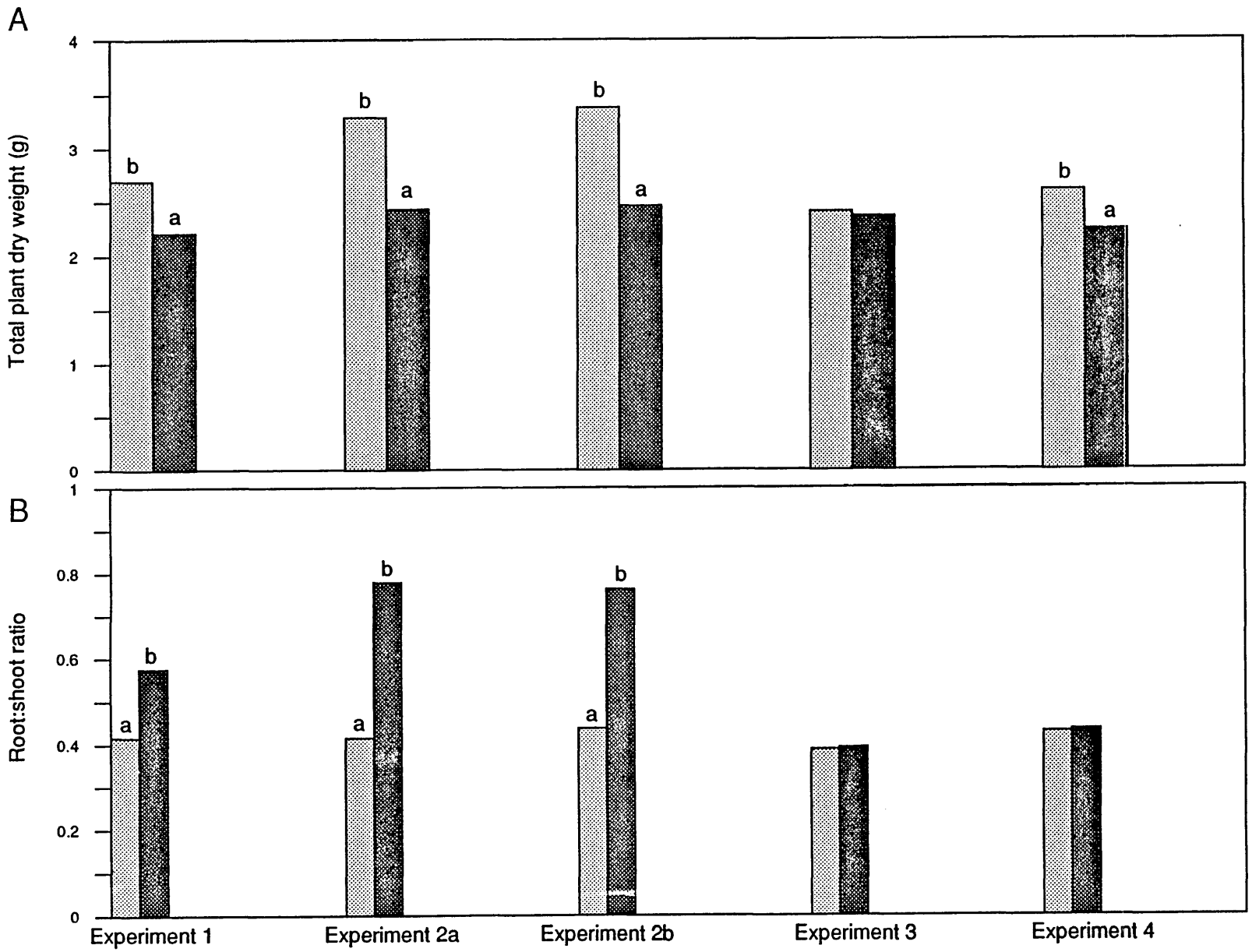
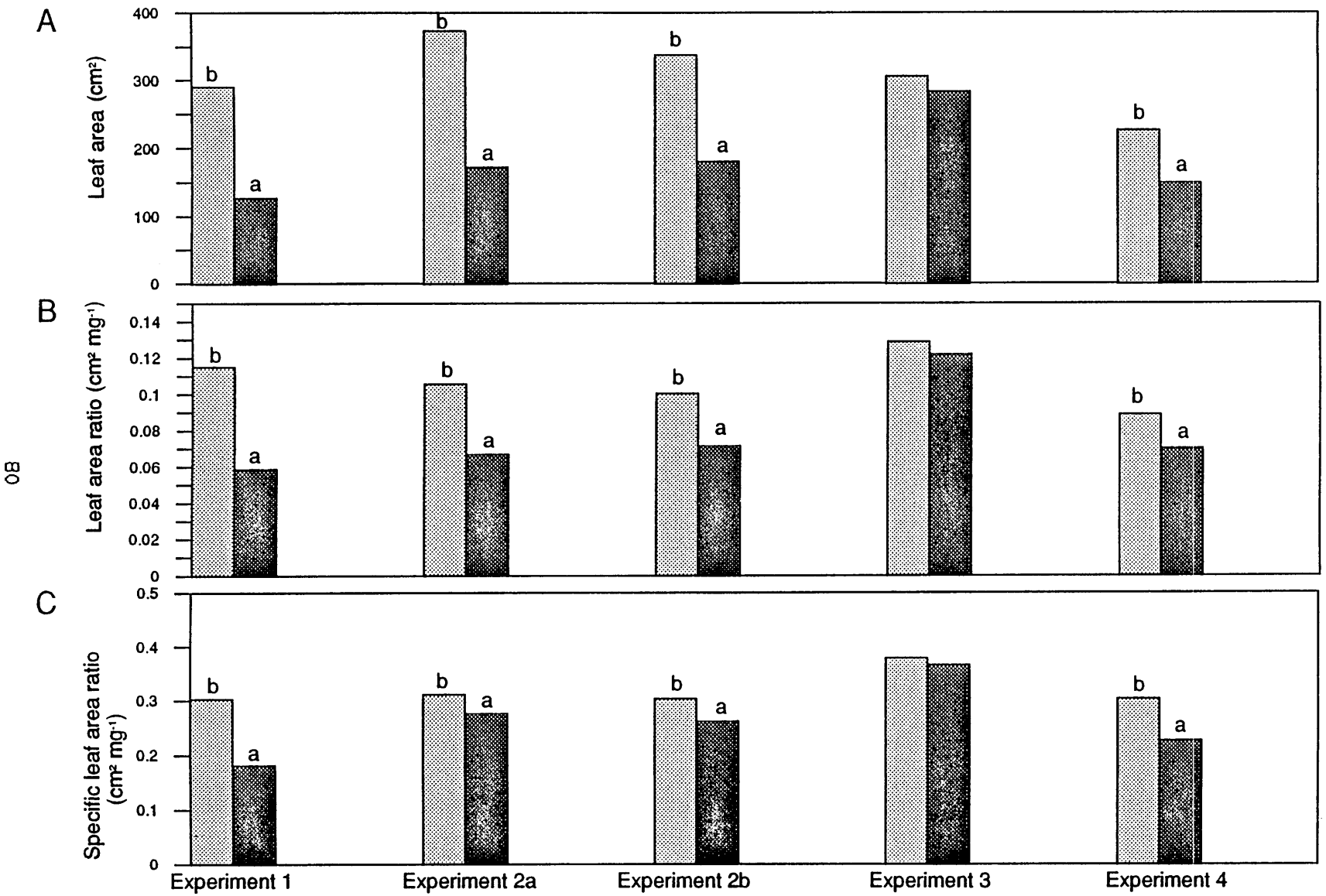

A
Total plant dry weight (g)
4
3
2
1
0
b
a
b
a
b
a
b
a
b
a
B
Root:shoot ratio
1
0.8
0.6
0.4
0.2
0
a
b
a
b
a
b
Experiment 1
Experiment 2a
Experiment 2b
Experiment 3
Experiment 4

Figure 2.9. The effect of water stress on leaf area, leaf area ratio, and specific leaf area ratio.

Plotted values represent the mean leaf area (A), leaf area ratio (B), and specific leaf area ratio (C) of well watered (▒) and water stressed (▓) plants, averaged across ozone fumigated and control treatments. Within each experiment entries not headed by the same letter are significantly different at $p<0.05$.


A
Leaf area (cm²)
400
300
200
100
0
B
Leaf area ratio (cm² mg⁻¹)
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
C
Specific leaf area ratio (cm² mg⁻¹)
0.5
0.4
0.3
0.2
0.1
0
b
a
Experiment 1
Experiment 2a
Experiment 2b
Experiment 3
Experiment 4

one week earlier, above ground growth was not affected by water stress. The root dry weight of water stressed plants was increased resulting in an increase (of 18%) in total plant dry weight, and an increase in the root:shoot ratio.

The only significant effect of water stress alone in experiment 3 was to increase the dry weight of dead leaves by more than 200% compared to the control plants.

4.6.2.2 The effect of ozone and ozone/water stress interactions

This description of the effects of ozone on the growth of *Vicia faba* will begin with experiments 1 and 3, as these were the most simple experiments, in which the timing of exposure to ozone and water stress was explored. Their simplicity meant that the ozone exposure regime was rather unrealistic (continuous fumigation with ozone day and night) but despite this, these experiments provide a useful starting point for investigation of plant responses to sequential (experiment 1) and simultaneous (experiment 3) exposure to ozone and water stress.

The results of experiments 2a and 2b will then be discussed, in which plants were exposed to ozone and water stress simultaneously, and plant response to both continuous ozone fumigation (experiment 2a) and to more realistic daily peaks of ozone (experiment 2b) was investigated.

Finally the results of experiment 4 will be described. The results from experiments 1, 2 and 3 were used to design this experiment, in which plants were exposed to daily peaks of ozone, and the timing of exposure to the two stresses was both sequential (treatment a) and simultaneous (treatment b).

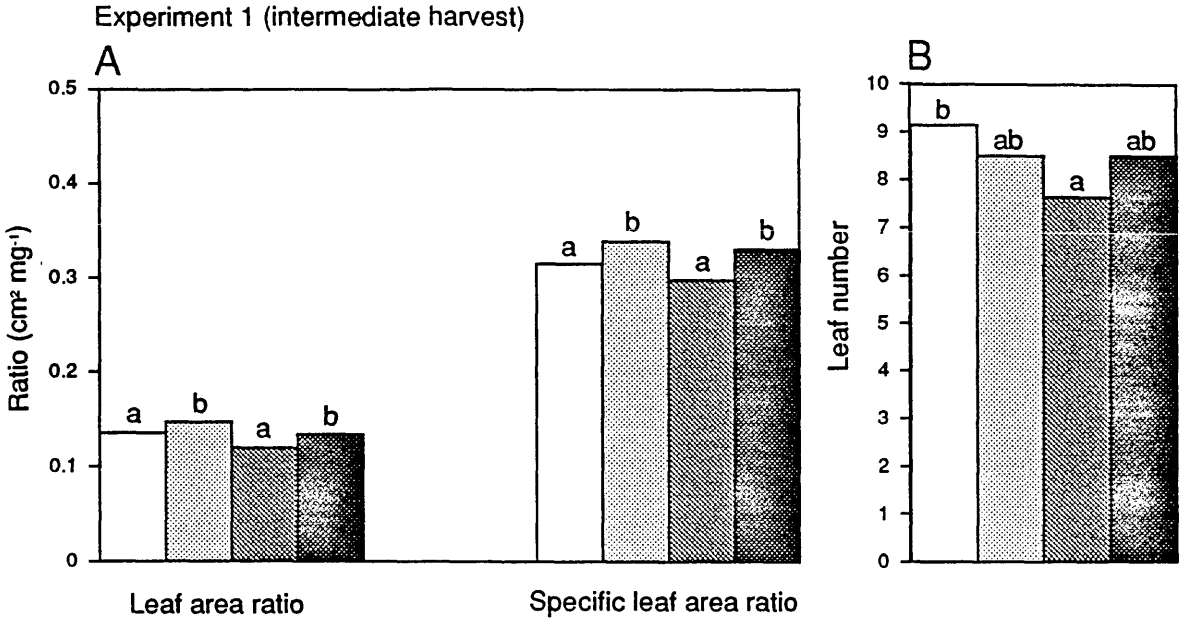
Experiments 1 and 3

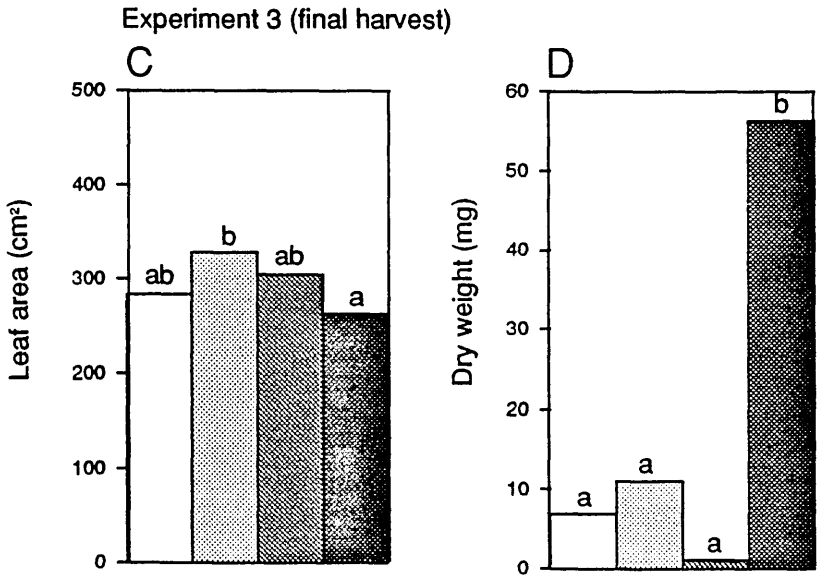
At the final harvest in experiment 1, there were no significant effects of ozone, or any significant ozone/water stress interactions on plant growth.

At the second intermediate harvest, one week after the end of the fumigation, there were significant increases in foliage growth in the ozone fumigated plants. These plants had an increased leaf area, resulting in significantly increased LAR and SLAR (Figure 2.10). Also, there was a significant ozone/water stress interaction on total leaf number. At this harvest water stress alone resulted in a significant reduction in leaf number. However, this reduction was only significant in the control plants; water stress had no effect on the number of leaves of plants that had been exposed to ozone (Figure 2.10). These effects of ozone were no longer significant at the end of the experiment.

Figure 2.10. The effect of ozone and water stress on plant growth (experiments 1 and 3).

Graphs represent the leaf area ratio and specific leaf area ratio (A) and the number of leaves (B) on plants at the intermediate harvest in experiment 1, and the leaf area (C) and weight of dead leaves (D) of plants at the final harvest in experiment 3. Plotted values represent means for well watered control plants ( ), well watered ozone fumigated plants ( ), water stressed control plants ( ), and water stressed ozone fumigated plants ( ). Within each graph entries not headed by the same letter are significantly different at $p<0.05$.


Experiment 1 (intermediate harvest)
A
B
Ratio (cm² mg⁻¹)
Leaf number
Leaf area ratio
Specific leaf area ratio


Experiment 3 (final harvest)
C
D
Leaf area (cm²)
Dry weight (mg)

Likewise, in experiment 3, the only effect of ozone was on leaf tissue (Figure 2.10). Ozone and water stress individually both increased the weight of dead leaves in this experiment; dead leaf dry weight was increased by 93% in ozone treated plants compared to those in filtered air. Also there was a significant interaction between the two stresses on this parameter; the weight of dead leaves was increased fivefold by water stress in ozone treated plants. There were no significant effects of ozone or water stress on live leaf dry weight, but there was a significant ozone/water stress interaction on live leaf area. Live leaf area was decreased by water stress in ozone treated plants, but not in control plants. Neither ozone nor water stress alone had a significant effect on leaf area.

Experiments 2a and 2b

Ozone had no effect on plant growth in experiment 2a. There were no significant ozone/water stress interactions on plant growth in either experiment 2a or b.

In experiment 2b, ozone significantly increased leaf area. The total leaf area of plants that had been exposed to ozone was 14% greater than that of the control plants. This increase in leaf area resulted in a significant increase in the leaf area ratio of ozone treated plants (Figure 2.11).

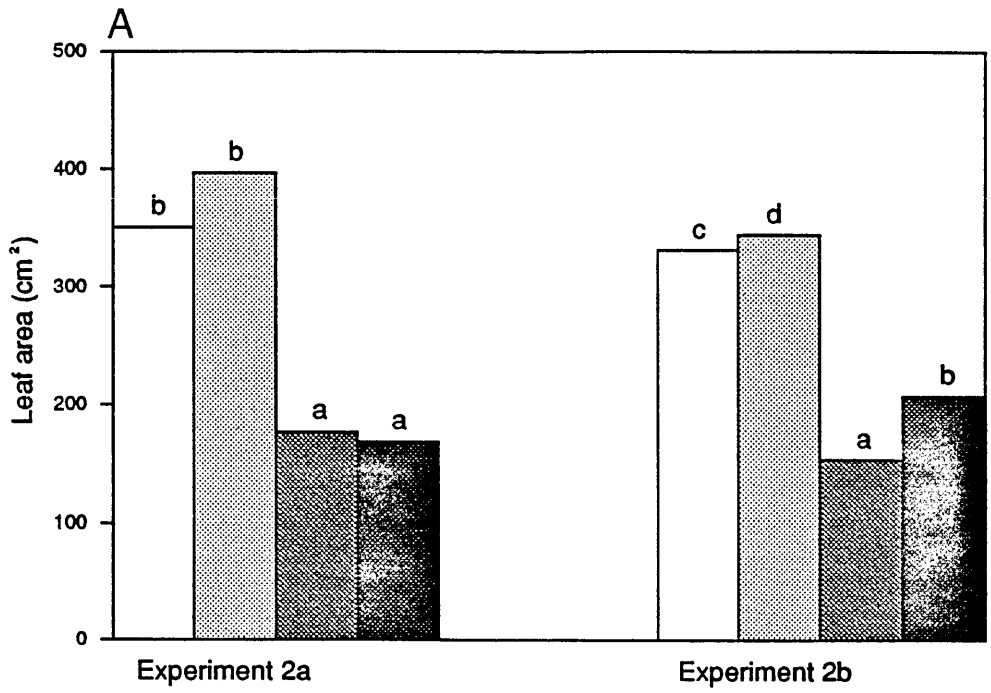
Experiment 4

In experiment 4, the final harvest in both treatments was after two weeks of water stress. In treatment a this was after a three week experiment, while in treatment b, this was after a two week experiment.

This was the only experiment in which total plant dry weight was affected by ozone. In both treatments total plant dry weight was significantly greater in the plants exposed to ozone compared to the controls (Figure 2.12). This increase was the result of a significant stimulation of above ground growth by ozone; leaf and stem dry weight were both significantly greater in the ozone treated plants in treatment B, and stem dry weight was significantly greater in the ozone treated plants in treatment A. There were also significant ozone/water stress interactions on plant growth in both treatments; ozone-induced increases in above ground and total plant dry weight were seen in fully watered plants only; there was no significant effect of ozone on the growth of water stressed plants.

Figure 2.11. The effect of ozone and water stress on leaf area and leaf area ratio (experiments 2a and 2b).

Plotted values represent the mean leaf area (A) and leaf area ratio (B) of plants in experiments 2a and 2b. Key as for Figure 2.10. Within each graph entries not headed by the same letter are significantly different at $p<0.05$.


A
500
400
300
200
100
0
Leaf area (cm²)
b
b
a
a
c
d
a
b
Experiment 2a
Experiment 2b

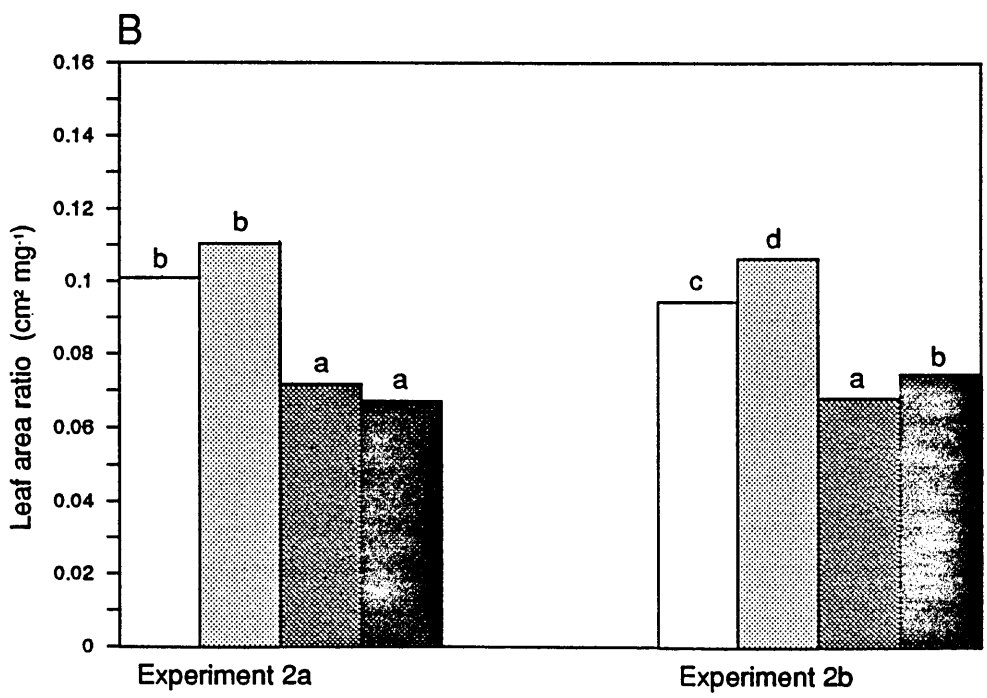
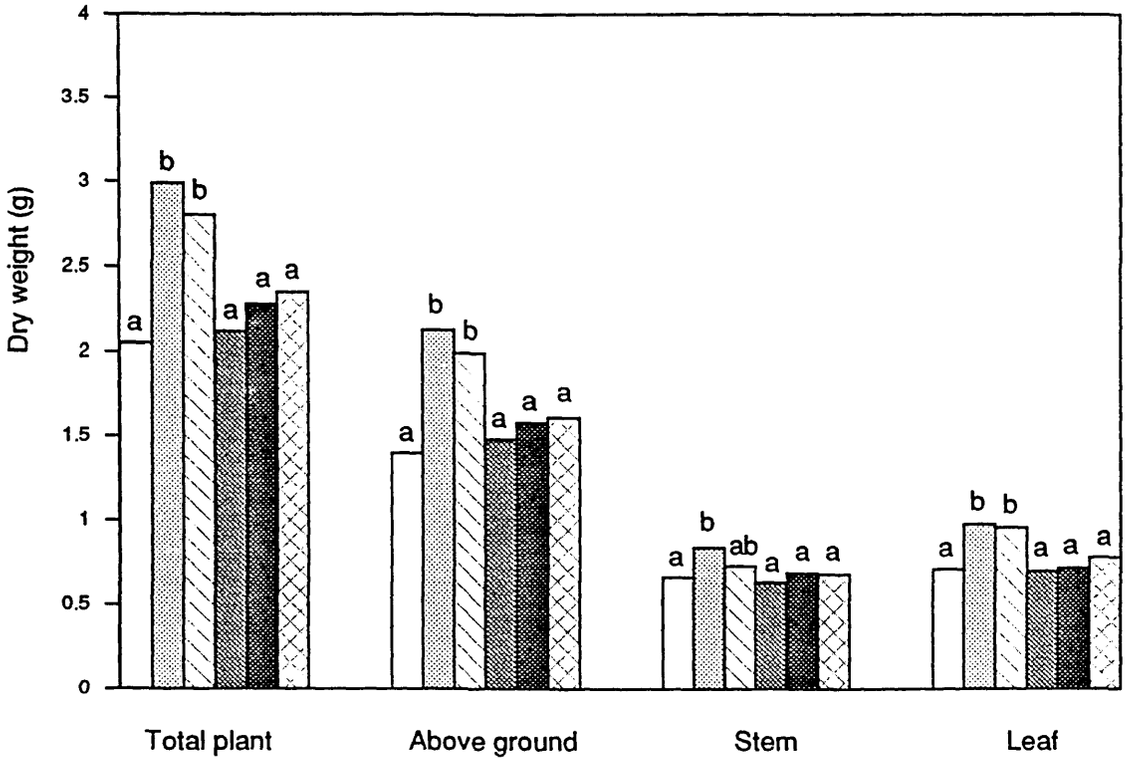

B
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Leaf area ratio (cm² mg⁻¹)
b
b
a
a
c
d
a
b
Experiment 2a
Experiment 2b

Figure 2.12. The effect of ozone and water stress on plant growth (experiment 4)



Plotted values represent mean total plant, above ground, stem amd leaf dry weight of well watered control plants ( ), well watered plants in treatment a ( ), well watered plants in treatment b ( ), water stressed control plants ( ), water stressed plants in treatment a ( ), and water stressed plants in treatment b ( ). Within each parameter entries not headed by the same letter are significantly different at $p<0.05$.

In the intermediate harvest, one week earlier, the number of leaves and leaf dry weight of plants in treatment a (but not treatment b) was significantly increased by ozone, but there were no significant ozone/water stress interactions. There was no effect of ozone on leaf number in either treatment a or b at the end of the experiment.

4.7 Gas exchange

4.7.1 Leaf conductance

Leaf conductance was measured in all experiments, by porometry in experiments 1, 2 and 3, and using the LCA-2 in experiment 4.

Water stress significantly reduced conductance in all experiments. Ozone significantly increased conductance in all experiments except experiment 2a, in which the effect of ozone was not significant. There was variation between experiments in the timing of increased conductance in relation to ozone exposure, and in the effect of water stress in modifying the response to ozone. These effects are discussed in detail below.

The effects of ozone and water stress on leaf conductance are summarised in Tables 2.12, 2.13 and 2.14.

4.7.1.1 The effect of water stress

Water stress resulted in considerable reductions in leaf conductance in all experiments. Water stress significantly reduced conductance from the seventh day of water stress onwards in experiment 1, from the sixth day of water stress onwards in experiment 2a and b, and from the third day onwards in experiment 4. Leaf conductance was measured on the fifth and eleventh days of the water stress episode in experiment 3, and was significantly reduced throughout the day (0700 to 1700) on both of these days. In experiment 1, from day 17 (the tenth day of water stress) onwards, severe soil water deficits resulted in almost total stomatal closure in some water stressed plants, making accurate determination of conductance impossible. Thus for days 17 and 21 of this experiment, the data for fully watered plants only are presented.

4.7.1.2 The effect of ozone and ozone/water stress interactions

Experiments 1 and 3

In experiments 1 and 3, leaf conductance was significantly increased by ozone. Water stress modified this response to ozone in experiment 3, but not in experiment 1.

Table 2.12

The effect of Ozone and Water stress on Leaf Conductance

Summary of Analysis of Variance

Experiments 1 and 3

Experiment	Day	0_3	WS	0_3*WS
1	4	0.70	0.05	1.41
	7	1.54	0.50	0.41
	11	**8.10** +	0.06	1.69
	14	**4.19** +	**18.53** -	0.01
	17	**4.39** +	n/a	n/a
	21	2.41	n/a	n/a
3	5	0.56	**20.21** -	1.37
	11	**11.56** +	**71.57** -	**13.44** *

Key as for Tables 2.9-2.11.

In experiment 1 the initial measurements of leaf conductance were used as covariates in the analysis. No covariates were used in experiment 3.

In experiment 1 the ozone fumigation was from day 1 to day 7 (inclusive) and plants were exposed to water stress from day 8 to day 21 inclusive. In experiment 3 the ozone fumigation was from day 1 to day 5 (inclusive) and plants were exposed to water stress from day 1 to day 11 inclusive.

The tabulated data for experiment 3 was that measured at 1000. For days 17 and 21 of experiment 1 data are presented for fully watered plants only.

Table 2.13

The effect of Ozone and Water stress on Leaf Conductance

Summary of Analysis of Variance

Experiments 2a and 2b

Experiment	Day	0_3	WS	0_3*WS
2a	2	2.79	0.76	0.73
	6	0.05	**88.82** -	0.01
	9	0.69	**91.55** -	0.01
	13	1.54	**43.19** -	0.12
2b	2	2.42	0.38	0.06
	6	**30.01** +	**170.7** -	**12.30** *
	9	0.51	**127.7** -	0.09
	13	0.02	**33.13** -	0.03

Key as for Tables 2.9-2.11.

The initial measurements of leaf conductance (made before the treatments began) were used as covariates in the analysis. In both experiments 2a and 2b the ozone fumigation was from day 1 to 6 inclusive, and plants were water stressed from day 1 to 13 inclusive.

Table 2.14

The effect of Ozone and Water stress on Leaf Conductance

Summary of Analysis of Variance

Experiment 4

Day	0_3	WS	0_3*WS
3	1.83	1.47	0.78
6	1.87	0.24	2.70
8	1.63	0.72	0.66
10	0.84	**5.96** -	0.54
13	0.18	**188.3** -	1.27
15	0.01	**164.8** -	0.31
17	0.04	**125.7** -	0.38
20	1.73	**78.98** -	**4.09** *

Key as for Tables 2.9-2.11.

The covariate used in the analysis was leaf age. Plants in treatment a were fumigated with ozone on days 1 to 7 inclusive, and in treatment b on days 8 to 15 inclusive. Plants were water stressed on days 8 to 20 inclusive. The data presented for day 20 are those obtained at the higher flow rate (500 ml min^{-1}).

In experiment 1 there were no ozone/water stress interactions; leaf conductance was increased by ozone in both fully watered and water stressed plants (Figure 2.13). These increases in response to ozone were not seen during the ozone fumigation, but were seen on the 4th, 7th and 10th days after the end of the ozone fumigation.

The significant increase in leaf conductance in experiment 3 was seen on one day only (day 11) and was restricted to the fully watered plants; there was no effect of ozone on the leaf conductance of water stressed plants (Figure 2.14). In this experiment leaf conductance was measured at 0700, 1000, 1400 and 1700. The leaf conductance of fully watered plants was increased by ozone throughout the day on day 11, but this increase was significant only at 1000 and 1700 (Figure 2.15).
As in experiment 1, leaf conductance was only increased by ozone after the ozone exposure; in this case the increase was seen six days after the end of the ozone fumigation.

Experiments 2a and 2b

There was no significant effect of ozone on leaf conductance in experiment 2a (Figure 2.16).

In experiment 2b ozone caused a significant increase in leaf conductance. As in experiment 3, this increase was seen on one day only and was restricted to the well watered plants; there was no effect of ozone on the leaf conductance of water stressed plants (Figure 2.17). The percentage increase in conductance in fully watered plants treated with ozone compared to fully watered controls was 70%. In experiment 2b, the significant ozone/water stress interaction was seen on the last day of the ozone fumigation.

Experiment 4

The results of experiments 1, 2 and 3 suggested that the incidence of ozone/water stress interactions on leaf conductance was dependant on the timing of exposure to the two stresses, and experiment 4 was set up to test this. Interactions were seen in experiments 2b and 3, in which plants were exposed to ozone and water stress simultaneously, but not

Figure 2.13. The effect of ozone and water stress on leaf conductance (experiment 1)

Plotted values represent the mean leaf conductance of plants in experiment 1. Leaf conductance is plotted against day of experiment. Key as for Figure 2.10. Within each day entries not headed by the same letter are significantly different at $p<0.05$.

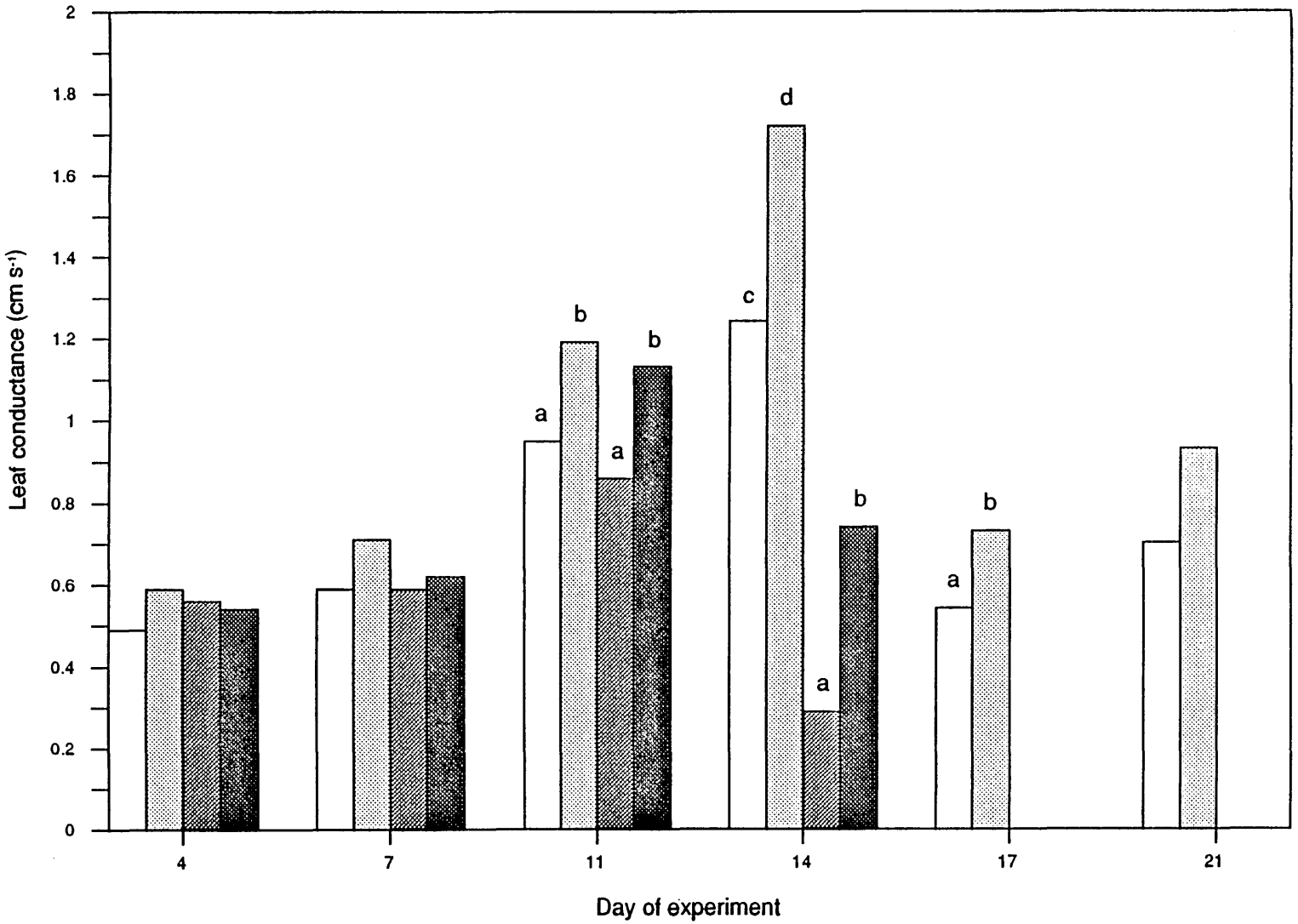

Leaf conductance (cm s-1)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
a
b
a
b
c
d
a
b
a
b
4
7
11
14
17
21
Day of experiment

Figure 2.14. The effect of ozone and water stress on leaf conductance (experiment 3)

Plotted values represent the mean leaf conductance of plants in experiment 3, measured at 1000. Leaf conductance is plotted against day of experiment. Key as for Figure 2.10. Within each day entries not headed by the same letter are significantly different at $p<0.05$.

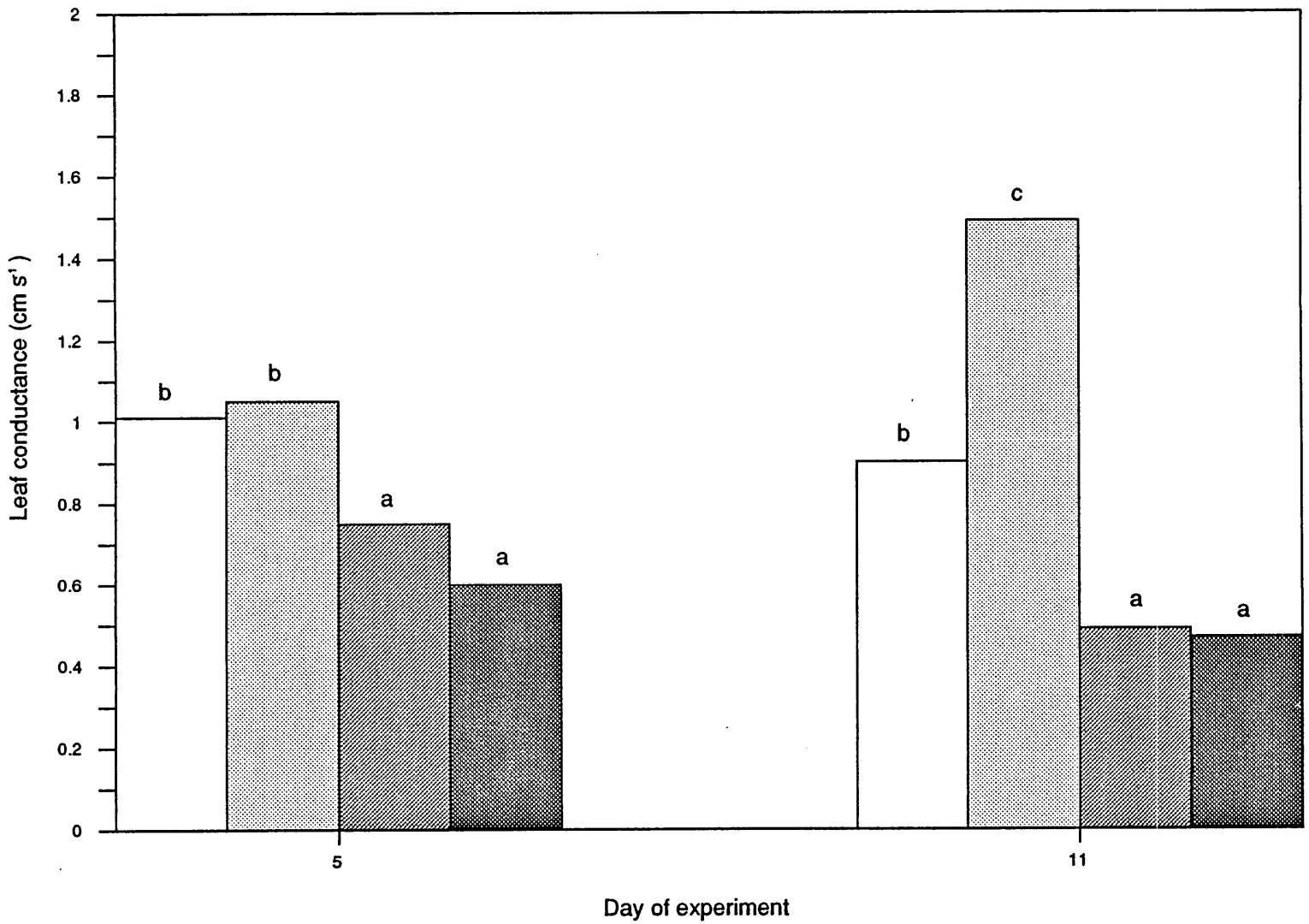
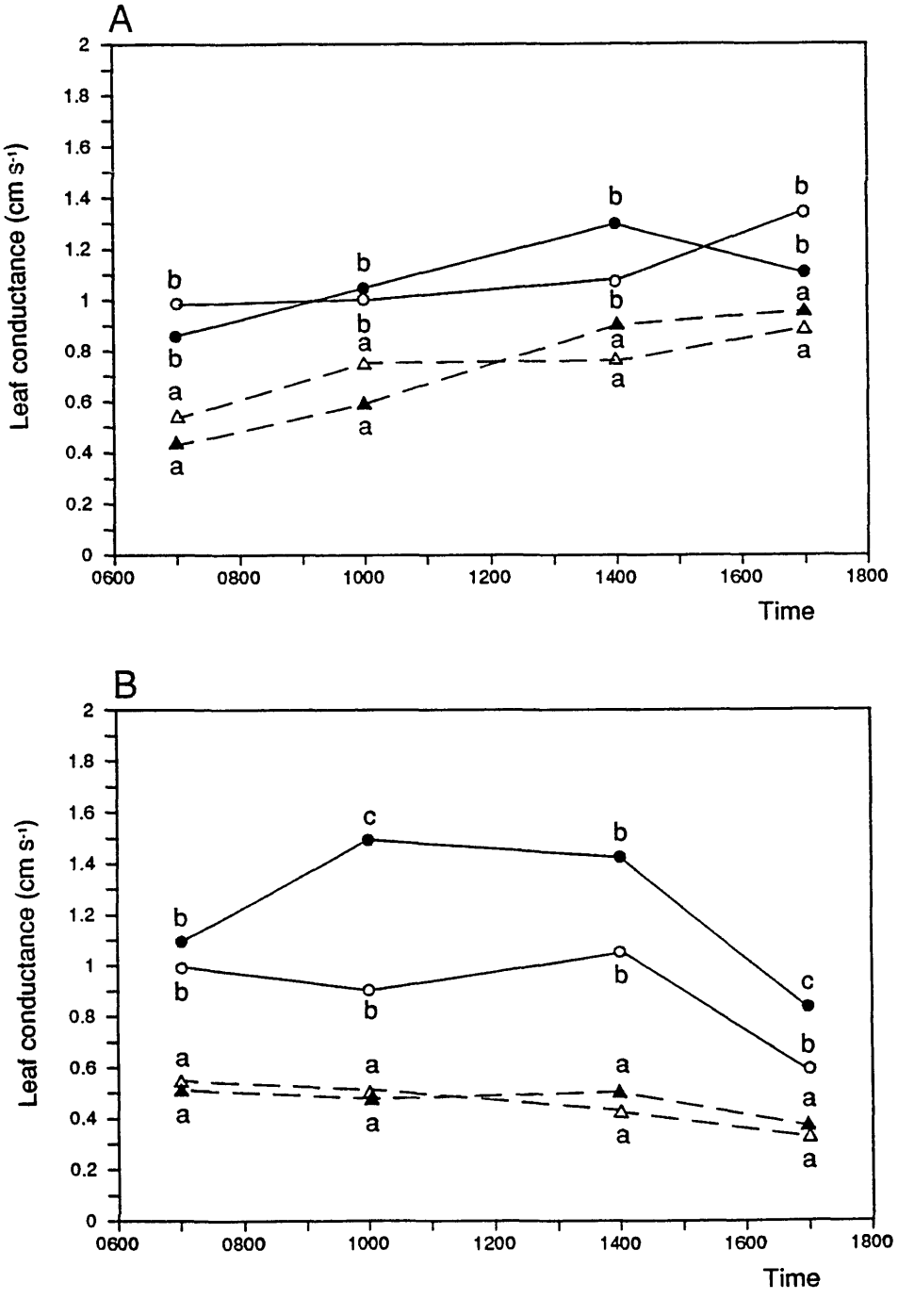

Leaf conductance (cm s⁻¹)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
b
b
a
a
b
c
a
a
5
11
Day of experiment

Figure 2.15. The effect of ozone and water stress on the daily time course of leaf conductance (experiment 3)



Leaf conductance is plotted against time of day on the fifth (A) and eleventh (B) day of the experiment. Plotted values represent means for well watered control plants (—○—), well watered ozone fumigated plants (—●—), water stressed control plants (-△-), and water stressed ozone fumigated plants (-▲-). At each time entries not headed by the same letter are significantly different at $p<0.05$.

Figure 2.16. The effect of ozone and water stress on leaf conductance (experiment 2a).

Plotted values represent the mean leaf conductance of plants in experiment 2a. Leaf conductance is plotted against day of experiment. Key as for Figure 2.10. Within each day entries not headed by the same letter are significantly different at $p<0.05$.

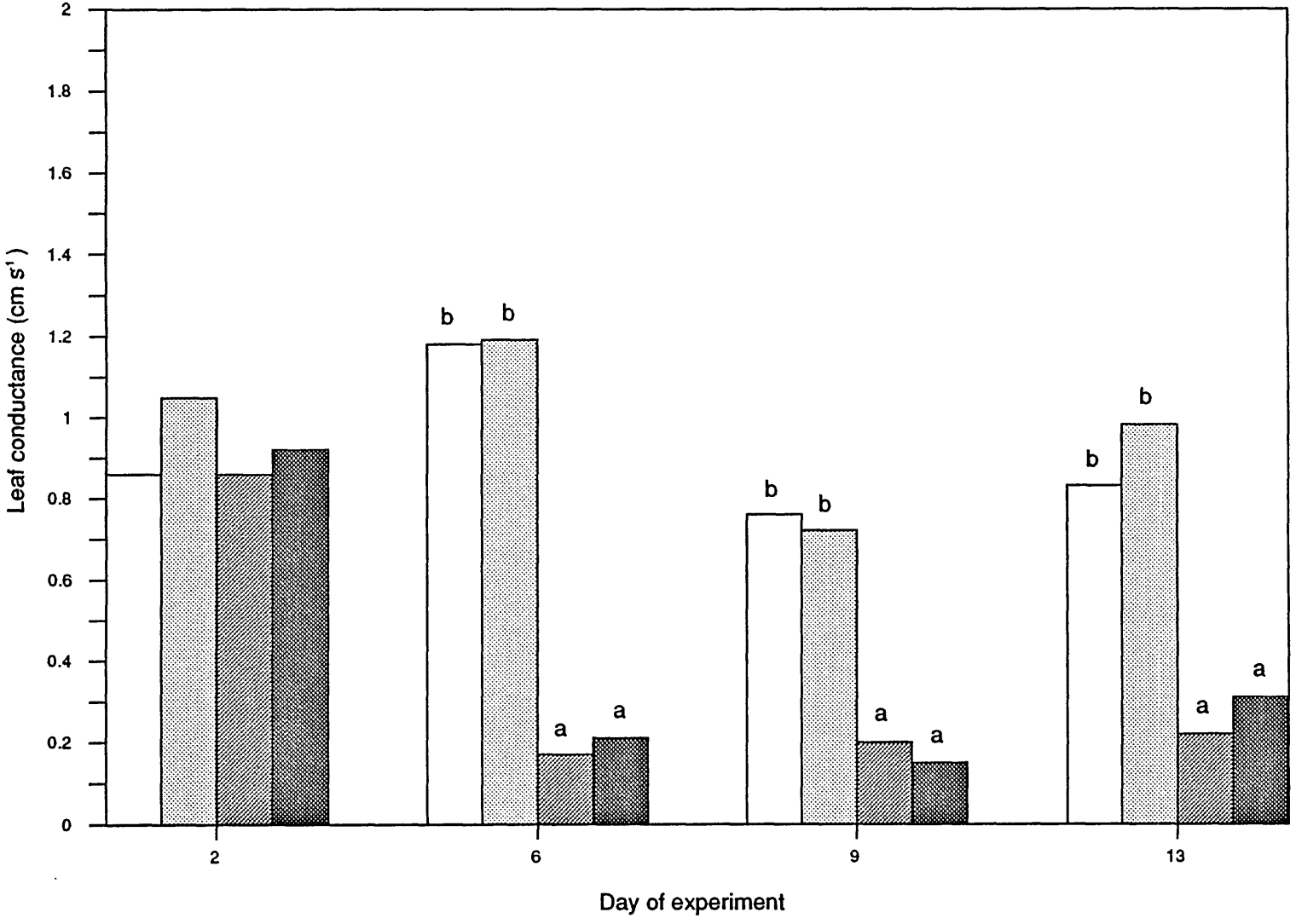
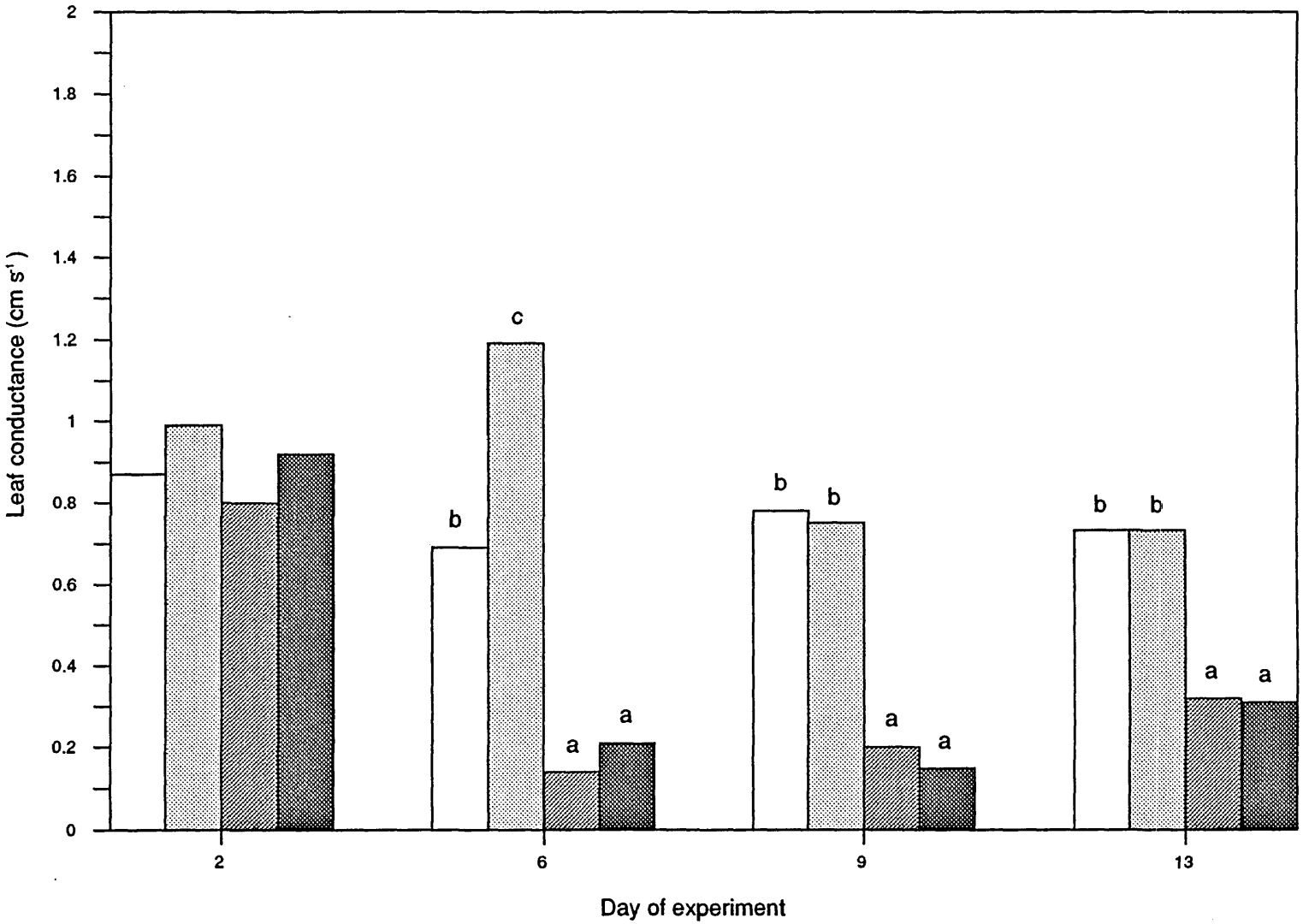

Leaf conductance (cm s⁻¹)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
b
b
a
a
b
b
a
a
b
b
a
a
2
6
9
13
Day of experiment

Figure 2.17. The effect of ozone and water stress on leaf conductance (experiment 2b).

Plotted values represent the mean leaf conductance of plants in experiment 2b. Leaf conductance is plotted against day of experiment. Key as for Figure 2.10. Within each day entries not headed by the same letter are significantly different at $p<0.05$.


Leaf conductance (cm s-1)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
b
c
a
a
b
b
a
a
b
b
a
a
2
6
9
13
Day of experiment

in experiment 1, in which exposure to ozone preceded exposure to water stress. In experiment 4 plants were exposed to ozone and water stress both simultaneously and sequentially.
However, in experiment 4, problems were encountered in obtaining a steady reading of leaf conductance using the LCA-2. These problems were thought to be due to humidification of the cuvette during measurements, and in an attempt to clarify this the last set of gas exchange measurements (day 20) were made with the flow rate to the cuvette increased from 300 ml min^{-1} to 500 ml min^{-1}. On these days measurements were also made (on the same plants) at the lower flow rate for comparison.

The only significant effect of ozone on leaf conductance was recorded at the higher flow rate on day 20. The parallel measurements made at the lower flow rate on the same day showed no significant effect of ozone on gas exchange, although the pattern of response to ozone and water stress was the same (Figure 2.18). It is possible therefore that ozone was altering gas exchange on other occasions during these experiments, and that these alterations were not detected due to the flow rate used.

Stomatal responses to ozone and water stress were the same in treatment a and b, i.e., the timing of exposure to the two stresses did not influence the stomatal response. Significant stomatal opening was seen in both treatments a and b, but was restricted to the fully watered plants; there was no affect of ozone on the leaf conductance of water stressed plants. This effect of ozone was seen on day 20, almost two weeks after the end of the ozone fumigation in treatment a, and 5 days after the end of the fumigation in treatment b (Figure 2.19).

4.7.2 Photosynthesis

In experiment 4 the LCA-2 portable IRGA was used to measure leaf conductance, net photosynthetic rate (P) and intercellular carbon dioxide (Ci) concentration. The effect of ozone and water stress on P and Ci is summarised in table 2.15. The equivalent leaf conductance data were summarised in table 2.14.

4.7.2.1 The effect of water stress

Water stressed plants had significantly reduced photosynthetic rates from the eighth day of water stress onwards in both treatments A and B. This followed a significant reduction in leaf conductance and Ci from the third day of water stress onwards (Figure 2.20).

Figure 2.18. The effect of ozone and water stress on leaf conductance (g_l), net photosynthetic rate (P) and intercellular CO_2 concentration (Ci) on day 20 of experiment 4.

On this day gas exchange measurements were made with a flow rate (from air supply unit to cuvette) of 300 and 500 ml min $^{-1}$. Plotted values represent the mean g_l, P and Ci at 500 ml min^{-1} (A) and 300 ml min-1 (B). Key as for Figure 2.12. Within each graph entries not headed by the same letter are significantly different at $p<0.05$.

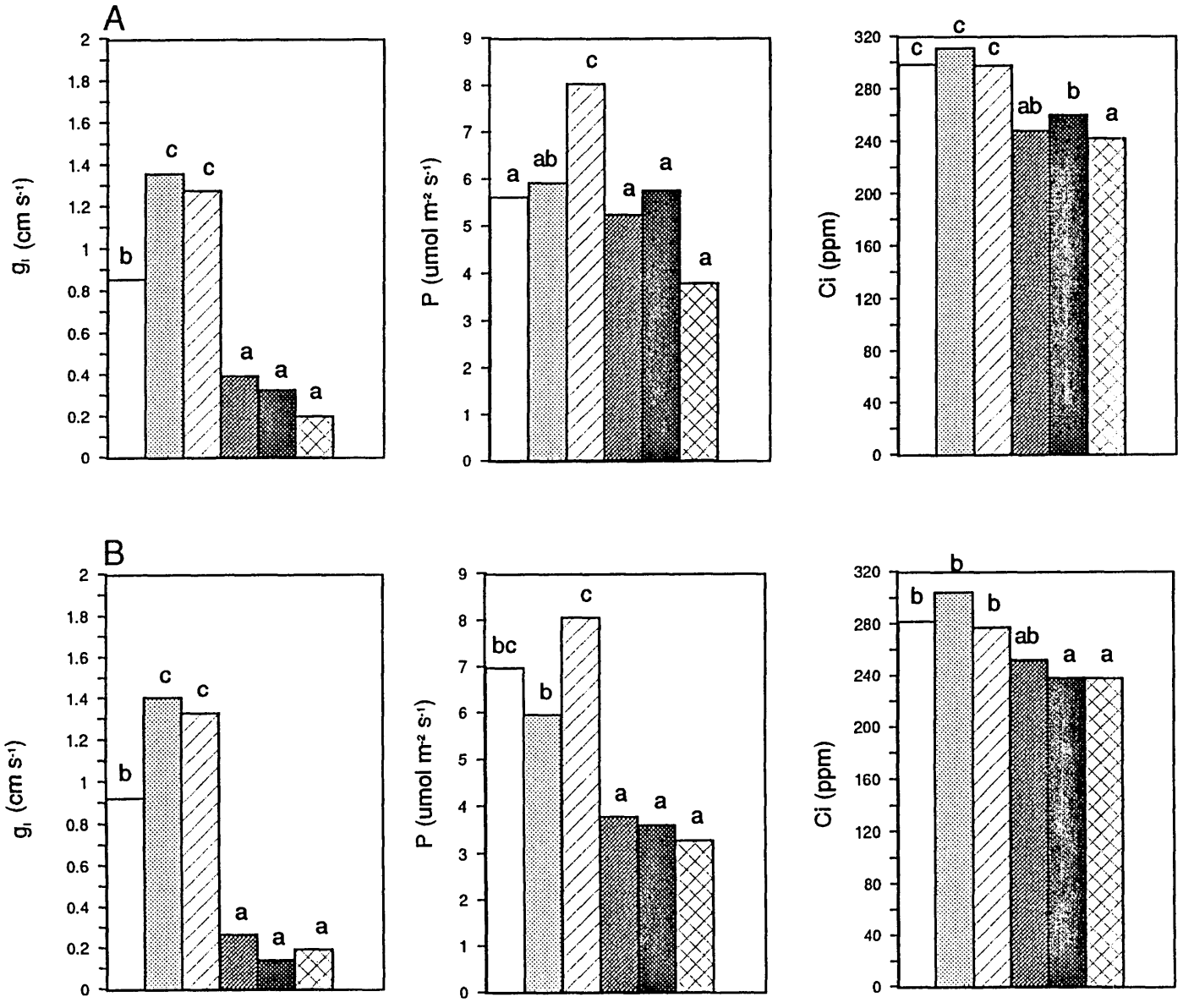

A
B
g_l (cm s^{-1})
P (umol m^{-2} s^{-1})
Ci (ppm)
b
c
c
a
a
a
a
ab
a
a
c
c
c
ab
b
a
bc
b
c
a
a
a
b
b
b
ab
a
a

Figure 2.19. The effect of ozone and water stress on leaf conductance (experiment 4)

Plotted vaues represent the mean leaf conductance of plants in experiment 4. Leaf conductance is plotted against day of experiment. Key as for Figure 2.12. For each day entries not headed by the same letter are significantly different at $p<0.05$.

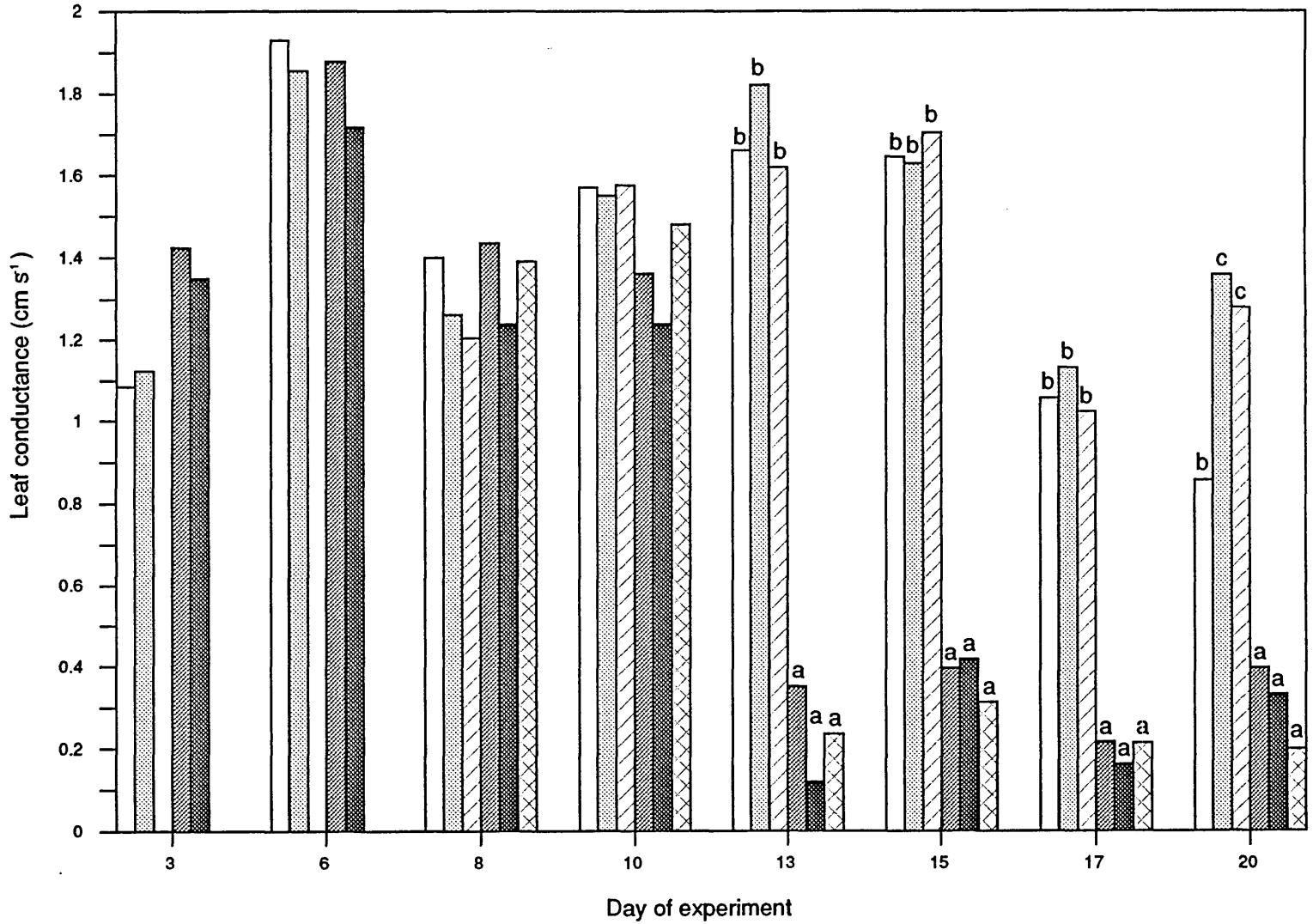

Leaf conductance (cm s-1)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
3
6
8
10
13
15
17
20
Day of experiment
b
b
b
b
a
a
a
b
b
b
b
a
a
a
b
b
b
a
a
a
b
c
c
a
a
a

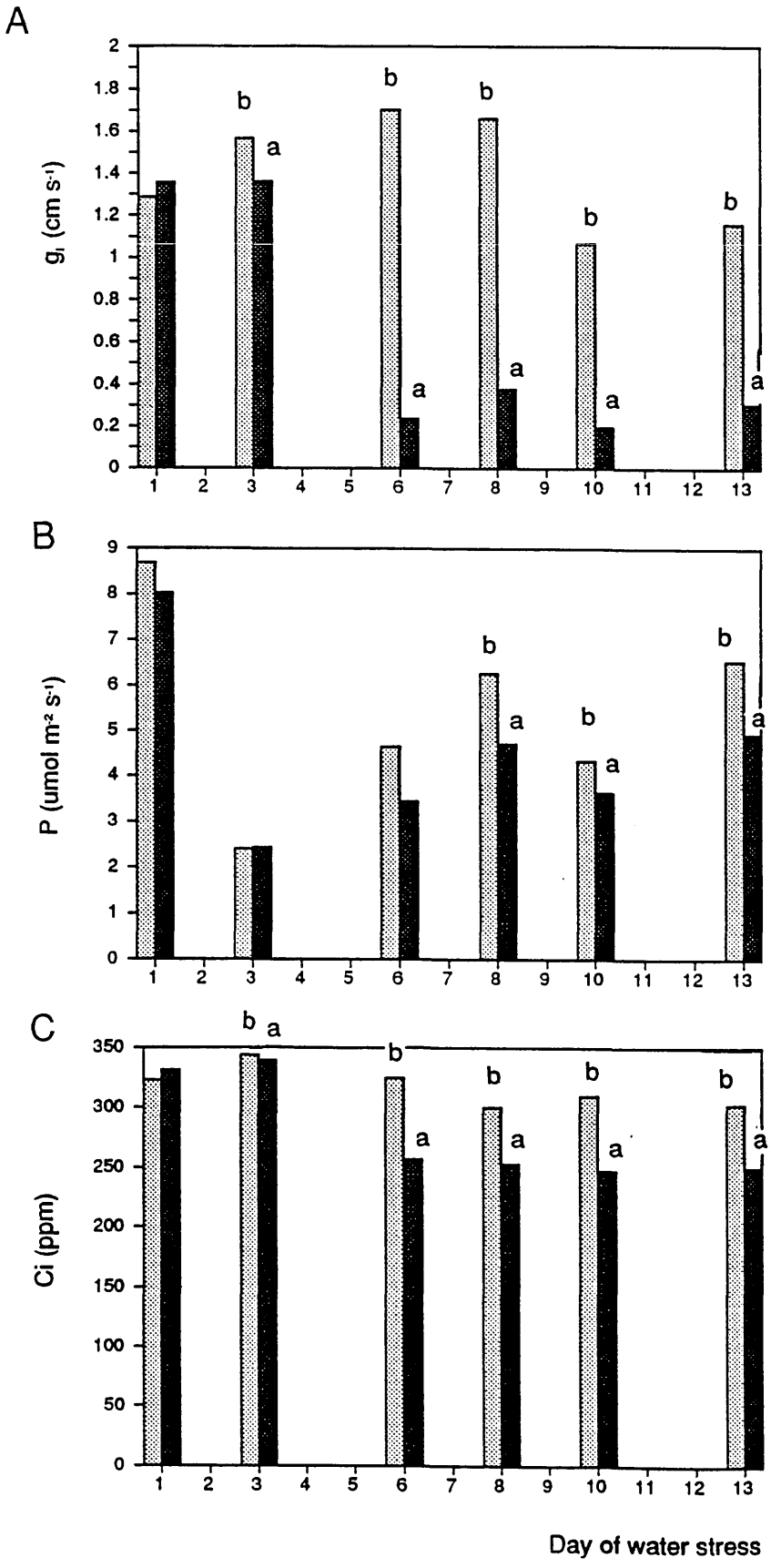
Table 2.15
The effect of Ozone and Water Stress on Gas Exchange
Experiment 4
Summary of Analysis of Variance

Day	Net photosynthesis			Intercellular CO_2 concentration		
	O_3	WS	O_3*WS	O_3	WS	O_3*WS
3	0.41	0.06	0.20	1.93	0.19	0.02
6	**3.82** -	0.13	0.41	0.34	1.92	1.32
8	0.22	1.70	0.80	0.31	2.53	1.78
10	0.35	0.01	0.18	2.17	**4.78** -	1.49
13	0.62	3.05	0.66	0.43	**76.01** -	2.04
15	1.26	**15.92** -	2.24	**4.53** #	**88.03** -	0.60
17	0.27	**6.47** -	1.03	0.60	**66.88** -	0.04
20	0.27	**7.69** -	**4.19** *	**4.12** #	**119.13** -	0.07

Tabulated values are variance (F) ratios. F ratios in bold type are significant at $p<0.05$. + indicates a significant increase in ozone (O_3) or water stress (WS), - indicates a significant decrease, and # indicates a significant increase and decrease. * represents a significant ozone/water stress interaction (O_3*WS). The covariates used in the analysis were leaf age and PAR.

Figure 2.20. The effect of water stress on leaf conductance (g_l), photosynthetic rate (P) and intercellular CO_2 concentration (Ci) in experiment 4.

g_l (A), P (B) and Ci (C) are plotted against day of water stress. Plotted values represent the mean of well watered (▒▒) and water stressed (▓▓) plants, averaged across ozone fumigated and control treatments. For each day entries not headed by the same letter are significantly different at $p<0.05$.

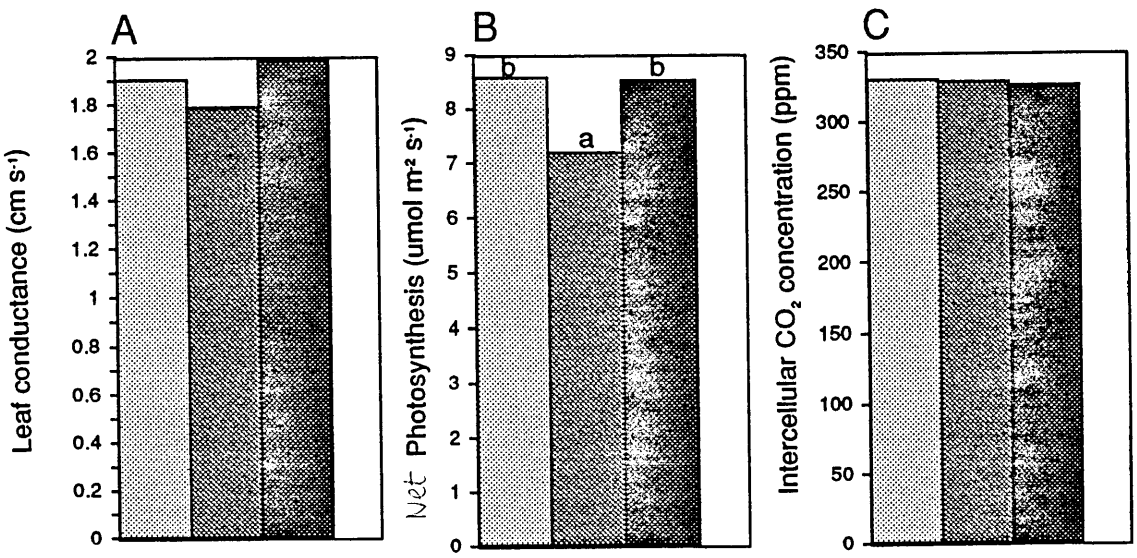

A
B
C
g_l (cm s^{-1})
P (umol m^{-2} s^{-1})
Ci (ppm)
Day of water stress

4.7.2.2 The effect of ozone and ozone/water stress interactions

On day 6 of the experiment there was a significant effect of ozone on P. This was before the plants in treatment b had been exposed to ozone, and before the onset of water stress. In treatment a, the effect of ozone was to reduce P of fumigated plants on one day during the ozone fumigation. This 16% inhibition of photosynthesis occurred on the sixth day of exposure to ozone and was not accompanied by any effect of ozone on leaf conductance or intercellular carbon dioxide concentration (Figure 2.21). There was no ozone-induced inhibition of photosynthesis on any day in treatment b.

In treatment b the only effect of ozone on photosynthetic rate was a significant ozone/water stress interaction on day 20, at the increased flow rate (Figure 2.18). This was the same day that the significant effect on ozone and water stress on leaf conductance was observed, and the nature of the effect was identical; fully watered plants which had been exposed to ozone had significantly increased photosynthetic rates compared to fully watered controls. There was no effect of ozone on the photosynthetic rate of water stressed plants.

Figure 2.21. The effect of ozone on leaf conductance (g_l), net photosynthetic rate (P) and intercellular CO_2 concentration (Ci) on day 6 of experiment 4.



Plotted values represent the mean g_l (A), P (B), and Ci (C), for well watered control plants ( ), well watered plants in treatment a ( ), well watered plants in treatment b ( ). Within each graph entries not headed by the same letter are significantly different at $p<0.05$.

5 Discussion

5.1 Ozone Fumigation

The duration of ozone episodes and the ozone concentrations administered in these experiments have frequently been exceeded during British summers. The duration of ozone exposures ranged between 5 and 8 days, and the overall mean ozone concentration of the fumigations ranged between 47 and 56 ppb. Monitoring data from a number of rural sites in the U.K. have revealed that the average daily ozone concentration exceeded 47 ppb for 7 or 8 consecutive days in three separate episodes in May, June and July 1989 (Warren Spring Laboratory 1989).

5.2 Water Stress

Withholding water from the soil is the most natural method of inducing water stress under greenhouse conditions but is also the most difficult to control (Krizek, 1985). The addition of a small quantity of water to a dry soil wets only a small soil volume; the remainder of the soil remains unwetted (Kramer, 1980) and therefore maintaining a uniform level of water deficit is extremely difficult. The aim of this experiment was to maintain a moderate level of soil moisture stress, but this was difficult to achieve and some wilting of lower leaves was observed in water stressed plants. The SWD used in these experiments is a convenient but crude measure of water stress. It describes only the bulk water content of the soil, and does not reveal this heterogeneity of soil water content. The water potential at the root surface is not known, since water is constantly being removed by the roots of transpiring plants (Krizek, 1985).

In very simple terms the movement of water through the soil/plant/atmosphere system occurs in response to a water potential gradient (Fitter & Hay, 1983). Therefore the soil water potential is a much more meaningful and useful measure of soil water stress than the SWD. Interestingly, in experiment 4 there was some suggestion in the soil water potential measurements, that exposure to ozone increased soil drying. In both treatments a and b, the soil water potential of water stressed treatments was consistently reduced by exposure to ozone, but this effect was not significant. There was no evidence of this effect in the SWD measurements made in this experiment.

5.3 Growth

Plant growth responses to water stress were fairly consistent, but more variation was seen in growth responses to ozone.

The effect of water stress on plant growth was generally to restrict foliage growth, reduce LAR and SLAR, and in some experiments to increase root dry weight and the root:shoot ratio. The reduction in total plant size resulting from soil water stress appeared to be more dependant on the duration of the stress than on the severity of the stress. The duration of soil water stress above an arbitrary threshold of severity (50% SWD) is closely related to the percentage reduction in total plant dry weight, but the maximum SWD is not (Table 2.16).

The most frequently observed effect of ozone on plant growth was to stimulate shoot growth; ozone fumigation had no effect on root growth in any experiment, and there was no significant effect of ozone on the root:shoot ratio. Ozone-induced increases in shoot growth in experiment 4 resulted in stimulations of total plant weight in both treatments a and b. This was the only experiment in which exposure to ozone altered total plant dry weight. Bennett *et al.* (1974) report that exposure of *Phaseolus vulgaris* to 30 ppb ozone resulted in significant increases in leaf and stem dry weight, compared to plants in filtered air. These authors also review several other reports of apparent increases in growth in response to fumigation with 20-50 ppb ozone, but many of these increases are not significant (at $p<0.05$).

There was considerable variation between these experiments in plant response to ozone and water stress. This is perhaps not surprising considering the differences between experiments in the timing of exposure to ozone and water stress, the severity of water stress, the ozone exposure regime, and the climatic conditions during each experiment. These differences between experiments are summarised in Table 2.17, for comparison with plant responses to ozone and water stress, which are summarised in Table 2.18.

There does appear to be some association between the ozone exposure regime and the growth response to ozone. In the two experiments in which plants were fumigated with daily peaks of ozone, increases in above ground growth were observed. The increase was in leaf area in experiment 2b, and shoot dry weight in experiment 4. There was no effect of ozone on plant growth at the final harvest in experiments 1, 2a and 3, in which fumigation with ozone was continuous. Likewise, observed growth stimulations in *P. vulgaris* followed daily, not continuous, fumigation with ozone (Bennett *et al.*, 1974).

The results of experiments 2a and 2b confirm that this difference between experiments in growth response to ozone was not the consequence of any other experimental conditions, since the only difference between these two experiments was in the ozone exposure regime, and increases in above ground growth were seen in experiment 2b, but not 2a.

Table 2.16

The Effect of Water Stess on Plant Growth

Reduction in Total Plant Dry Weight at the Final Harvest

Experiment	% reduction in dry weight	Duration of water stress	Maximum SWD (%)
3	2	6	79
1	14	8	85
4	14	8	55
2a	25	10	75
2b	27	10	75

"Duration of water stress" refers to the number of days when the soil water deficit (SWD) exceeded a threshold of 50 %. For example, the soil water deficit of water stressed treatments was greater than 50% for ten days preceding the final harvest in experiments 2a and 2b.

Table 2.17
Summary of experimental treatments and conditions

Tabulated values for air temperature (T_a) and relative humidity (RH) are means ± standard deviation, measured at 1200 and at 1400. The "percentage reduction due to WS" represents the percentage reduction (due to water stress) in total plant dry weight at the final harvest, and is included as an indication of water stress severity.

Table 2.18
Summary of the effect of ozone and water stress on leaf conductance and above-ground growth at the final harvest

+ indicates a significant increase in ozone (O_3) or water stress (WS), and - indicates a significant decrease. * represents a significant ozone/water stress interaction (O_3*WS) and ns indicates that there was no significant treatment effect.

Table 2.17

Exp.	Timing of exposure to O_3 and WS	Ozone exposure regime	Time of year	Mean midday T_a (°C)	Mean midday RH (%)	% reduction due to WS
1	sequential	continuous	February	17 ± 3	49 ± 7	14
2a	simultaneous	continuous	June	27 ± 5	25 ± 8	25
2b	simultaneous	daily peaks	June	27 ± 5	25 ± 8	27
3	simultaneous	continuous	September	21 ± 8	43 ± 7	2
4 a b	sequential simultaneous	daily peaks	March	25 ± 5	34 ± 9	14

Table 2.18

Exp.	Growth			Leaf conductance		
	WS	O_3	O_3*WS	WS	O_3	O_3*WS
1	-	ns	ns	-	+	ns
2a	-	ns	ns	-	ns	ns
2b	-	+	ns	-	+	*
3	ns	ns	*	-	+	*
4 a	-	+	*	-	ns	*
b	-	+	*	_	ns	*

Differences in growth response to daily and continuous fumigations may simply have been the result of differences in ozone dose. Daily peaks of ozone were administered between 0900 and 1700, whereas the fumigation greenhouse received additional lighting from 0600 to 2100, and so when fumigation was continuous ozone uptake may have occurred over most of this 15 hour period (depending on the day length, and therefore the time of year). Heagle, Heck *et al.* (1987) report that tobacco yield in plots receiving proportional (to ambient ozone concentrations) additional ozone for 12 hd^{-1} (1000 to 2200) was 10% less than in those receiving proportional addition for 7 hd^{-1} (1000 to 1700).

Neither the ozone exposure regime nor the timing of exposure to ozone and water stress can explain the differences between experiments in the occurrence of ozone/water stress interactions on plant growth. There were no ozone/water stress interactions on growth in experiments 1, 2a and 2b, but there were significant ozone/water stress interactions on shoot growth in experiment 4, and leaf area in experiment 3. In experiment 3 live leaf area was decreased by water stress in ozone treated plants, but not in control plants. It is probable that this interaction on leaf area was the consequence of increased death of leaves in plants exposed to both ozone and water stress in this experiment. Likewise, in experiment 4 above ground dry weight was decreased by water stress in ozone treated plants, but not in control plants. In experiment 4 this interaction was seen in plants exposed to ozone and water stress sequentially (treatment a) and simultaneously (treatment b).

There is some evidence to suggest that the severity of water stress was important in determining the occurrence of ozone/water stress interactions on growth. In Table 2.16 the reduction in plant dry weight at the final harvest is used as an indication of the severity of water stress. In experiments 3 and 4, in which ozone/water stress interactions on growth occurred, this reduction was relatively small. However, the fact that significant interactions were seen in only two experiments makes it difficult to assess the importance of water stress severity in determining the occurrence of interactions, especially as environmental conditions during each experiment were different. It is well known that plant sensitivity to air pollution is strongly influenced by environmental variables (Mclaughlin & Taylor, 1981), and environmental conditions will also be important in determining the severity of the water stress.

5.4 Gas Exchange

The most consistent effect of ozone in these experiments was to increase leaf conductance. This observation contradicts much of the published literature, which reports

that ozone causes a decrease in conductance. One explanation for this contradiction may be the relatively low ozone concentrations used in these experiments; Black (pers. comm.) found that ozone increased conductance of *Vicia faba* at low concentrations but decreased it at high concentrations.

Exposure of *Vicia faba* to ozone resulted in increased leaf conductance in all experiments except 2a. The increases were not seen on all days, and were observed several days after the ozone fumigation except in experiment 2b when conductance increased on the last day of the ozone exposure.

Increased leaf conductance is unlikely to have resulted from changes in cuticular transpiration, since ozone at low concentrations is not thought to alter the water permeability of plant cuticles (Kerstiens & Lendzian, 1989), and conductance measurements were generally made at mid day, when the cuticular component of leaf conductance would probably be low. Changes in stomatal density would be expected to alter leaf conductance. However, stomatal density was assessed in experiment 1 and no effect of ozone fumigation was found (Table 2.19). Furthermore, gas exchange measurements were always made on fully expanded leaves, and the stomatal density of these would not be expected to change greatly over the course of the experiment.

Ozone is known to accelerate leaf senescence, and evidence for this was provided by an increase in the weight of dead leaves in ozone fumigated plants in experiment 3. The same leaf was used for conductance measurements throughout experiments 1, 2 and 3, so it is possible that increased conductance in response to ozone in the later stages of these experiments was the consequence of accelerated senescence in ozone fumigated plants. However, the conductance of ozone fumigated plants did not increase as the experiment progressed, and in experiment 4 (in which the same leaf was not used for conductance measurements throughout the experiment), increased conductance was observed in young leaves at the end of the experiment.

Therefore, it appears most likely that increased leaf conductance was the result of stomatal opening in response to ozone.

Water stress significantly reduced stomatal conductance in all experiments. There was no clear threshold of SWD for stomatal closure.

Table 2.19

Mean values of adaxial and abaxial stomatal density

Measurements made at the final harvest in experiment 1

Leaf surface	Treatment			
	Well watered control	Well watered ozone	Water stressed control	Water stressed ozone
Adaxial	26	25	32	34
Abaxial	38	39	44	49

Tabulated values are mean number per field of view at magnification x 10.
10 replicate counts per area were made for each surface of 1 leaf of 14 plants per treatment. F ratios for water stress, ozone and the interaction term were 38.8, 1.07 and 2.64 respectively for the adaxial surface, and 21.29, 2.80 and 1.58 respectively for the abaxial surface. The effect of water stress was significant at $p<0.001$.

There was variation between experiments in the incidence and nature of ozone/water stress interactions on conductance. In experiments 2b, 3 and 4, exposure to ozone had no effect on the stomatal conductance of water stressed plants; ozone-induced increases in conductance were seen in the well watered plants only. Only in experiment 1 did exposure to ozone result in significantly increased conductance in both well watered and water stressed plants. In this experiment exposure to ozone preceded exposure to water stress, while in experiments 2b, 3 and treatment b of experiment 4, plants were water stressed during the ozone fumigation. These results suggest that the timing of exposure to ozone and water stress is important in determining the incidence of ozone/water stress interactions on conductance. Reduced pollutant uptake by water stressed plants may explain the ozone/water stress interactions following simultaneous exposure to ozone and water stress. However, in treatment a of experiment 4 plants were exposed to water stress after exposure to ozone, and a significant ozone/water stress interaction on conductance was observed.

Comparisons between the conductance data obtained with the LCA-2 in experiment 4, and those obtained with the porometer in experiments 1, 2 and 3 must be made with caution, especially as technical problems were encountered with the LCA-2. In a comparison of diffusive conductances obtained by porometer measurements and by calculation from gas exchange data, Black & Black (1979) report an extremely good agreement for *Vicia faba*. However, such an agreement is dependant on gas exchange measurements being made under constant conditions. This appeared not to be the case in the present measurements of conductance with the LCA-2, humidification of the cuvette appeared to occur during measurements, which was thought to lead to stomatal opening. Therefore the timing of exposure to ozone and water stress cannot be dismissed as an determinant in the incidence of ozone/water stress interactions on conductance.

There is some evidence to suggest that the severity of plant water stress also influenced the incidence of ozone/water stress interactions on conductance. Stomatal closure due to water stress was less in experiment 1, than in treatment a of experiment 4. In experiment 1, on the days that ozone increased conductance, the percentage reduction in conductance due to water stress was at most 65 %. In experiment 4, on the day that the ozone/water stress interaction on conductance occurred, the percentage reduction in conductance due to water stress was 75 %.

Environmental factors such as temperature and humidity are known to influence the effects of air pollutants on conductance (Winner *et al.*, 1988), and differences between experiments in environmental conditions during ozone exposure might explain some of

the variability in gas exchange responses to ozone and water stress. *Vicia faba* exhibits direct stomatal responses to humidity, and Black & Unsworth (1980) have demonstrated a humidity-SO_2 interaction on the stomata of this species. They observed that when the vapour pressure deficit (vpd) was low, and stomata were open, exposure to SO_2 induced rapid and irreversible increases in stomatal conductance, but at high vpd, stomatal conductance decreased with exposure to SO_2.

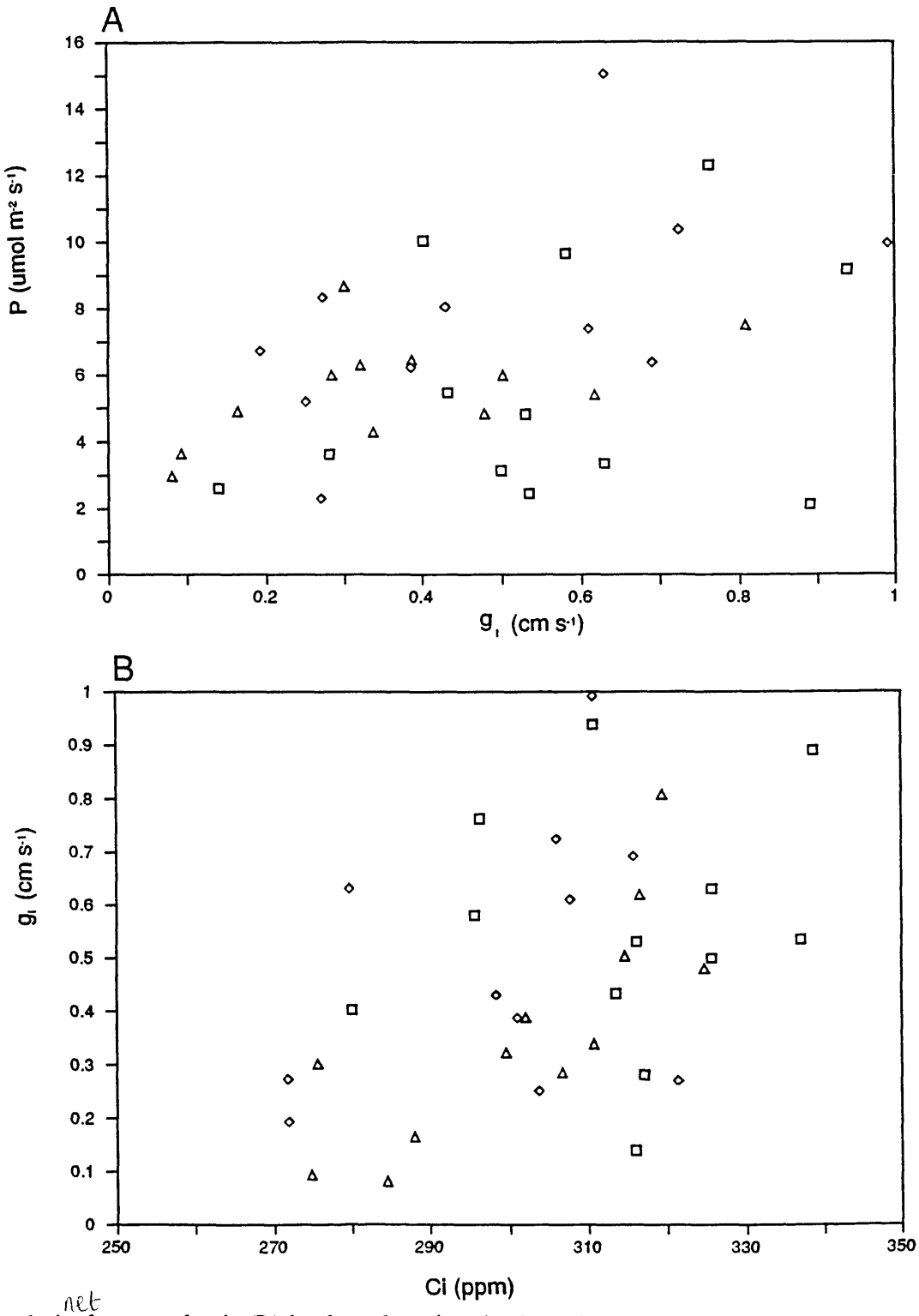
Photosynthesis

Stomatal opening in response to ozone may allow greater rates of carbon dioxide uptake, and this could explain growth stimulations in response to ozone. On the other hand, effects of ozone on stomatal conductance may be indirect effects of ozone-induced changes in net photosynthesis.

Photosynthesis was measured in experiment 4 only, and exposure to ozone resulted in both stimulation and inhibition of A_{net}. In treatment b a significant ozone/water stress interaction was observed on A_{net}, stomatal conductance and growth; ozone-induced increases in A_{net}, conductance, and total plant dry weight were seen in well watered plants only, but ozone had no effect on the growth or gas exchange of water stressed plants. Examination of the gas exchange data for day 20 of this experiment, on which effects of ozone on gas exchange were seen, suggests that the direct effect of ozone was on the stomata, and that this stomatal opening allowed greater rates of CO_2 exchange (Figure 2.22). Ozone may initially increase mesophyll activity resulting in a decrease in Ci and the opening of stomata. On the other hand, ozone may be having a direct impact on the stomatal apparatus. If stomatal conductance was altered via an effect of ozone on photosynthesis there would be a negative relationship between conductance and Ci. However, this was not the case.

The A_{net} of plants in treatment a was significantly reduced by exposure to ozone on one day only, the sixth day of the ozone fumigation. Black *et al.* (1982) observed reductions in the photosynthetic rate of *Vicia faba* L. cv Dylan, exposed to ozone concentrations of 50 ppb or more (up to 300 ppb) for four hours, and complete recovery after 20 hours following exposure to concentrations less than 90 ppb. These authors conclude that reductions in net photosynthesis cannot be explained purely by decreases in stomatal conductance. This may also have been the case in this experiment, as on the day that A_{net} was inhibited there was no effect of ozone on leaf conductance. However, the conductance data from this experiment must be interpreted with caution due to the problems with the LCA-2.

Figure 2.22. The effect of ozone on the gas exchange of well watered plants (day 20, experiment 4).



In graph A, net photosynthesis (P) is plotted against leaf conductance (g_l), and in graph B g_l is plotted against intercellular CO_2 concentration (Ci). Values for individual plants are plotted; well watered control plants (△), well watered plants in treatment a (□) and well watered plants in treatment b (◇).

5.5 Visible Ozone Injury

The visible injury assessments provide some evidence that simultaneous exposure to ozone and water stress results in less ozone damage than sequential exposure to the two stresses. In experiments 3 and 4, plants that were water stressed during the ozone episode (i.e. treatment b of experiment 4) had significantly less visible ozone injury than plants that were well watered. In both of these experiments water stress resulted in significant reductions in stomatal conductance during the ozone fumigation, and therefore reduced pollutant uptake may explain the reduced ozone damage in the water stressed plants. Stomatal closure has been associated with a reduction in leaf injury in some studies (for example, Butler & Tibbitts, 1979), but in others there was no correlation between changes in stomatal aperture and the amount of ozone induced leaf injury (for example, Olszyk & Tibbitts, 1981a; Miller & Davis, 1981).

However, in experiment 3 simultaneous exposure to ozone and water stress resulted in greater increases in the weight of dead leaves than resulted from exposure to either stress alone. This effect of ozone and water stress on leaf death might account for the reduced number of injured leaves in water stressed plants in this experiment.

This was the only example of ozone having an adverse effect on *V. faba*. It is interesting to note that the mean mid day air temperature during experiment 3 was 21 °C, and Miller & Davis (1981) report that leaves of *Phaseolus vulgaris* are more sensitive to ozone injury when exposed at 21 °C, than at 16, 27 or 32 °C. McLaughlin & Taylor (1981) report that foliar uptake of ozone by *P. vulgaris* was enhanced three- to fourfold by an increase in relative humidity from 35 to 75%. Thus for the same exposure concentration plants exposed to ozone in more humid air may experience a greater internal flux of ozone than those in less humid air. The mean mid day relative humidity during experiment 3 was 43 $\pm$ 7%. This was greater than the equivalent mean during experiments 2a, 2b and 4, but less than that for experiment 1, in which there was no evidence of ozone having an adverse effect on *V. faba*.

To summarise, ozone at realistically low concentrations had a significant impact on both the growth and gas exchange of *Vicia faba*.

The data from these experiments provide some evidence that exposure to ozone may increase plant sensitivity to water stress. Increases in shoot growth and leaf conductance

in response to ozone would be expected to reduce the capacity of fumigated plants to control water loss to the atmosphere, and to reduce their capacity to conserve water in the soil.

In experiments 3 and 4 there was some evidence of exposure to ozone increasing plant sensitivity to water stress. Ozone-induced increases in shoot growth in experiment 4 were seen in well watered but not water stressed plants, and in experiment 3, exposure to ozone and water stress resulted in greater increases in the weight of dead leaves than resulted from exposure to either stress alone. On the other hand, in experiments in which exposure to ozone increased the leaf conductance or leaf area of water stressed plants (experiments 1 and 2b respectively) there was no obvious detrimental effect of ozone on the growth of water stressed plants. However, plant water stress and soil water potential were not measured in these experiments, so it is difficult to assess the effect of stomatal opening and increased shoot growth on soil water availability and plant water stress.

While the timing of exposure to ozone and water stress appeared to influence the degree of visible leaf injury, and possibly the effect of ozone and water stress on gas exchange, the differences between experiments in growth responses to ozone and water stress cannot be explained by the timing of exposure to the two stresses. Other studies of plant responses to ozone and water stress have indicated that the concentration of ozone and the severity of water stress are both important factors in determining the nature and incidence of ozone/water stress interactions (Heggestad *et al.*, 1985; Temple, Kupper *et al.*, 1988). There is some evidence that the ozone exposure regime is an important factor in explaining growth differences between these experiments, and that the severity of water stress is important in determining the incidence of ozone/water stress interactions on gas exchange, and perhaps growth. However, it is difficult to assess the effect and importance of the severity of the water stress, because of lack of accurate data on plant and soil water relations.

Chapter 3

Effects of Ozone and Water Stress on *Fagus sylvatica*

1 Introduction

Over the past 20 years there has been a dramatic increase in the numbers of diseased and dying trees in forests throughout central and north-west Europe (Krause *et al.*, 1986; Prinz *et al.*, 1982; Blank, 1985). *Fagus sylvatica* is a major component of European forest ecosystems and there is concern about its health on a European scale (Ashmore *et al.*, 1985). There is considerable controversy over the cause of European forest decline, and over the role of atmospheric pollution in this decline. One of the ways in which air pollution is believed to be involved is through increasing the sensitivity of trees to a variety of biotic and abiotic stresses, including drought and frost (McLaughlin, 1985).

In the U.K., soil water is considered an important limiting factor to beech growth on many soils, in particular shallow rendzinas and coarse sands (Brown, 1953). Lonsdale *et al.* (1989) have demonstrated that at a number of sites in southern Britain twig extension growth of *F. sylvatica* has not recovered the rates measured before the 1975/1976 drought. European beech is considered to be relatively insensitive to ozone (Guderian 1985), however there is very little experimental data concerning ozone-sensitivity of beech. In a filtration experiment in south-east England, Ashmore (1984) observed no significant effect of ambient ozone on leaf chlorosis of *F. sylvatica*, but leaves of European ash (*Fraxinus excelsoir*) and silver birch (*Betula pendula*) showed significantly more chlorosis in unfiltered, compared to filtered air. However, fumigation of *F. sylvatica* with ozone for 42 days resulted in foliar injury on shaded plants exposed to 75 ppb, and on all plants exposed to 150 ppb (Prinz 1983).

The experiments presented in chapter 2 demonstrate that ozone at low concentrations has a significant impact on both the growth and gas exchange of *Vicia faba*, and provide some evidence that exposure to ozone may increase plant sensitivity to water stress. There are numerous reports in the literature of ozone at concentrations below 150 ppb altering both the growth and gas exchange of tree seedlings. Reduced shoot growth following exposure to ozone has been reported for several tree species (Kress & Skelly, 1982; Reich *et al.*, 1984), and in the few studies in which root growth has been measured there is some evidence that it is more severely impacted than shoot growth (for example, Hogsett *et al.*, 1985). Low concentrations of ozone are reported to cause both increases and decreases in conductance and photosynthesis in trees (Reich *et al.*, 1986; Freer Smith & Dobson, 1989; Reich & Lassoie, 1984), and an effect of ozone on gas exchange is one mechanism by which ozone might alter the sensitivity of trees to water stress.

This study with *F. sylvatica* was designed to determine dose-response relationships over a range of ozone concentrations typical of different British summers. In the experiments with *Vicia faba*, plants were exposed to only one ozone concentration (approximately 50 ppb), making the results applicable (in a strict sense) to just one level of ozone. Dose-response data are of value since they allow inference of response to a range of ozone concentrations. This is of particular interest since there is evidence in the literature to suggest that the nature of ozone/water stress interactions on for example, crop yield, is highly dependant on the ozone concentration to which the plants are exposed (Heggestad *et al.*, 1985). At seasonal mean ozone concentrations below 80 ppb, Heggestad and colleagues observed more than additive reductions in the yield of *Glycine max* exposed to ozone and soil moisture stress, compared to those plants exposed to either stress alone. In contrast, at ozone concentrations above 80 ppb, water stress appeared to protect the plants from ozone-induced yield losses.

In this experiment beech saplings were exposed to ozone and water stress intermittently over a four month period, and gas exchange and non destructive growth measurements were made both during this period, and nine months later, in the spring of the following year, when a final destructive harvest was made. Several experiments with tree seedlings have shown that some effects of fumigation with ozone, particularly growth effects, are not apparent until the spring after pollutant exposure (Peterson *et al.*, 1989).

The plants were exposed to episodes of elevated ozone and simultaneous plant water stress, followed by recovery periods. In this way it was hoped that any cumulative effects of successive exposure to ozone and water stress episodes could be assessed, as well as the trees' capacity to recover between episodes. Intermittent 'recovery' periods are thought to be critically important in governing physiological and growth responses to chronic levels of ozone stress (Tingey & Taylor 1982), and intermittent exposure to ozone is typical of summertime conditions in the U.K., where ozone is highly episodic in occurrence (UK PORG 1987).

2 Materials and methods

2.1 Experimental design

The experimental design is summarised in Figures 3.1 and 3.2. The ozone/water stress treatments spanned ten weeks from 28th June to 7th September 1988, and consisted of three two week ozone and water stress episodes, with two week 'recovery' periods in between.

The timing of stress episodes and 'recovery' periods, and the timing of the measurements made in the exposure period, are shown in Figure 3.2. Gas exchange measurements and measurements of pre dawn and midday leaf water potential were made at the end of each episode and each recovery period. Gas exchange measurements were made on four or five occasions throughout the day from 0600 to 2000. Non destructive growth measurements and visible assessments were made throughout the exposure period, and autumn leaf fall was monitored from mid August to its completion in December. The effect of exposure to ozone and water stress on spring bud burst in the following year was assessed, and then the final gas exchange and non destructive growth measurements, and the final destructive harvest, were made in June 1989, nine months after the end of exposure to ozone and water stress.

2.2 Ozone exposures

The ozone exposure regime was based on ozone data from rural monitoring stations in the U.K. The ozone exposures were designed to represent a range of typical U.K. conditions, in terms of mean summertime concentrations, maximum hourly means, and number of hours above various threshold ozone concentrations (60, 80, 100, and 120 ppb).

The control received typical background concentrations throughout the experiment. The remaining 5 treatments received this concentration during the recovery periods, but were exposed to elevated ozone concentrations during the three two week ozone episodes (Figure 3.3). The ozone peaks were gradually increased day by day as the ozone episode progressed, and then gradually decreased again. The duration of the daytime ozone peak was 8 hours (approximately 0900 to 1700).

The peaks were based on seven diurnal patterns (Table 3.1). The target ozone concentrations for the day time peaks varied from 30 to 150 ppb, and the night time concentrations from 15 to 45 ppb. The number of days or nights at each of these concentrations, was gradually increased from treatment 2 through to treatment 6. The intended number of days at each ozone exposure pattern during a 28 day cycle for each

Figure 3.1
Summary of Experiment

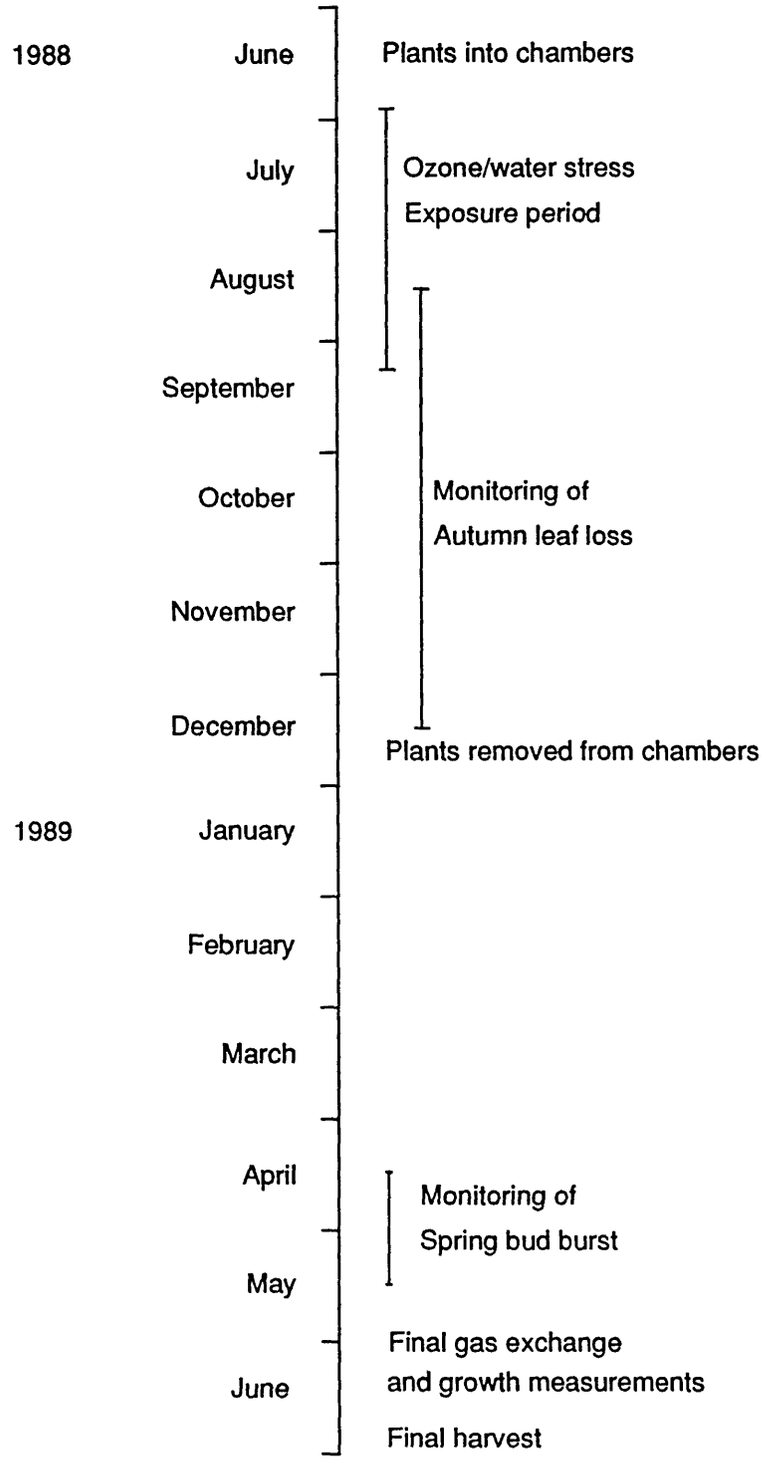
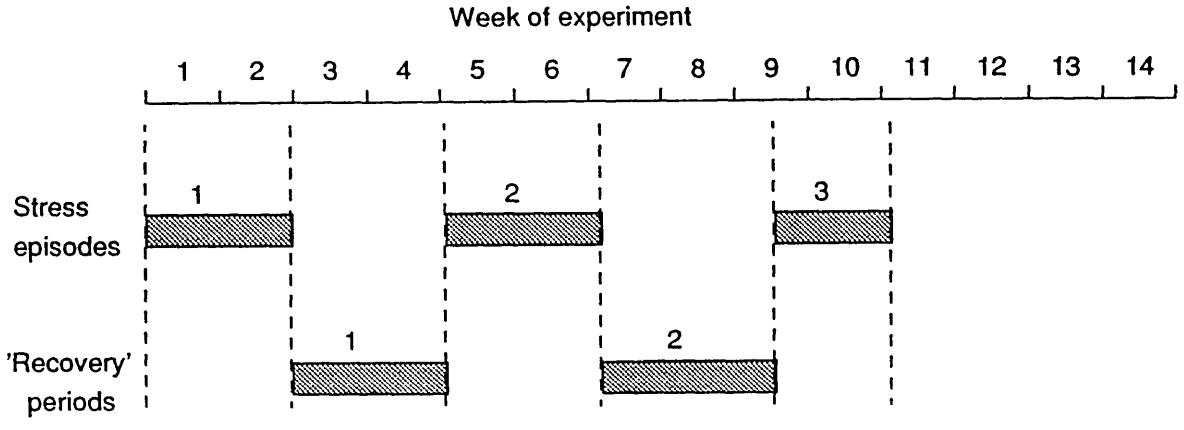
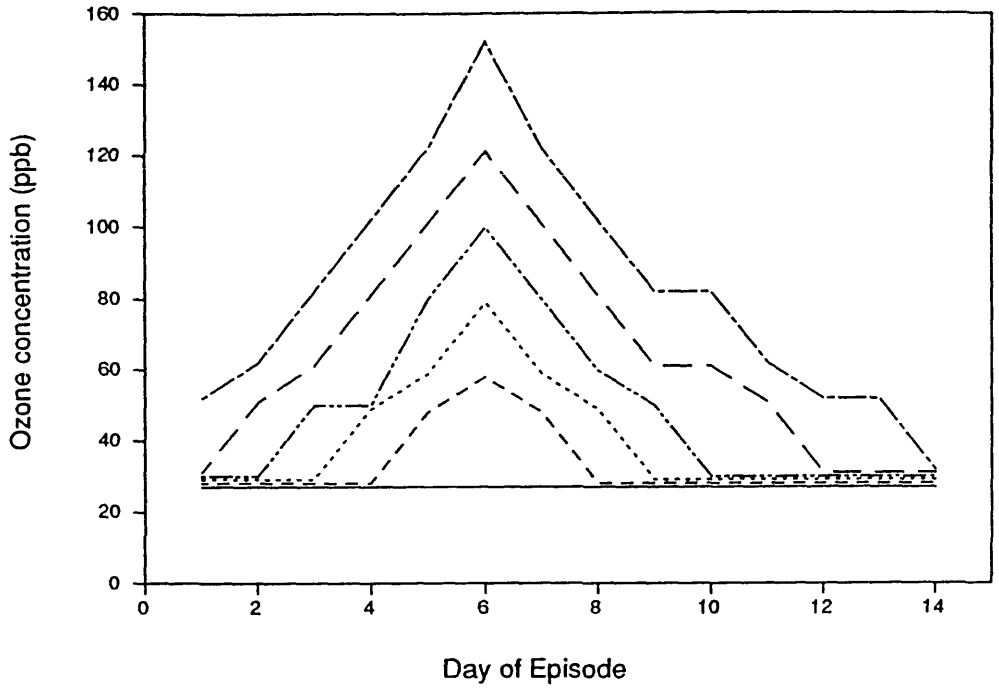


Figure 3.2. Timing of episodes and 'recovery' periods.



Gas exchange measurements were made at the end of each episode and each 'recovery' period (i.e. at the end of week 2,4,6,8 and 10). Non destructive growth measurements were made in weeks 1 (initial measurements), 4,8 and 13. Visual assessments of tree health were made in weeks 4,6,8,10,12 and 14.

Figure 3.3. Target ozone concentrations for each treatment during an episode.



Ozone concentration is plotted against day of episode. Plotted values are 8 h daily mean concentrations for treatment 1 (——), treatment 2 (- - -), treatment 3 (·····), treatment 4 (·——·), treatment 5 (— —) and treatment 6 (-——-).

Table 3.1
Diurnal Patterns For Ozone peaks

Pattern	Ozone Concentration (ppb)		
	Day	Night	Mean
A	30	15	20
B	50	20	30
C	60	30	40
D	80	35	50
E	100	40	60
F	120	45	70
G	150	45	80

Table 3.2
Number of Days at Each Target Pattern in each Four Week Cycle

Pattern	Treatment					
	1	2	3	4	5	6
A	28	25	23	21	18	15
B		2	2	3	2	3
C		1	2	1	3	2
D			1	2	2	3
E				1	2	2
F					1	2
G						1
Mean ozone conc. (ppb)	20.0	21.4	23.2	25.4	29.6	34.3

treatment is shown in Table 3.2. The 28 days include a 15 day 'recovery' period at pattern A for all treatments. The 28 day target mean ozone concentration for each treatment is also shown in Table 3.2.

In this way six ozone doses were administered, of increasing mean ozone concentration, with increasingly higher day and night maximum concentrations, and with increasing numbers of hours above threshold ozone concentrations.

Ozone peaks were only administered when the prevailing weather conditions were consistent with those for naturally occurring high levels of ozone, i.e. not in very wet or windy weather.

2.3 Fumigation system

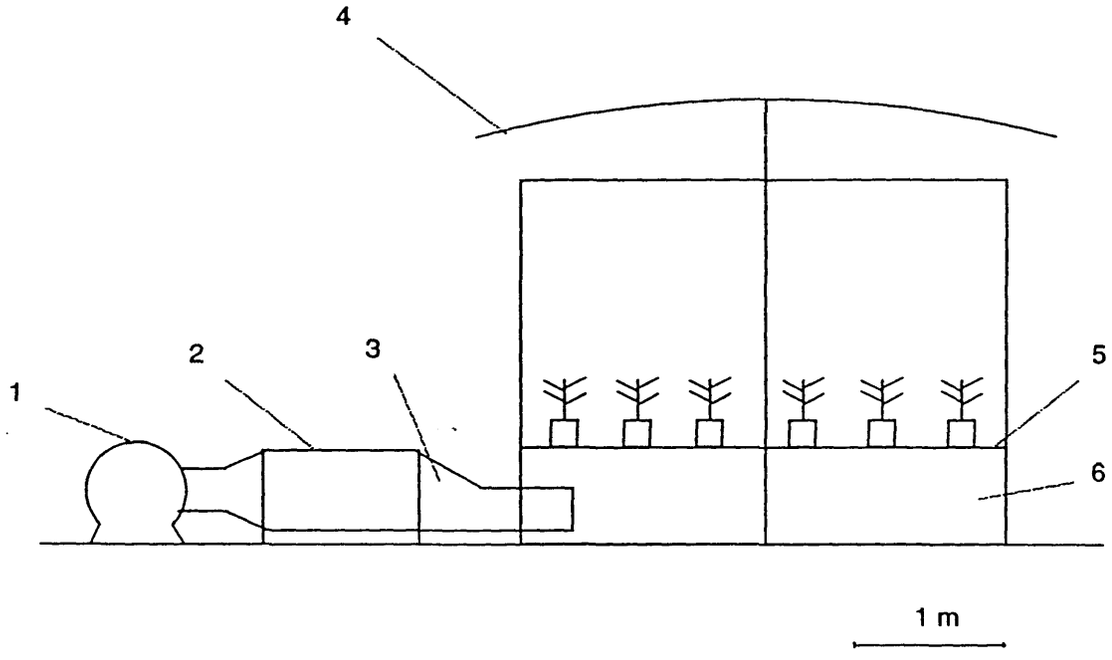
The plants were fumigated in six semi-open top outdoor chambers (Figure 3.4). The chamber design and characteristics are summarised by Ashmore *et al.*, (1986). The chambers had a diameter of 3.30 m, were 2.70 m high and had a volume of 19.70 m^3. The walls were made of heavy duty corrugated PVC sheeting ('Novolux', ICI) attached to a stainless steel pipe frame. The roof was made of heavy duty polythene sheeting stretched over a stainless steel frame, and fixed 15 cm above the top of the chamber walls. The chambers had a raised perforated wooden floor 50 cm above ground level, on which the plants were seated. The chambers were ventilated at a constant rate with charcoal filtered air. The air was pumped in to the base of the chamber and distributed via perforated polythene manifolds around the circumference and across the centre of the chamber. Air flow through the chambers averaged 1.53 $m^3 s^{-1}$, and the filters achieved 70-80 % removal of ozone from the chambers.

Shading was placed around the chamber walls, and the roof was sprayed with "Cool Glaze" to prevent the chambers from heating up on warm days. As a result light intensity (PAR) was reduced by between 20 and 50% inside the chambers, depending on the level of incident radiation. Air temperatures inside the chamber were between 1 and 6 °C higher than those outside.

2.3.1 Ozone generation

Ozone was generated from air by an electric discharge generator (LABO Ozoniser, Ozotech, Sussex), and then was piped, via a set of control valves, to each chamber. The ozone was introduced into the air flow at the inlet at the base of the chamber, and the manifolds and perforated floor ensured that the ozone and air were well mixed and evenly distributed across the chamber.

Figure 3.4. Design of ozone fumigation chambers



(1) Centrifugal fan; (2) Charcoal filter box; (3) Ozone inlet; (4) Roof; (5) False floor; (6) Air mixing sub chamber (from Ashmore *et al.*, 1986).

The ozone was passed through a water trap to remove any contaminant N_2O_5 before it entered the chambers. The water in the trap was changed every 3-4 days. Measurements of the rate of acidification of the trap water (Ainsworth, pers. comm.) indicate that this would have precluded any N_2O_5 or nitric acid vapour entering the chambers.

2.3.2 Ozone monitoring

The ozone concentration in each chamber was monitored with a Dasibi 1003-AH UV photometer, and recorded on a chart recorder. A multichannel solenoid valve sampler was used to sample air sequentially from each chamber; each chamber was sampled for approximately 8 minutes each hour. These samples were drawn via PTFE tubing from the centre of each chamber, at plant height (40 cm above the raised wooden floor). Line loss along these chamber sample lines was calculated by comparing the output of an analyser placed in the chamber with that at the end of the sampling system. Line loss was found to be approximately 10 % and a correction factor was applied to all readings.

2.4 Plants

Two-year old saplings of *F. sylvatica* were potted up in November 1987. The saplings were grown in 17 cm diameter shrub pots containing approximately 4 litres of a peat/sand (3:1) mixture, containing a slow release NPK fertiliser (Osmocote) and a NPK fertiliser mix with trace elements (based on UCD1 standard mix, Matkin & Chandler, 1957). The plants were then overwintered outside.

In order to ensure a similar distribution of sapling sizes in each experimental treatment, the basal diameter and shoot length of each sapling were measured in March 1988, before the experiment began. These data were used to select groups of 13 trees of similar dimensions; the 13 trees were then assigned at random to one of 12 treatment groups, or to an initial harvest group. Each experimental treatment contained 16 replicate saplings, which were not all the same size, but the range of sizes was similar in each treatment.

On 20th June 1988 two treatment groups were placed in each of the six chambers, and the ozone and water stress treatments began on 28th June. Of the two treatment groups placed in each chamber (and therefore in each ozone regime) one was to be water stressed during the stress episodes, and one was to be kept fully watered throughout the experiment.

In order to minimize chamber effects the treatments were rotated among the chambers every two weeks, so that during the 12 week exposure period each treatment spent 2 weeks in each chamber.

2.5 Soil water stress

In order to study the effect of simultaneous and episodic exposure to ozone and water stress, half of the plants in each ozone treatment were kept fully watered with tap water throughout the experiment, the other half were water stressed during the ozone episodes by witholding water, and fully watered during the recovery periods.

Initially, attempts were made to monitor soil water stress with resistance blocks (see chapter 2, section 2.5.2 for details). Blocks were buried in the pots 10 cm below the soil surface in eight water stress plants per treatment, and one fully watered plant per treatment. Readings were taken from the blocks every three days throughout each water stress period. Unfortunately the blocks were found to be insufficiently sensitive to changes in soil water potential in this soil type, and meter readings did not fall with decreasing soil water content. This may be due to the high peat content of this soil (75%), resulting in a poor interface for water movement between soil and block.

Instead, soil water stress was monitored at regular intervals during each stress episode by weighing each water stressed plant, soil and pot. In this way estimates of the Soil Water Deficit were made, see chapter 2, section 2.5.1 for details of calculations.

2.6 Plant water stress

2.6.1 Measurement of leaf water potential.

Leaf water potential was measured with a pressure chamber (see chapter 2, section 2.6.1 for details of apparatus). Measurements were made on one day at the end of the first and second stress episodes and at the end of each of the recovery periods, the same days on which gas exchange measurements were made. Leaf water potential was not measured at the end of the third episode due to a lack of availability of suitable leaves on which to make measurements. In order to seal a leaf into the pressure chamber it had to have a petiole of at least 1 cm. By this stage in the experiment there were not enough leaves which had a long enough petiole, and were sufficiently healthy for measurements to be made.

Leaf water potential was measured before dawn and at mid day. Measurements were made on 24 leaves, one leaf from a sample of 4 plants in each water stressed treatment. A different set of 4 plants was sampled at dawn and at mid day. These were not the same trees that were used for gas exchange measurements.

A young sun leaf was selected, its position on the branch and within the canopy was noted, and a score of 'leaf condition' was made (see section 2.9). Attempts were made to choose only healthy leaves. The leaf was cut from the tree *in situ* in the fumigation chamber and sealed into a plastic bag, containing moist filter paper, until it was placed in the pressure chamber. Damp filter paper was placed inside the pressure chamber in an attempt to reduce evaporative water loss from the leaves during measurement.

The time interval between cutting the leaf from the plant and making the measurement was minimized, and was always less than 3 minutes.

Each set of measurements took approximately two hours, so to minimize differences between treatments due to the timing of sampling, two circuits of the chambers were made, taking leaves from two plants from each chamber on each circuit.

2.6.2 Measurement of relative leaf water content.

As described in chapter 2 (section 2.6.2), the relative water content (RLWC) of leaves was calculated from fresh and dry weight data. Relative water contents were measured in water stressed and fully watered plants before dawn and at mid day.
The leaves cut from the plant for water potential measurement were weighed immediately on removal from the pressure chamber, oven dried, weighed again, and the relative water content determined. Water content measurements were also made on 4 fully watered plants from each treatment at dawn, and at mid day. Leaves were cut from two of the watered plants in each chamber at the same time as the water stressed plants were sampled, each time a circuit of the chambers was made. The position of these leaves on the branch and within the canopy was noted. Leaf water content measurements were also made at the end of the third episode.

The leaf area of each leaf used in these measurements was determined before the leaves were oven-dried. This enabled the specific leaf area ratio of each leaf to be calculated.

2.7 Gas exchange measurements

The LCA-2 Leaf Chamber Apparatus was used to measure carbon dioxide assimilation and transpiration of beech leaves.

Measurements were made one day after the end of each ozone and water stress episode, and at the end of each 'recovery' period, coinciding with days on which measurements of pre dawn and midday plant water status were made. On these days the plants were always experiencing background ozone concentrations in all chambers.

On each day, four or five sets of measurements were made throughout the day, starting at 0700, 1000, 1300, 1600, and sometimes 1900, (depending on weather conditions and incident light levels).

Measurements were made on one leaf from each of 6 trees per treatment (i.e. a total of 72 leaves). Leaves were selected, marked with coloured cotton, and their position on the branch and within the canopy was noted. Only first flush, never lammas, leaves were selected, and attempts were made to choose only healthy leaves. A score of 'leaf condition' for each of the leaves selected was made (see section 2.9 for details). On each of the measurement days, gas exchange measurements were made on the same 72 selected leaves throughout the day.

Measurements were made on the plants *in situ* in the chambers, with the inlet tube to the Air Supply Unit suspended outside the chamber. The flow rate from the Air Supply Unit to the cuvette was maintained at 300 ml min^{-1}. Leaves were sealed into the cuvette and a measurement recorded within one minute. Each set of measurements took approximately two hours, therefore to minimize differences between treatments due to the timing of sampling, two circuits of the chambers were made, measuring leaves from six plants from each chamber (three plants from each treatment) each time.

1989 Gas Exchange Measurements

Before the final harvest of the plants in June 1989 a final set of gas exchange measurements was made. Measurements were made on one day only, on one selected leaf of 6 plants per treatment at 0700, 1000, 1300 and 1600 hours.

2.8 Growth measurements

2.8.1 Non destructive

Non destructive growth measurements were made on all the plants before the experiment began and then at approximately monthly intervals throughout the growing season. The following parameters were measured:

1. Total number of leaves
2. Number of lammas leaves
3. Basal diameter
4. Previous years shoot length
5. Current year shoot length
6. Current year lammas shoot length

The basal diameter was measured with calipers at the same marked place on the trunk each time, at approximately 2 cm above the soil surface.
A final set of growth measurements was made in June 1989, once the initial flush of growth was complete.

2.8.2 Destructive harvests

An initial harvest of 32 trees was made in June 1988. The final destructive harvest of all the trees was made in June 1989, once the spring bud burst and initial flush of growth was complete. The dry weight of the leaves was determined; the shoot was partitioned into each year of growth, dried and weighed; and the roots were washed thoroughly, dried and weighed. In the initial harvest (where there was some lammas growth) the lammas leaves and stem were weighed separately. At the final harvest the leaves were counted and one sixth of them were sampled for leaf area measurement. Attempts were made to select a representative sample of leaves, in terms of leaf size. From this sample the total leaf area was calculated.

2.9 Visual assessments

Visual assessments of all the trees were made at approximately two week intervals (from 22.7.88 to 3.10.88) in an attempt to quantify observed damage symptoms.

These assessments consisted of observing the plants *in situ* in the chambers, and making, for each tree, overall scores of chlorosis and necrosis. The scores were made on a subjective scale of 0 to 10, and attempts were made to score the plants on days when the weather was settled, so that scoring was not confused by sudden changes in sunlight.

'Leaf condition' scores were made on the individual leaves selected for each set of gas exchange and leaf water potential measurements. The same assessments of chlorosis and necrosis were made, but in this case scores were based only on the leaf on which measurements were to be made.

2.10 Aphid control

Throughout the experiment the trees in all chambers were repeatedly attacked by the beech woolly aphid (*Phyllaphis fagi*). In an attempt to control this pest the trees were sprayed on four occasions during June and July 1988 with a systemic insecticide (Rapid, ICI), and the leaves were individually wiped with tissues soaked in a weak solution of detergent.

2.11 Autumn leaf fall

The trees were left in the chambers throughout the Autumn, and Autumn leaf loss was monitored from 25.8.88, until it ceased in mid December. Every 2 to 7 days the fallen leaves from each tree were collected, counted, oven dried and weighed. When leaf loss had ceased, the remaining leaves were pulled off each tree (on 15.12.88), counted, oven dried and weighed.

2.12 Spring bud burst

Bud burst was monitored on seven dates from its beginning in late April, through to its completion on 24.5.89. For each tree an assessment of the stage of bud burst was made on a scale of 0 to 10. The assessments were based on the number of buds burst, and the degree of bud opening.

3 Data analysis

(See Appendix 1)

The data were analysed by two-way analysis of variance using the Genstat 4 package. The ozone concentrations used in the analysis were the cumulative (from the start of the experiment) mean concentrations expressed as eight hour daily means. The ozone concentrations from the end of the ten week exposure period were used in the analysis of all data collected after this day.

As this was a designed dose-response study, as part of the analysis of variance, all treatment sums of squares were partitioned into linear and quadratic components in order to test for the existence of linear and quadratic trends between ozone concentration and plant response. The ozone/water stress interaction term was also partitioned into linear and quadratic components in order to test whether trends between ozone concentration and plant response were dependant on the level of water stress.

Before performing the analysis the data were examined to ensure that they were normally distributed. There was no evidence of non-normal distribution in gas exchange, water stress and visual assessment data.

However, some of the growth data were subjected to logarithmic transformations to ensure that they were normally distributed. Percentage data (autumn leaf fall) were subjected to angular transformations. For some data (lammas growth and bud burst score) no transformation was appropriate to normalise the data, so chi^2 analysis was used.

The covariates used in the analysis of variance are outlined in the following sections. Only covariates which were not significantly affected by ozone or water stress were used. Whenever covariates were used all values presented are adjusted means.

3.1 Soil water stress

Soil water stress data were analysed using the total shoot length of each plant as a covariate. Total shoot lengths were measured in weeks 4, 8 and 13 of the experiment, and the measurement made most recently after each SWD measurement was used in the analysis. In using this covariate, the assumption was made that shoot length was a good indication of total plant weight. This assumption is based on regression analysis performed on the initial and final harvest data (Figure 3.5).

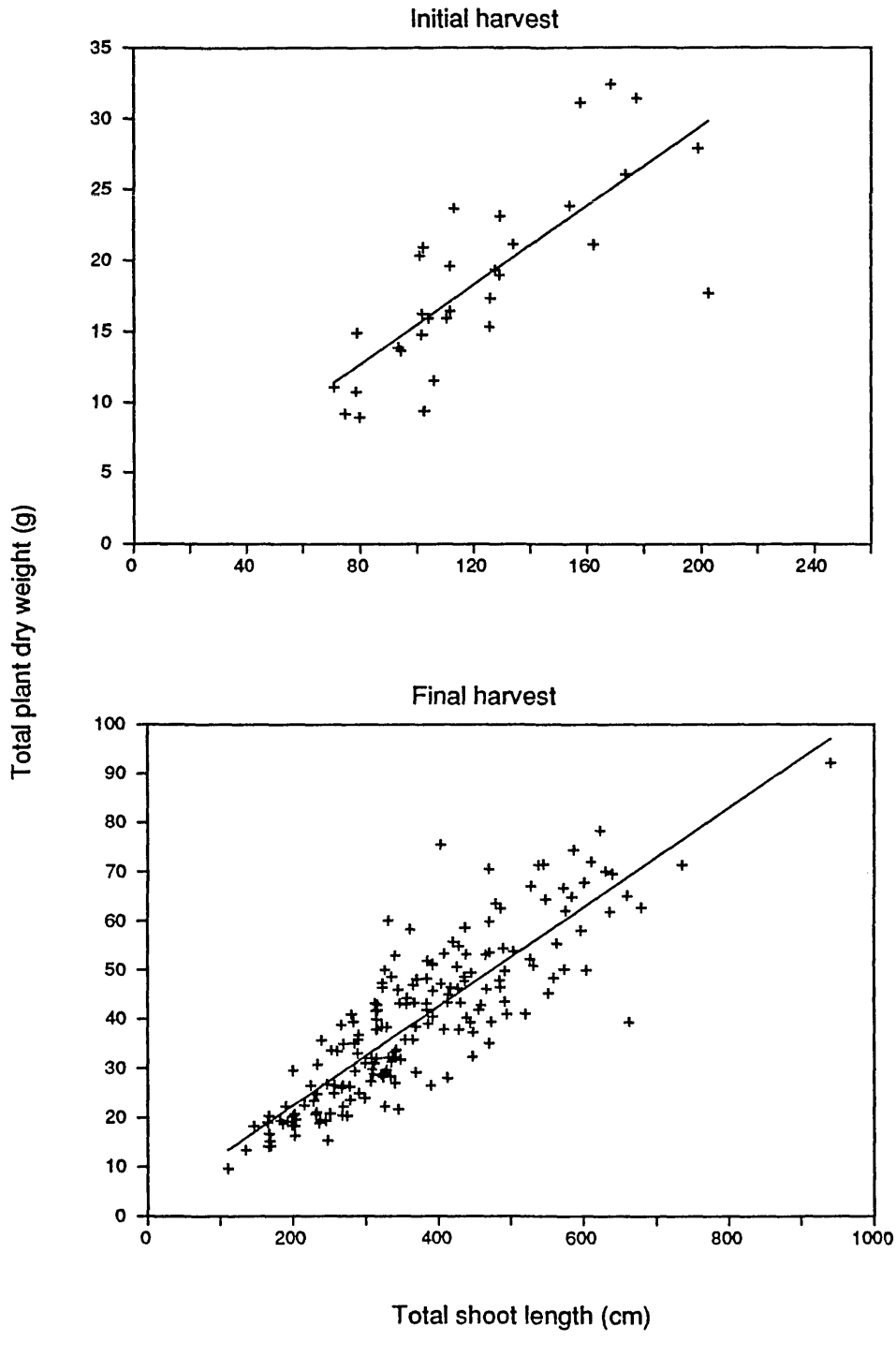
3.2 Plant water stress

No covariates were used in the analysis of relative leaf water content (RLWC). Attempts were made to use the total tree shoot length (at the time of measurement), the position of the leaf within the canopy, and a score of leaf health (chlorosis and necrosis), as

Figure 3.5. The correlation between total plant dry weight and total shoot length at the initial and final harvests.

Total plant dry weight for each plant is plotted against total shoot length. The equations for the fitted lines and corrlation coefficients (r) are:

Initial harvest: Dry weight = 0.14 x shoot length + 1.47 (r=0.774)
Final harvest: Dry weight = 0.10 x shoot length + 2.17 (r=0.853)


Initial harvest
35
30
25
20
15
10
5
0
0
40
80
120
160
200
240
Final harvest
100
90
80
70
60
50
40
30
20
10
0
0
200
400
600
800
1000
Total plant dry weight (g)
Total shoot length (cm)

covariates in the analysis, but none were significant. Plant size, leaf position and leaf chlorosis had no significant effect on leaf water potential; the only significant covariate for leaf water potential was the score of leaf necrosis, and this was used in the analysis.

Analysis of the RLWC data was first performed on all plants, to investigate effects of both ozone and water stress, and secondly was restricted to investigating the effect of ozone on the water stressed plants only.

3.3 Gas exchange measurements

Gas exchange measurements were analysed using the appropriate ozone data from the end of each episode and 'recovery' period. These ozone data are presented in Figure 3.8.

On each measurement day gas exchange measurements were made at four or five times throughout the day. These four or five sets of data were analysed separately. For all sets of data, measurement of PAR made by the LCA-2 for each gas exchange measurement was used as a covariate in the analysis of variance. A score of leaf chlorosis was also used as a covariate, except for data from the end of episode 1 and that collected in 1989, when leaf chlorosis was not scored.

3.4 Growth measurements

After each harvest the dry weight and leaf area data obtained were used to calculate the total plant dry weight; the total above ground dry weight and the root:shoot ratio (RSR). The leaf area data were used to calculate the leaf area ratio (LAR) and specific leaf area ratio (SLAR).

In the anova of the non-destructive growth data, the initial 1988 growth measurements, made before the experiment began, were used as covariates. The final harvest data was analysed using the initial total shoot length (measured before the experiment began) as a covariate. In this analysis the assumption has been made that total shoot length is a good indication of total plant dry weight. This assumption is based on regression analysis performed on the initial harvest data (Figure 3.5).

Chi2 Test on Lammas Growth

A chi^2 test was used to analyse the effects of ozone and water stress on lammas growth. For each set of 1988 non-detructive measurements, the mean number of trees (averaged over all treatments) with some lammas leaves or shoots was calculated. A chi^2 test was performed on the number of trees in each treatment above and below this mean number.

3.5 Visual assessments

Visual assessments were quantified in the form of two scores; a score for chlorosis (the mean of scores for different types of chlorotic damage) and a score for necrosis (the mean of different types of necrotic damage, including mechanical damage to the leaves). At each assessment the two scores for each leaf or tree were analysed for effects of ozone and water stress. No covariates were used in this analysis.

3.6 Autumn leaf fall

The dry weight and number of leaves recorded on each collection day were summed for each week of the collection, and were then converted into the percentage of the total number, or dry weight, of leaves on the tree. The cumulative percentages were then analysed for the effects of ozone and water stress. No covariates were used in this analysis.

3.7 Spring bud burst

The effect of ozone and water stress on bud burst was analysed using a Chi^2 test. The mean bud burst stage (over all treatments) on each assessment day was calculated. A Chi^2 test was then performed on the number of trees in each treatment above and below this mean stage. This test was repeated for each assessment day.

4 Results

4.1 Ozone exposures

The actual ozone concentrations for each treatment during the three episodes, and the two recovery periods, are shown in Figure 3.6. The concentrations are expressed as 8 hour daily means. The maximum hourly mean concentrations during each episode are shown in Figure 3.7. The relatively poor weather conditions of summer 1988, together with equipment failure, meant that the actual ozone concentrations were below the target ones.

Table 3.3 shows the 8 hour daily mean ozone concentrations for each treatment for each episode and for each recovery period. In Table 3.4 and Figure 3.8, the cumulative means (from the beginning of the experiment) are shown for the end of each episode and recovery period. Again, the concentrations are expressed as 8 hour daily means.

4.2 Soil water stress

Half of the plants in each treatment were water stressed by witholding water during the stress episodes. During the first and second stress episodes the water stressed plants were watered once, five days before the end of the episode, to prevent the development of excessively severe water stress. This was not necessary during the third episode.

The maximum soil water deficits were recorded at the ends of the episodes. The soil water deficit at the end of each episode is shown in Table 3.5. Ozone had no significant effect on the Soil Water Deficit at any stage in the experiment.

4.3 Plant water relations

Plant water stress measurements were made before dawn and at mid day at the end of each stress episode and each 'recovery' period, i.e. on the same days as the gas exchange measurements were made. The relative leaf water content of both well watered and water stressed plants was measured, while leaf water potential measurements were made on water stressed plants only.

4.3.1 Relative leaf water content

The effect of ozone and water stress on the relative leaf water content of well watered and water stressed plants is summarised in Table 3.6.

Figure 3.6. The daily mean ozone concentration during each episode and recovery period.

Ozone concentration is plotted against week of experiment. Plotted values are 8 h daily mean concentrations for each treatment. Key as for Figure 3.3.

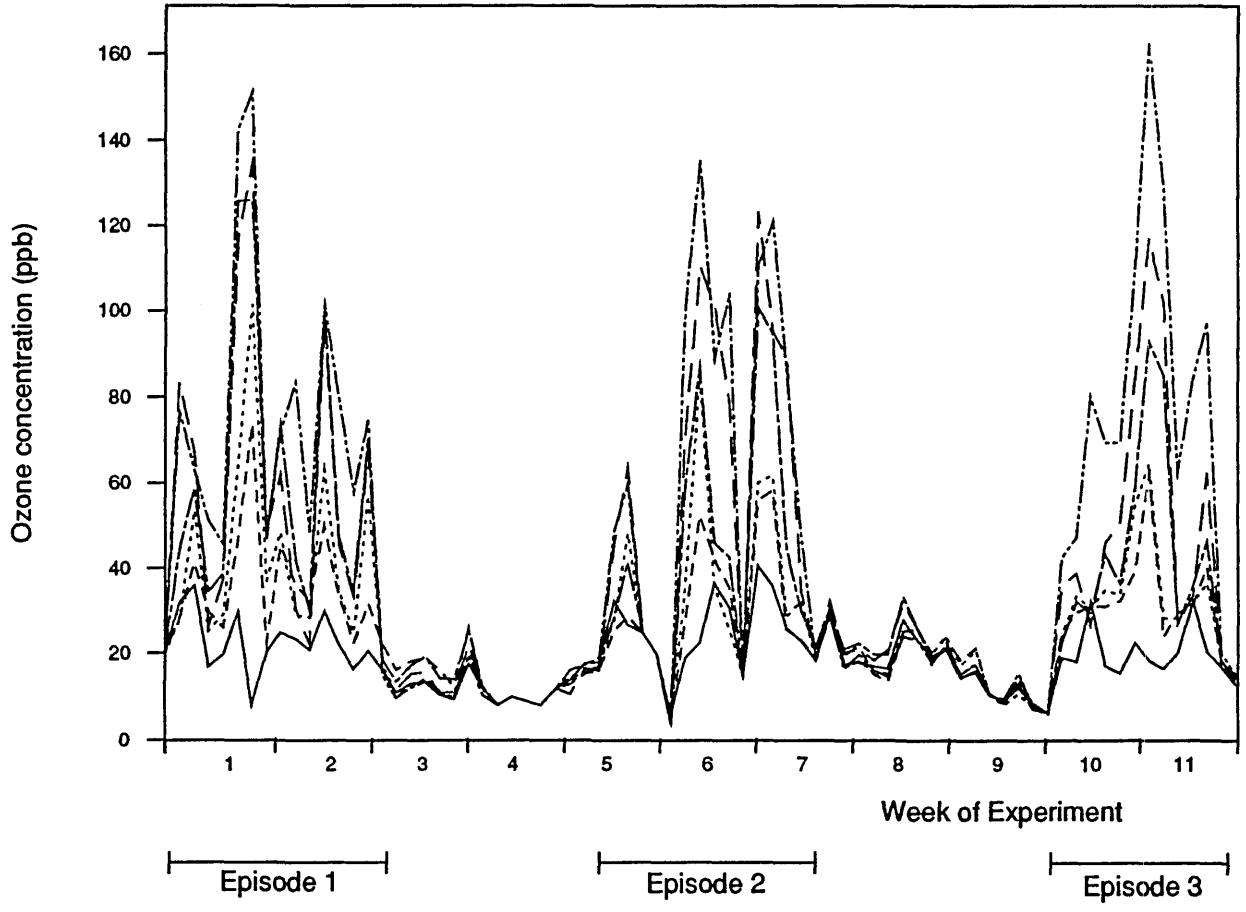
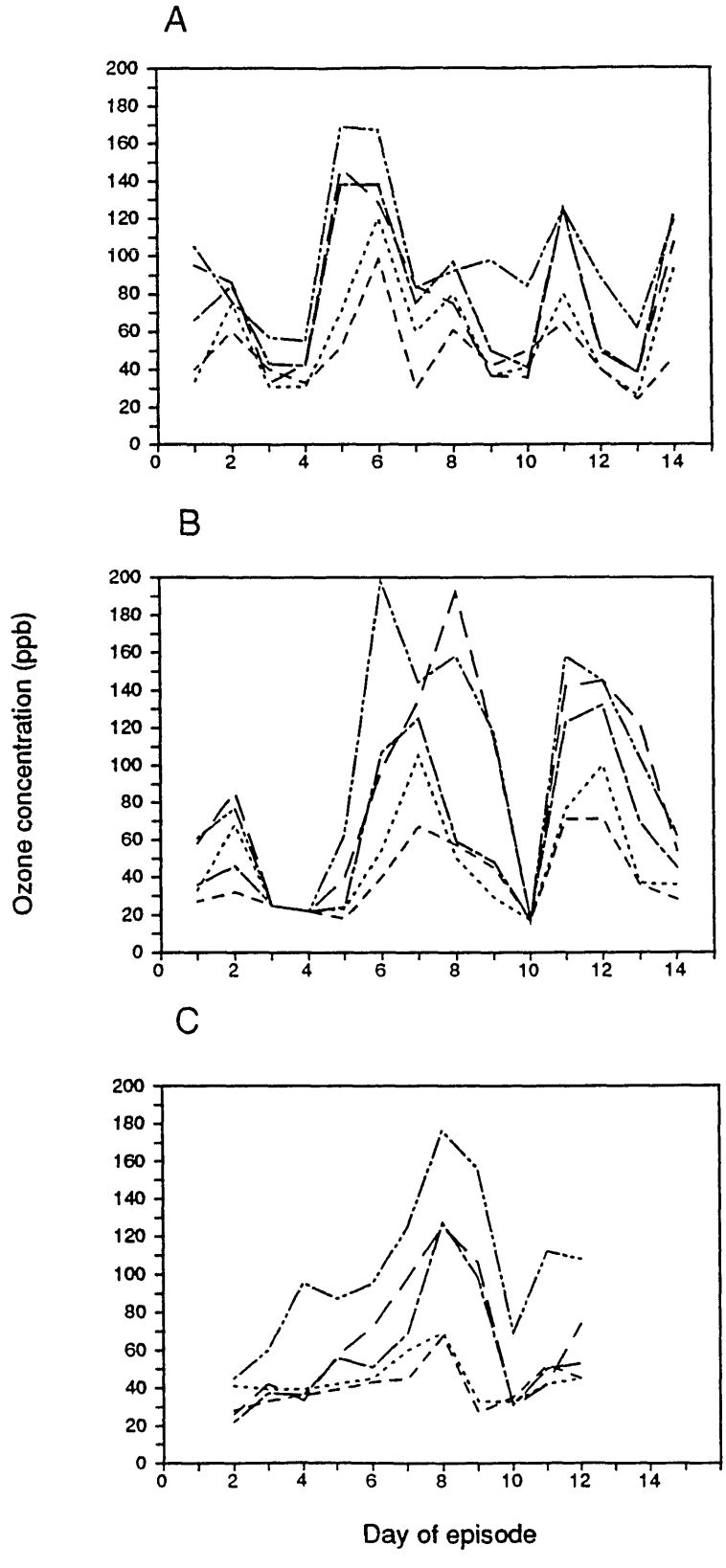

Ozone concentration (ppb)
0
20
40
60
80
100
120
140
160
1
2
3
4
5
6
7
8
9
10
11
Week of Experiment
Episode 1
Episode 2
Episode 3

Figure 3.7. The maximum hourly mean ozone concentrations during each episode.



Ozone concentration is plotted against day of episode for episode 1 (A), episode 2 (B), and episode 3 (C). Plotted values are maximum hourly mean concentrations for each treatment. Key as for Figure 3.3.

Figure 3.8. Cumulative mean ozone concentrations for each treatment.

Plotted values are the cumulative mean 8 h daily mean ozone concentrations for each treatment at the end of each episode and each recovery period.

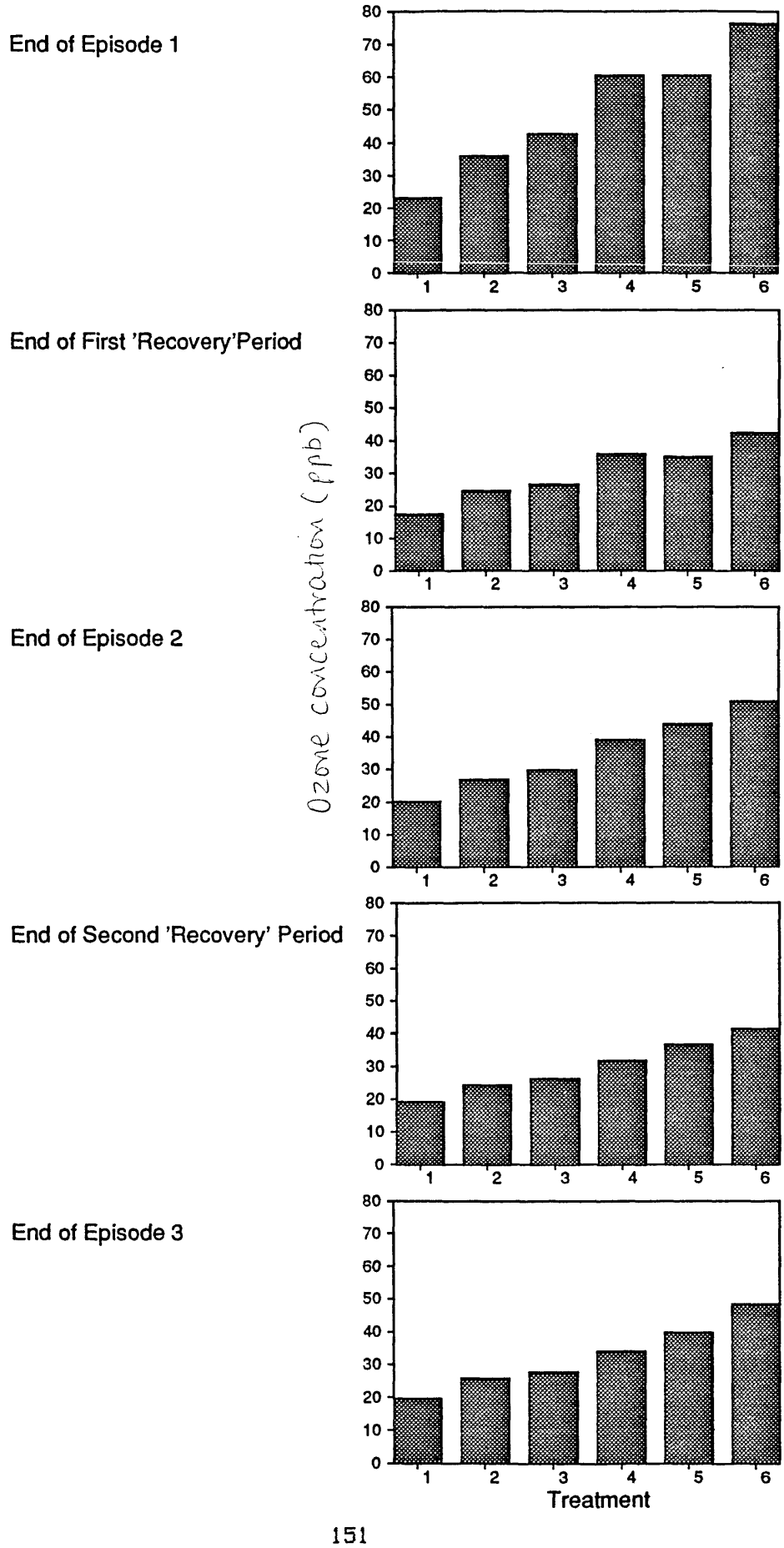

End of Episode 1
End of First 'Recovery'Period
End of Episode 2
End of Second 'Recovery' Period
End of Episode 3
Ozone concentration (ppb)
0
10
20
30
40
50
60
70
80
1
2
3
4
5
6
Treatment

Table 3.3

Mean Ozone Concentration For Each Episode and Each 'Recovery' Period

Period	Treatment					
	1	2	3	4	5	6
Episode 1 (Weeks 1 & 2)	23.3	36.0	42.8	60.5	60.6	76.4
'Recovery' 1 (Weeks 3 & 4)	12.5	14.7	12.5	14.3	12.8	12.7
Episode 2 (Weeks 5 & 6)	25.7	31.4	36.2	45.6	62.8	69.3
'Recovery' 2 (Weeks 7 & 8)	16.9	17.7	17.1	18.5	18.1	16.8
Episode 3 (Weeks 9 & 10)	21.2	34.1	36.5	46.6	56.7	86.8

Tabulated values represent 8 hour daily (0900-1700) mean ozone concentrations (in ppb).

Table 3.4
Cumulative Mean Ozone Concentrations at the end
Of Each Episode and Each 'Recovery' Period

	Treatment					
	1	2	3	4	5	6
Episode 1 (Week 2)	23.3	36.0	42.8	60.5	60.6	76.4
'Recovery' 1 (Week 4)	17.5	24.6	26.6	35.9	35.1	42.4
Episode 2 (Week 6)	20.1	26.8	29.7	39.0	43.9	51.0
'Recovery' 2 (Week 8)	19.2	24.3	26.2	31.8	36.7	41.5
Episode 3 (Week 10)	19.5	25.8	27.8	34.1	39.8	48.4

Tabulated values represent 8 hour daily (0900-1700)mean ozone concentrations (in ppb), averaged over the experimental period.

Table 3.5

Soil Water Deficit (SWD) at the End of Each Episode

Epsiode	SWD at end of episode (%)
1	37
2	41
3	32

The covariate used in the analysis was total shoot length.

Table 3.6
The Effects of Ozone and Water Stress on Relative Leaf Water Content
Summary of Analysis of Variance

Measurements were made at dawn and at mid day at the end of each episode (weeks 2, 6 and 10) and each 'recovery' period (weeks 4 and 8).

Tabulated values represent variance (F) ratios. LIN indicates a linear relationship with ozone (O_3) or a linear ozone/water stress interaction (O_3*WS). QUAD indicates a quadratic relationship with ozone or a quadratic ozone/water stress interaction. F ratios in bold type are significant at $p<0.05$. + represents a significant increase in water stressed (WS) plants, - represents a significant decrease in water stressed plants, and * represents a significant relationship with ozone, or a significant ozone/water stress interaction.

	Time	WS	O_3		O_3*WS	
			LIN	QUAD	LIN	QUAD
Episode 1 (Week 2)	Dawn	1.04	0.01	0.01	0.19	0.43
	Midday	2.93	0.49	3.09	3.33	0.29
'Recovery' 1 (Week 4)	Dawn	1.51	3.91	**4.67** *	3.34	0.18
	Midday	1.14	0.60	0.24	2.05	0.01
Episode 2 (Week 6)	Dawn	1.54	0.84	0.38	2.56	2.42
	Midday	0.85	0.01	2.87	0.73	0.36
'Recovery' 2 (Week 8)	Dawn	0.66	0.19	0.01	0.04	0.06
	Midday	**22.86** +	3.48	0.97	**5.92** *	1.04
Episode 3 (Week 10)	Dawn	0.30	0.50	1.26	0.25	1.32
	Midday	0.80	0.87	0.17	3.00	1.09

4.3.1.1 The Effect of Water Stress

There was no significant effect of water stress on the pre dawn or mid day leaf water content at the end of each stress episode. The relative leaf water content of water stressed plants was reduced at mid day, but not significantly so. At the end of each 'recovery' period, two weeks after exposure to water stress, the mid day relative leaf water content of plants that had been water stressed was greater than that of the plants that had not been water stressed. This increase was significant at the end of the second 'recovery' period, after two stress episodes (Figure 3.9).

4.3.1.2 The Effect of Ozone and Ozone/Water Stress Interactions

There was no effect of ozone on the relative leaf water content at the end of each ozone episode. At the end of both of the 'recovery' periods, there was a significant decrease in RLWC with increasing cumulative mean ozone concentration (Figure 3.10). This effect was seen in the pre dawn measurements at the end of the first 'recovery' period, and in the mid day measurements at the end of the second. At the end of the first 'recovery' period this significant decrease was seen both in plants that had, and had, not been water stressed. However, at the end of the second 'recovery' period there was a significant ozone/water stress interaction on RLWC; the linear decline in RLWC with increasing ozone concentration was seen in the plants that had been water stressed only.

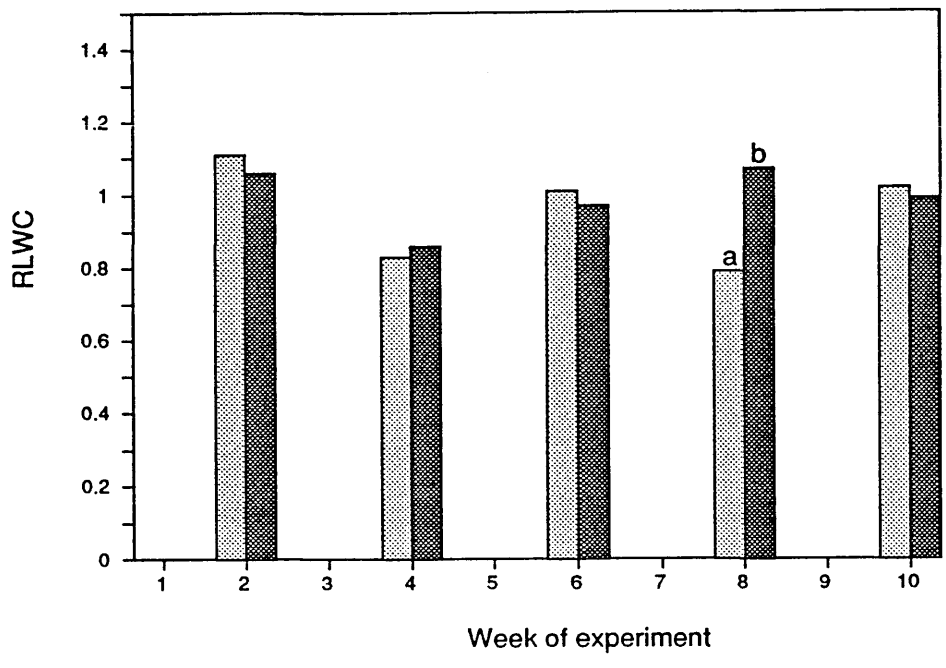
4.3.2 Water stressed plants: the effect of ozone on plant water relations

When the analysis of variance was restricted to the water stressed plants, significant effects of ozone on plant water stress were seen at the end of episodes 1 and 2, and at the end of each recovery period. These significant effects of ozone are summarised in Figure 3.11, and the analysis of variance of the water stressed plants is summarised in Table 3.7.

At the end of the first ozone episode there was a significant linear decrease in mid day relative leaf water content with increasing ozone concentration. There was also a significant effect of ozone on RLWC two weeks after the end of the episode, at the end of the first recovery period. At this time the effect was seen before dawn, and not at mid day. This was the only occasion when an effect of ozone on pre dawn plant water stress was seen.

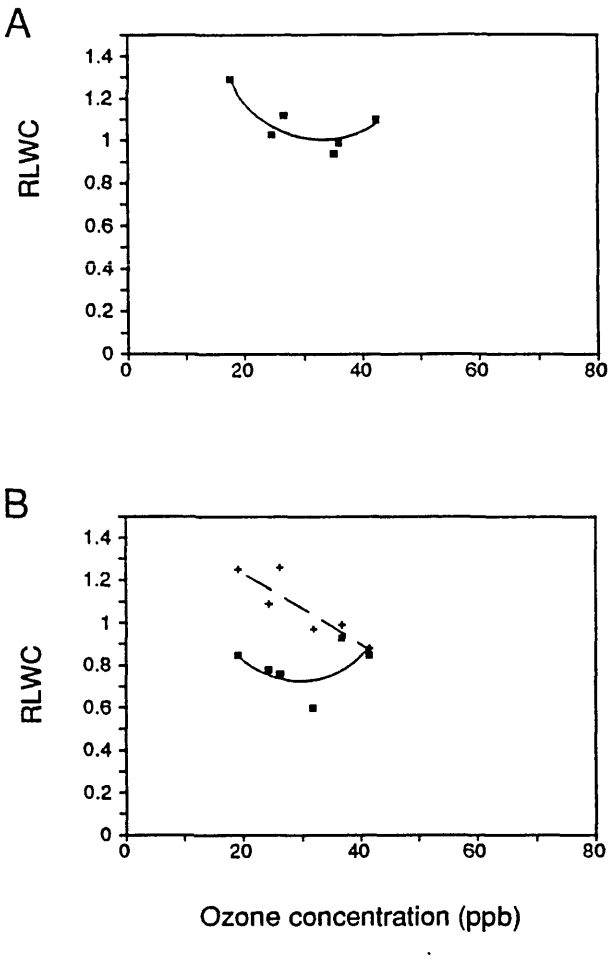
At the end of the second ozone episode there was a significant quadratic relationship between mid day leaf water potential and ozone concentration; leaf water potential was

Figure 3.9. The effect of water stress on mid day relative leaf water content.



Mid day relative leaf water content (RLWC) is plotted against week of experiment. Plotted values are means for well watered ( ) and water stressed ( ) plants, averaged across all ozone treatments. For each week entries not headed by the same letter are significantly different at $p<0.05$.

Figure 3.10. The effect of ozone and water stress on relative leaf water content.



Relative leaf water content (RLWC) is plotted against cumulative mean ozone concentration.

A. The effect of ozone on *pre*dawn RLWC (week 4).

Plotted values (■) represent treatment means averaged across both water stress treatments. The solid line represents the fitted relationship between RLWC and ozone concentration.

B. Ozone/water stress interaction on RLWC at mid day (week 8).

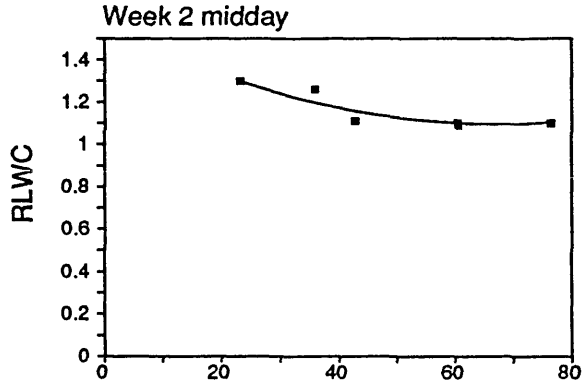
Plotted values represent treatment means for well watered (■) and water stressed (+) plants. The solid line represents the fitted relationship between RLWC and ozone concentration for well watered plants, the broken line represents the fitted relationship for water stressed plants.

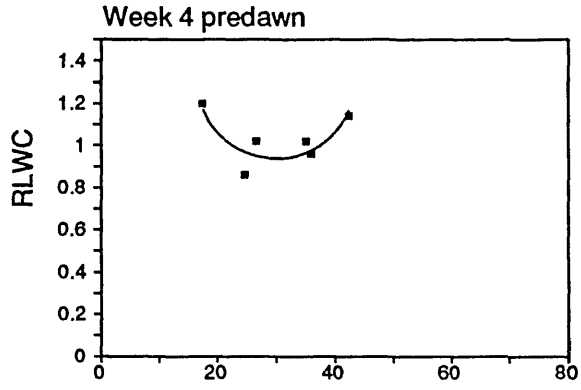
Figure 3.11. The effect of ozone on the relative leaf water content and leaf water potential of water stressed plants.

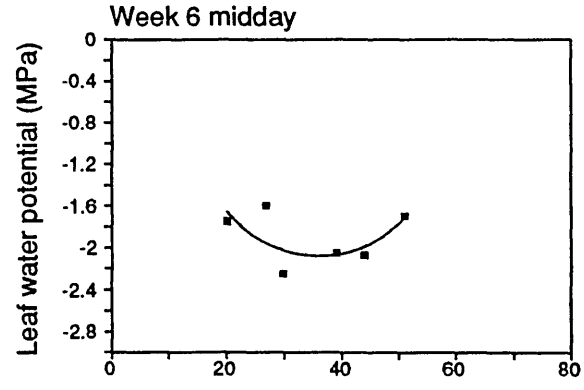
Relative leaf water content (RLWC) and leaf water potential are plotted against cumultive mean ozone concentration. Plotted values (■) represent the treatment means for water stressed plants; solid lines represent the fitted relationship for water stressed plants.

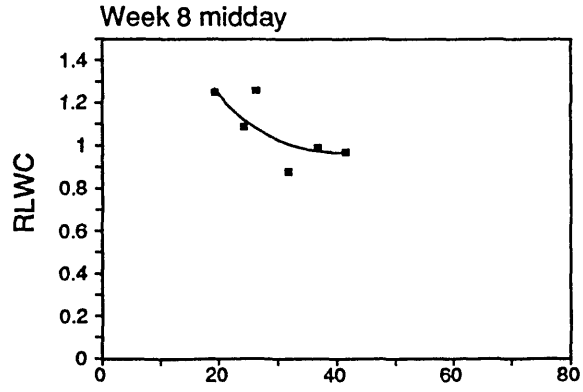
Relative Leaf Water Content

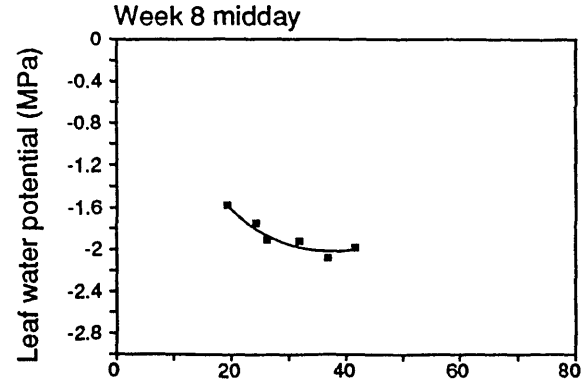
Leaf Water Potential











Ozone concentration (ppb)

Table 3.7

The Effect of Ozone on The Relative Leaf Water Content and Leaf Water Potential of Water Stressed Plants. Summary of Analysis of Variance

	Time	Relative Leaf Water Content		Leaf Water Potential	
		LIN	QUAD	LIN	QUAD
Episode 1 (Week 2)	Dawn	0.21	0.44	0.03	0.35
	Midday	**6.93** *	1.62	0.65	1.32
'Recovery' 1 (Week 4)	Dawn	0.03	**9.07** *	0.83	0.62
	Midday	0.20	0.08	0.92	3.70
Episode 2 (Week 6)	Dawn	2.47	1.85	1.54	0.08
	Midday	0.98	2.17	0.20	**5.61** *
'Recovery' 2 (Week 8)	Dawn	0.07	0.36	0.71	1.01
	Midday	**4.69** *	0.58	**7.29** *	1.55
Episode 3 (Week 10)	Dawn	0.02	2.43	---	---
	Midday	2.43	0.73		

Leaf water potential was not measured at the end of the third episode (week 10). The covariate used in the analysis of leaf water potential was the score of leaf necrosis. No covariates were used in the analysis of relative leaf water content. Tabulated values represent variance (F) ratios. LIN indicates a linear relationship with ozone, and QUAD indicates a quadratic relationship with ozone. F ratios in bold type are significant at $p<0.05$ and * represents a significant relationship with ozone.

decreased at intermediate ozone concentrations, but not at the highest ozone dose. Two weeks later, at the end of the second recovery period, there was a significant linear decrease in both leaf water potential and RLWC with increasing ozone dose.

There was no effect of ozone on the relative leaf water content at the end of the third ozone episode, and leaf water potential was not measured at this stage.

4.4 Plant gas exchange

The effects of ozone and water stress on leaf conductance (g_l), photosynthetic rate (P), and intercellular CO_2 concentration (Ci) are summarised in Tables 3.8, 3.9 and 3.10 respectively.

4.4.1 Effects of water stress

There was little effect of water stress at the end of the first episode (week 2). However at the end of the second and third episodes (weeks 6 and 10 respectively) water stress significantly reduced g_l, P and Ci at various times throughout the day.

At the end of weeks 4 and 8, two weeks after the trees had been water stressed, there were no significant differences in g_l, P or Ci between trees that had, and had not, been water stressed.

4.4.2 Effects of ozone

The effect of ozone on leaf conductance

Significant relationships between ozone and g_l were seen at the end of the first episode, the first 'recovery' period and the third episode (Figure 3.12).

At the end of the first episode, after a two week exposure to ozone, g_l increased with increasing mean ozone concentration. This effect of ozone was seen at mid day (1300) and in the afternoon (1600). Two weeks later, at the end of the first 'recovery' period, the opposite response to ozone was seen, a linear decrease in g_l as ozone concentration increased. At the end of the third episode g_l was increased at the intermediate ozone concentrations (approximately 30 to 40 ppb ten week mean), but decreased again at highest concentration. The g_l at the highest ozone dose (48 ppb ten week mean) was not significantly different from that in the control treatment.

Tables 3.8, 3.9 and 3.10
The Effect of Ozone and Water Stress on Plant Gas Exchange
Summary of Analysis of Variance

Table 3.8 Leaf Conductance

Table 3.9 Net Photosynthetic Rate

Table 3.10 Intercellular CO_2 Concentration

Key as for Table 3.6.

The covariates used in the analysis were PAR and a score of leaf chlorosis, except for the data from the end of episode 1, when leaf chlorosis was not scored, and PAR alone was used as a covariate.

Table 3.8 Leaf Conductance

Week	Time	WS	O_3		O_3*WS	
			LIN	QUAD	LIN	QUAD
2	0700	0.20	1.97	0.28	5.33 *	3.62
	1000	8.06 +	4.01	0.22	4.44 *	1.42
	1300	0.51	4.45 *	0.25	1.48	0.29
	1600	0.70	5.29 *	0	0.53	2.09
	1900	3.57	0.76	1.12	0.04	0.68
4	0700	0.69	1.84	0.63	1.78	0.12
	1000	0.26	1.06	0.31	2.45	0
	1300	0.01	0.83	0.05	2.23	0.02
	1600	2.50	4.60 *	0.03	0.03	2.51
	1900	3.82	0.06	2.02	0.46	0.59
6	0700	2.83	0.46	0.05	2.29	1.84
	1000	9.50 -	0.33	0.02	1.90	4.08 *
	1300	17.91 -	0.01	1.33	20.3	1.46
	1600	10.71 -	2.37	1.17	2.12	0.81

Table 3.8 Continued
Leaf Conductance

Week	Time	WS	O_3		O_3*WS	
			LIN	QUAD	LIN	QUAD
8	0700	0	0.36	0.68	8.93 *	2.59
	1000	0.06	0.98	0.26	6.77 *	9.93 *
	1300	0.96	0.45	0.12	3.98	9.96 *
	1600	0.40	0.21	1.24	5.17 *	5.25 *
	1900	0.29	1.36	0.10	3.84	5.73 *
10	0700	8.58 -	0.28	4.65 *	3.76	4.97 *
	1000	16.17 -	0.05	18.73 *	0.96	1.50
	1300	7.93 -	0.06	8.93 *	2.60	2.74
	1600	16.00 -	2.76	0.02	0.69	0.68

Table 3.9 Photosynthesis

Week	Time	WS	O_3		O_3*WS	
			LIN	QUAD	LIN	QUAD
2	0700	0.08	0.05	11.12 *	0.22	1.59
	1000	1.60	9.09 *	1.15	5.39 *	1.27
	1300	3.79	0.77	0.01	0.70	0.42
	1600	2.00	5.23 *	0.18	0.67	0.15
	1900	3.26	3.71	7.60 *	0	1.18
4	0700	0.22	0	0.21	0.77	0.07
	1000	1.45	0.96	0.26	0.55	0
	1300	1.16	1.43	0	0	0.61
	1600	0.07	0.23	1.93	0.25	0.95
	1900	0.02	10.90 *	0	0.24	0.76
6	0700	0	0.60	0	0	6.34 *
	1000	6.62 -	0.01	1.02	1.86	0.54
	1300	12.13 -	0.24	0.01	0.14	0.77
	1600	4.68 -	5.63 *	0.25	0.38	0.34

Table 3.9 Continued

Photosynthesis

Week	Time	WS	O_3		O_3*WS	
			LIN	QUAD	LIN	QUAD
8	0700	0	0.58	0.36	0.03	0.48
	1000	3.71	3.51	0.80	1.69	0.26
	1300	1.74	1.23	0.50	1.99	2.71
	1600	0.41	3.98	1.90	**7.43 ***	0.28
	1900	0	**6.14 ***	3.85	0	0.04
10	0700	0.08	0.06	0.90	0.13	0.11
	1000	3.06	**4.81 ***	**13.19 ***	1.61	0.06
	1300	2.30	0.06	**8.62 ***	0.08	2.28
	1600	**6.60 -**	**4.38 ***	2.78	2.14	0.19

Table 3.10 Intercellular CO_2 Concentration

Week	Time	WS	O_3		O_3*WS	
			LIN	QUAD	LIN	QUAD
2	0700	0.93	0.32	16.09 *	1.04	0.66
	1000	0.52	10.11 *	0.25	1.86	0.31
	1300	5.92 -	0.07	1.25	0.02	0.17
	1600	1.16	3.12	0.08	0.13	0.07
	1900	0.12	0.53	1.35	0.02	3.87
4	0700	0.09	0.02	0.55	0.02	0.96
	1000	8.64 -	24.91 *	5.57 *	0.32	1.26
	1300	2.08	3.25	0.45	0.68	1.66
	1600	3.12	0.21	1.56	0	0.08
	1900	2.57	3.02	1.07	0.38	2.30
6	0700	7.48 -	0.25	0.01	0.02	2.54
	1000	0.78	16.34 *	0.78	0.09	3.16
	1300	8.52 -	0	1.10	1.98	0.04
	1600	5.28 -	0.06	2.91	1.08	0.29

Table 3.10 Continued
Intercellular CO_2 Concentration

Week	Time	WS	O_3		O_3*WS	
			LIN	QUAD	LIN	QUAD
8	0700	0.06	2.21	0.38	1.29	2.83
	1000	2.63	0.14	1.18	1.09	4.98 *
	1300	0.27	1.03	0.02	1.60	5.92 *
	1600	1.12	3.38	0.14	0.54	1.09
	1900	0.02	2.47	1.17	0	0.10
10	0700	1.56	0	0.20	2.42	1.34
	1000	4.55 -	4.39 *	2.33	7.11 *	4.69 *
	1300	4.77 -	3.62	2.19	6.19 *	2.54
	1600	0	1.69	1.52	3.70	0.01

Figure 3.12. The effect of ozone on leaf conductance.

Leaf conductance (g_l) is plotted against cumulative mean ozone concentration.

Figure 3.13. The effect of ozone on net photosynthesis.

Photosynthesis (P) is plotted against cumulative mean ozone concentration.

Figure 3.14. The effect of ozone on intercellular CO_2 concentration.

Intercellular CO_2 concentration (Ci) is plotted against cumulative mean ozone concentration.

Gas exchange measurements were made at 0700, 1000, 1300, 1600 and 1900 at the ends of weeks 2, 4 and 8, and at 0700, 1000, 1300 and 1600 at the end of weeks 6 and 10. Plotted values (■) represent treatment means averages across both water stress treatments. The solid lines represent the fitted relationship between g_l, P or Ci and ozone concentration.

Figure 3.12: Leaf Conductance

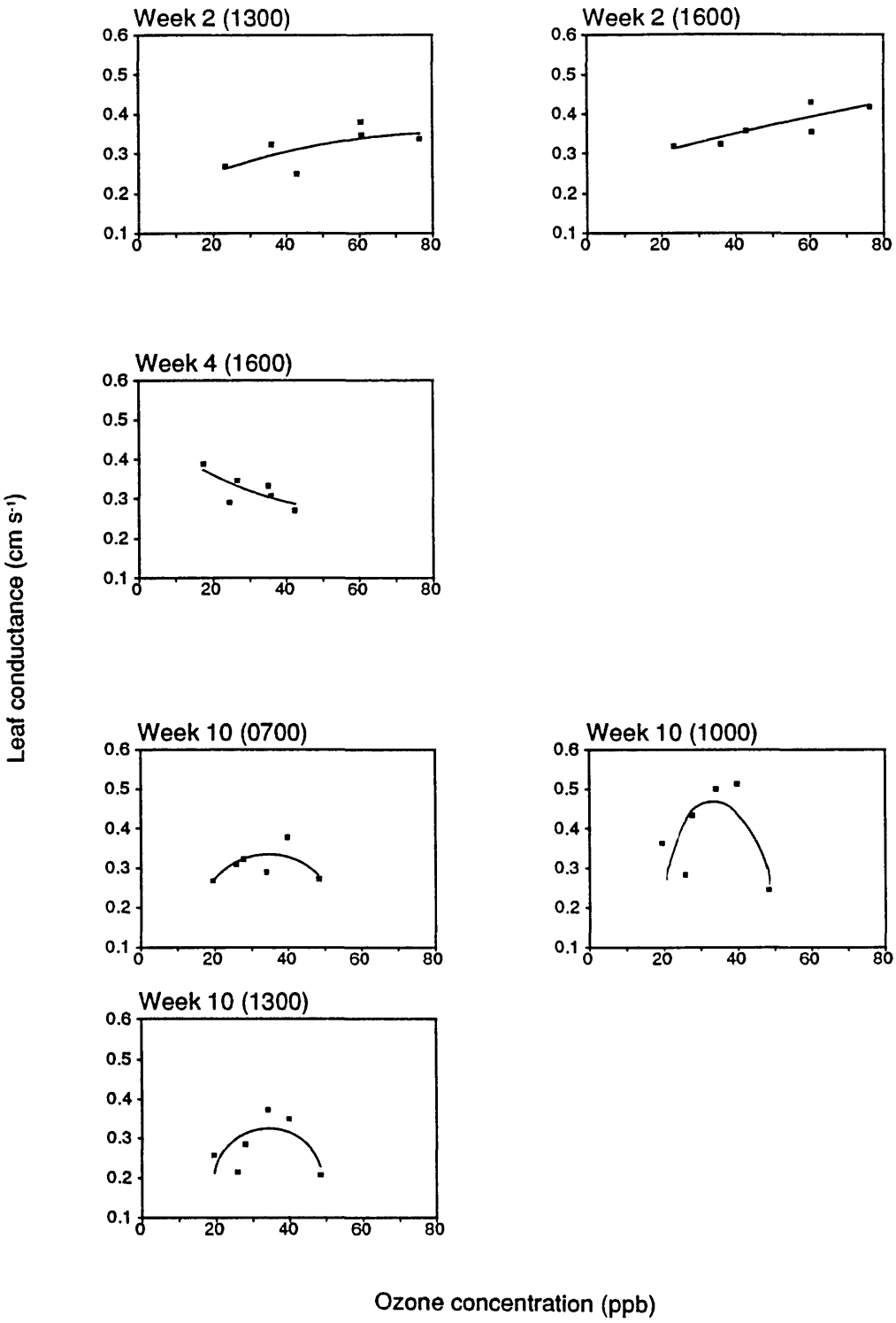


Figure 3.13: Photosynthesis

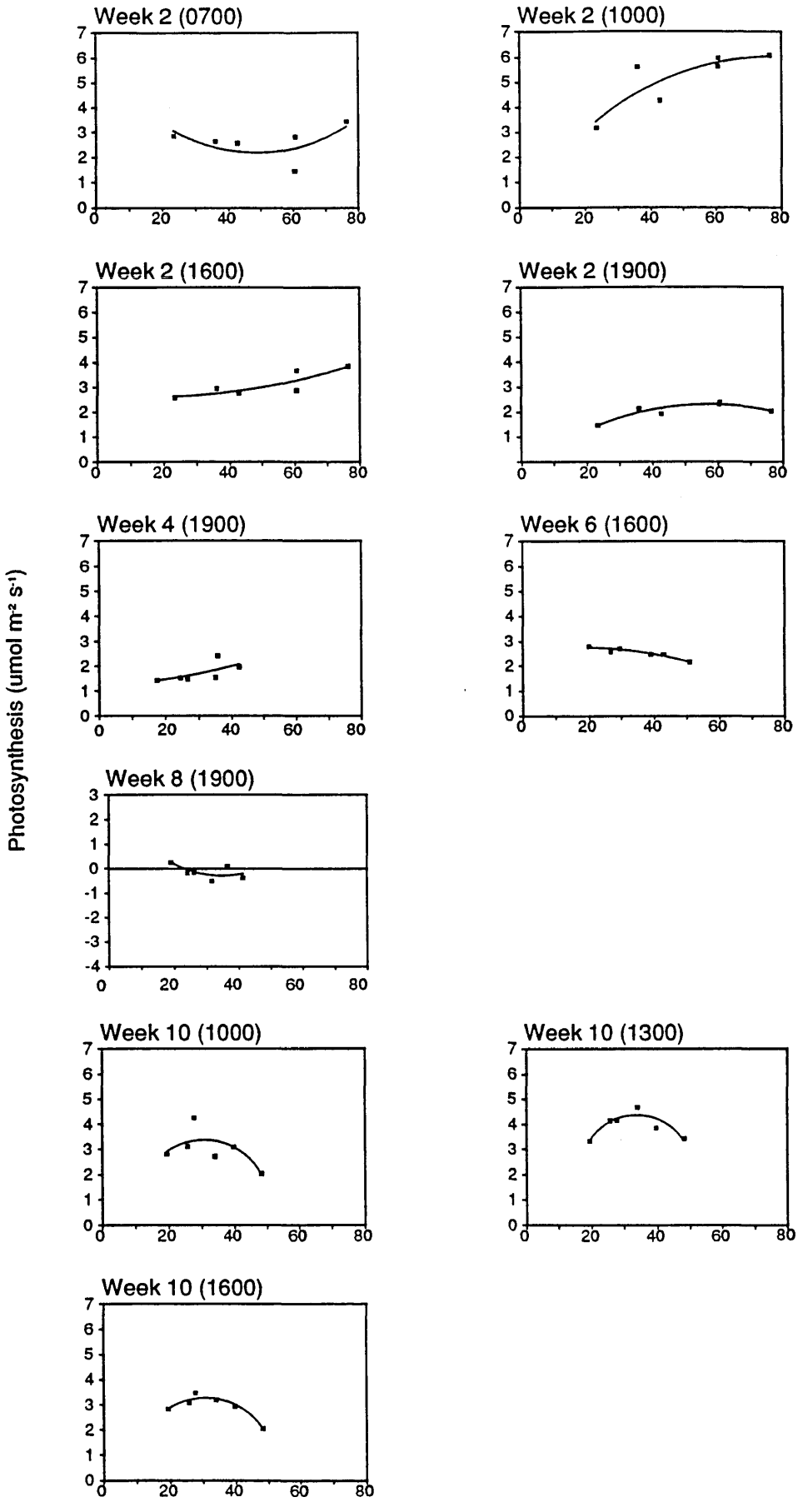
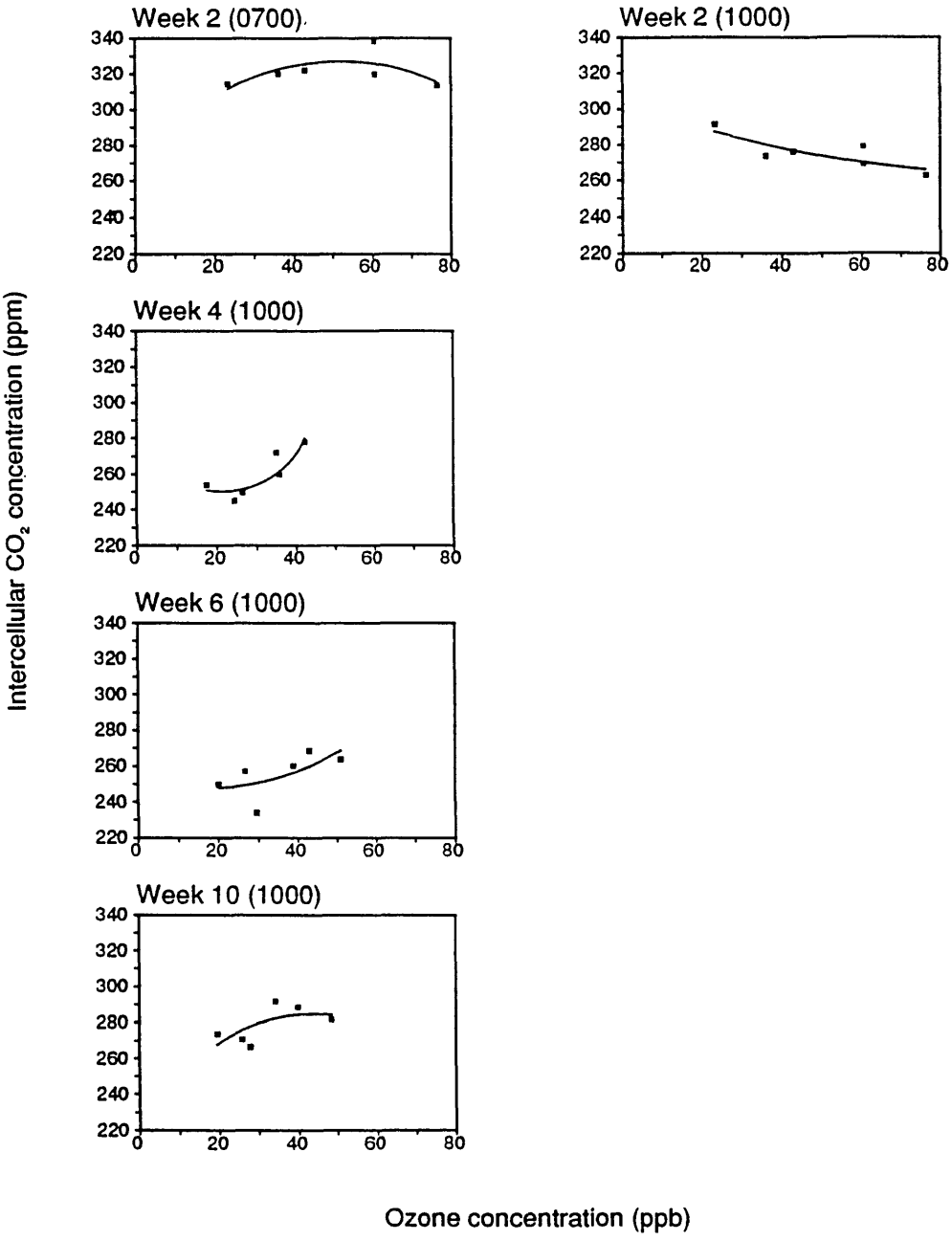


Figure 3.14: Intercellular carbon dioxide concentration



The graphs in Figure 3.12 clearly illustrate the change in the nature of the relationship between ozone concentration and g_l as the experiment progressed. Up until the end of week 4, the relationship was a linear one (although the direction of the linear effect varied), however, at the end of the experiment, the relationship was quadratic.

The effect of ozone on net photosynthetic rate

Significant effects of ozone on P were seen at the end of all three episodes and both 'recovery' periods. These significant effects are illustrated in Figure 3.13. These graphs show that, like g_l, the relationship between P and ozone was linear at the start of the experiment and quadratic at the end.

At the end of the first episode, the rate of photosynthesis increased at 1000 and 1600 as ozone concentration increased. The response to ozone was less clear early in the morning (0700) and in the early evening (1900). The same linear relationship between ozone concentration and P was seen at 1900 in week 4, two weeks after the end of the ozone episode, at the end of the first 'recovery' period.

At the end of the second episode, and two weeks after this episode (at the end of week 8) there was a significant linear decrease in P as ozone concentration increased. The decrease was seen at 1600 at the end of the episode, and at 1900 two weeks later. At 1900 on this day, light levels were low, so respiration exceeded photosynthesis.

At the end of the third episode, P was increased at the intermediate ozone concentrations, but not at the highest ozone concentration. There was some suggestion of an inhibition of P at the highest ozone dose; at 1000 and 1600 the value of P in the highest ozone treatment was less than that of the control treatment, but this reduction was not significant (at $p<0.05$).

The effect of ozone on intercellular CO2 concentration

The measurements of Ci may help to elucidate the mechanism by which ozone is altering g_l and P.

Significant relationships between ozone and Ci were seen at the end of all three episodes, and two weeks after the end of episode 1 (Figure 3.14). In all cases there was a linear relationship between ozone concentration and Ci at 1000.

At the end of episode 1, Ci decreased as ozone concentration increased. At this time, P was showing the opposite linear trend with ozone, and there was no significant effect of ozone on g_l. Two weeks after episode 1, and at the end of episodes 2 and 3, Ci increased as ozone dose increased. At this time at the end of the first 'recovery' period and episode 2 there was no effect of ozone on g_l or P, and at the end of episode 3, g_l and P were both showing a quadratic relationship with ozone.

4.4.3 Interactions between ozone and water stress

Water stress was seen to modify the effect of ozone on plant gas exchange at the end of each episode. No significant ozone/water stress interactions were seen at the end of the first 'recovery' period. However, significant interactions between ozone and water stress were seen after the second 'recovery' period,

Ozone/water stress interactions on leaf conductance

Significant ozone/water stress interactions on g_l were seen in the morning measurements (at 0700 or 1000) at the end of all three episodes, and throughout the day at the end of the second 'recovery' period (Figure 3.15).
At the end of the first episode, the linear increase in g_l in response to ozone was seen only in the water stressed plants; the fully watered plants showed no response to ozone.

At the end of the second episode, and 2 weeks after this episode, plants that had been water stressed showed a significant increase in g_l as ozone dose increased. Plants that had been fully watered throughout the experiment showed the opposite response to ozone; a decrease in g_l as the ozone dose increased. At the end of the episode and in some of the measurements made two weeks later, these trends were not continued into the highest ozone treatments. The greatest difference in g_l between plants that had, and had not, been water stressed was seen in the control ozone treatment. At the end of the episode, in the control ozone treatment, the g_l of water stressed plants was 58% less than that of well watered plants in the same treatment. Two weeks later, between 1000 and 1600, plants that had previously been water stressed (but had been fully watered for two weeks before these measurements were made), showed 40 to 50 % reductions in g_l compared to plants that had been fully watered throughout the experiment.

After the third episode the increase in g_l seen in the intermediate ozone treatments was restricted to the water stressed plants. In contrast, there was evidence of a linear decrease in g_l with increasing ozone dose in the fully watered plants.

Figure 3.15. Ozone/water stress interactions on leaf conductance.

Leaf conductance (g_l) is plotted against cumulative mean ozone concentration.

Figure 3.16. Ozone/water stress interactions on net photosynthesis.

Photosynthesis (P) is plotted against cumulative mean ozone concentration.

Figure 3.17. Ozone/water stress interactions on intercellular CO_2 concentration.

Intercellular CO_2 concentration (Ci) is plotted against cumulative mean ozone concentration (in ppb).

Gas exchange measurements were made at 0700, 1000, 1300, 1600 and 1900 at the ends of weeks 2, 4 and 8, and at 0700, 1000, 1300 and 1600 at the end of weeks 6 and 10. Plotted values represent treatment means for well watered (■), and water stressed (+) plants. The solid lines represent the fitted relationship between g_l, P or Ci and ozone concentration for well watered plants; the broken lines represent the fitted relationship for water stressed plants.

Figure 3.15: Leaf Conductance

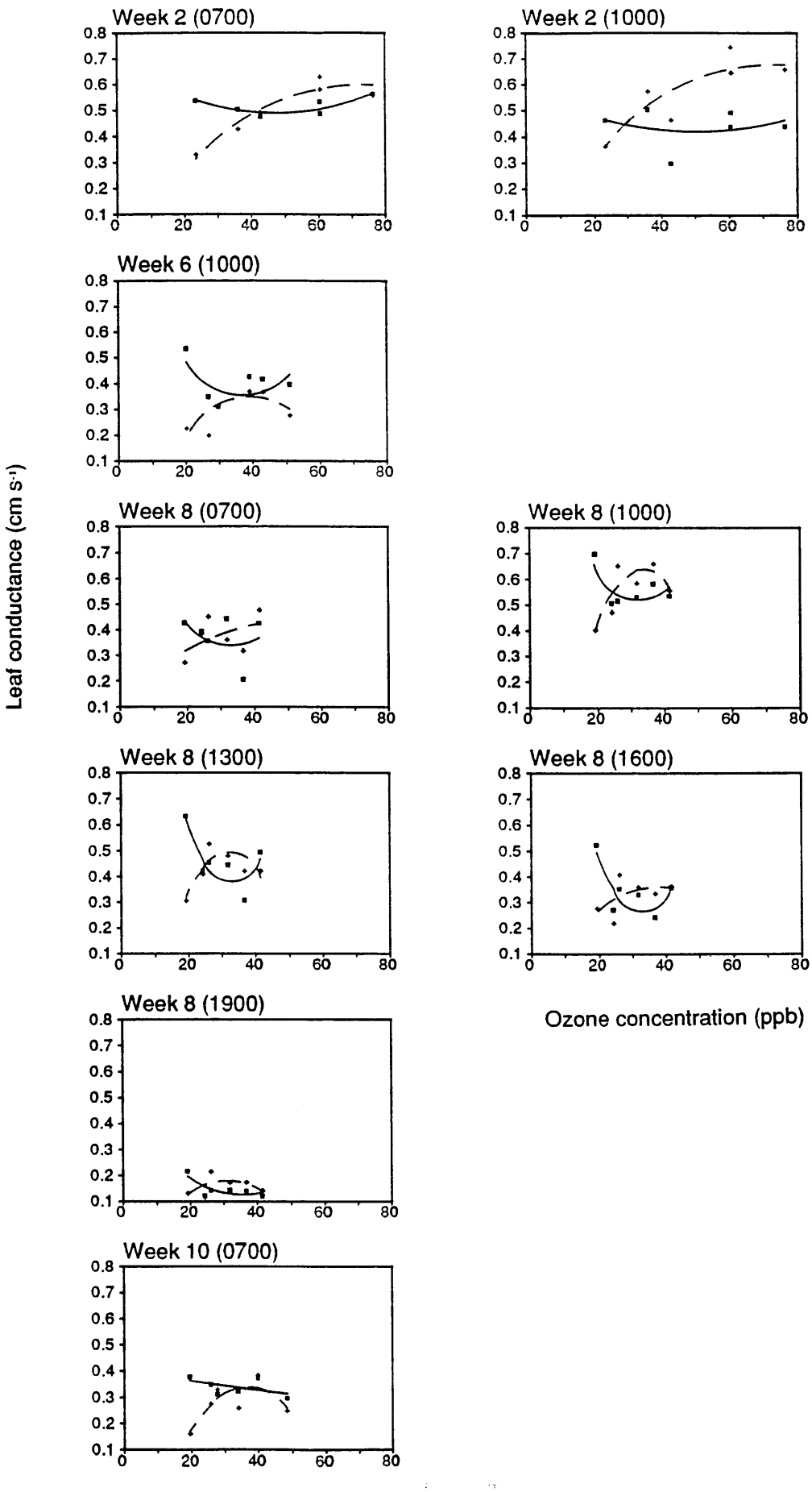


Figure 3.16: Photosynthesis

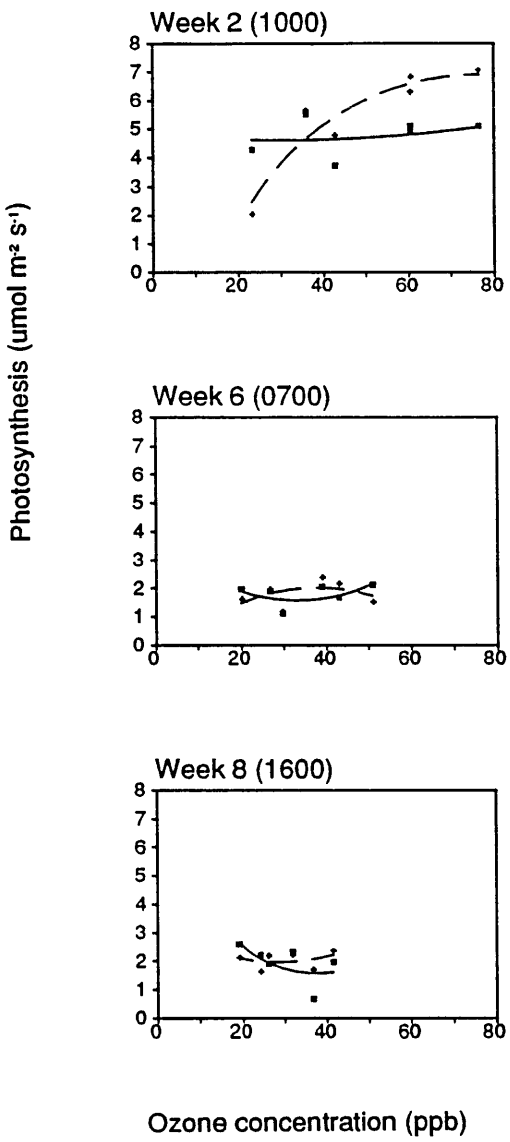
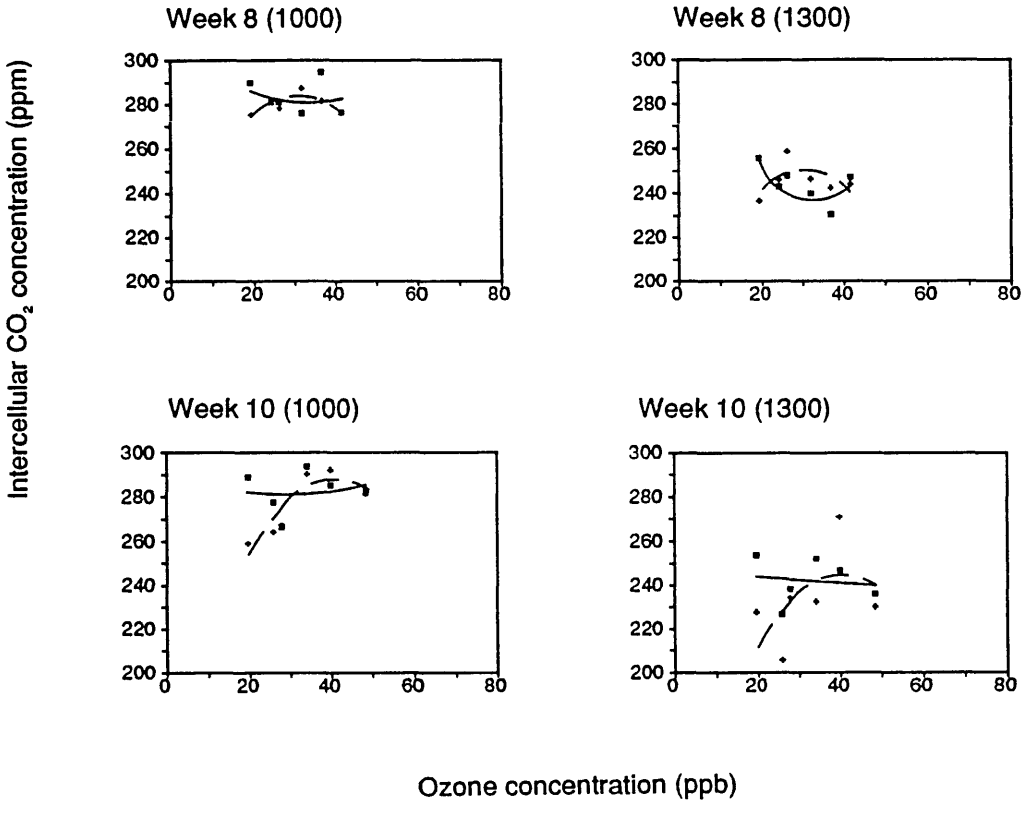


Figure 3.17: Intercellular carbon dioxide concentration



Ozone/water stress interactions on net photosynthetic rate.

A modifying effect of water stress on P was seen at the end of episodes 1 and 2, and two weeks after episode 2 (Figure 3.16).

At the end of episode 1 the linear increase in P as ozone dose increased was restricted to the water stressed plants; the fully watered plants showed little response to ozone.

At 0700, at the end of the second episode, P was increased in water stressed plants and decreased in well watered plants in the intermediate ozone treatments. Two weeks later, at the end of the second 'recovery' period, plants that had been fully watered throughout the experiment showed significant decreases in P at 1600 as ozone dose increased; the plants that had previously been water stressed showed little response to ozone.

There were no significant ozone/water stress interactions on P at the end of the third episode.

Ozone/water stress interactions on intercellular CO2 concentration

Figure 3.17 illustrates the significant relationships between ozone, water stress and Ci. Significant ozone/water stress interactions on Ci were seen, at 1000 and 1300, just before and at the end of the third episode.

At the end of the second 'recovery' period, trees that had not been water stressed showed a decrease in Ci as ozone dose increased. This is the same response to ozone as was seen in the measurements of g_l and P in the fully watered plants on this day. Plants that had been water stressed showed a quadratic relationship between ozone and Ci. Ci increased in intermediate ozone treatments, but not in the highest ozone treatment. The Ci of plants in the highest ozone treatment did not differ significantly from that of control plants. The relationship between ozone and g_l in plants that had been water stressed was the same at these times on this day.

Two weeks later, after the third episode, the Ci of fully watered plants showed very little response to ozone. However, in the water stressed plants there was a linear increase in Ci with increasing ozone dose, but this trend was not continued into the highest ozone treatment.
g_l showed a similar response to ozone and water stress at this stage in the experiment, as illustrated in Figure 3.15. There were no ozone/water stress interactions on P at this stage.

4.4.4 Gas exchange measurements in 1989

Gas exchange measurements were made in June 1989, nine months after the ozone and water stress treatments had ended. Exposure to ozone and water stress in 1988 had no significant effect on the conductance and photosynthesis of the plants in 1989 (Table 3.11).

4.5 Plant growth

4.5.1 Non-destructive growth measurements in 1988 and 1989

Analysis of variance on the initial growth measurements showed that there were no significant differences in plant size between the treatment groups before the experiment began.

In analysing these non destructive growth measurements particular attention was paid to the effects of ozone and water stress on lammas growth, as the majority of this lammas growth occurred during the ten week experimental period. In contrast, the first flush of growth was largely complete before the experimental treatments began.

The effects of ozone and water stress on growth in 1988 and 1989 are summarised in Tables 3.12 and 3.13, respectively.

4.5.1.1 The effect of water stress

There were no significant effects of water stress in the first set of growth measurements. These measurements were made in week 4 of the experiment, after the first stress episode and recovery period.

From the end of the second recovery period (week 8 of the experiment) onwards, there was a reduction in the basal area of plants that had been water stressed, compared to those that had not (Figure 3.18). This reduction in basal area was small (4 to 6%) but significant. The photosynthetic rate of water stressed plants was also significantly reduced from week 8 onwards. This effect of water stress on basal area was also evident in the 1989 growth measurements.

Growth measurements made in the eighth week of the experiment, after two water stress episodes, indicate a transitory significant increase (of 9%) in the current year shoot length of plants that had been water stressed. This resulted in a significant increase in the total

Table 3.11

The Effect of Ozone and Water Stress on Leaf Conductance (g_s), Net photosynthetic Rate (P) and Intercellular CO_2 Concentration (Ci) Measurements made in 1989. Summary of Analysis of Variance

Key as for Table 3.6.

The covariate used in the analysis was PAR. No measurements were made at 1600 due to equipment failure.

	Time	WS	O_3		O_3*WS	
			LIN	QUAD	LIN	QUAD
g_i	0700	0.96	0.58	0.54	1.23	0.60
	1000	1.74	1.15	0.09	1.79	0.02
	1300	0.13	0.04	0	0.39	0.97
	1900	1.03	0.01	0.27	0.34	1.54
P	0700	0.33	0.09	0.88	0.23	0.18
	1000	1.21	3.35	0.11	2.66	0.17
	1300	0.25	0.12	0	0.23	0
	1900	0.13	2.61	3.94	1.63	1.96
Ci	0700	0.21	0.16	0.45	0.51	1.12
	1000	1.09	3.91	0.03	2.12	0.19
	1300	0.30	0.12	0.02	0.22	1.02
	1900	0.04	5.20 *	2.11	2.32	3.79

Table 3.12
1988 Growth Measurements
Summary of Analysis of Variance

Key as for Table 3.6.

The covariates used in the analysis were the initial non destructive measurements, made before the treatments began.

Date	Parameter	WS	O_3		O_3*WS	
			LIN	QUAD	LIN	QUAD
Week 4 25.7.88	Basal area	1.67	2.47	0.53	0.09	2.87
	Total leaf number	2.80	0.03	0.78	2.09	2.35
	Lammas leaf number	2.54	0.14	1.40	1.56	2.34
	'0'year (1988) shoot length	2.81	2.05	0.25	0.29	0.98
	Total shoot length	2.83	1.91	0.32	0.30	0.99
Week 8 17.8.88	Basal area	7.65 -	0.01	0.24	0.01	0.02
	Total leaf number	1.37	0.42	0.99	0.50	**4.90** *
	Lammas leaf number	1.21	0.69	2.30	0.19	**5.48** *
	'0'year (1988) shoot length	**6.47** +	0.02	0.12	0.02	3.74
	Total shoot length	**6.01** +	0.02	0.15	0.03	**4.20** *
Week 13 21.9.88	Basal area	**8.22** -	0.02	1.55	0.45	0.09
	Total leaf number	0.01	0.67	0.01	0.09	1.99
	Lammas leaf number	0.94	0.61	**5.51** *	0.23	**4.25** *
	'0'year (1988) shoot length	2.95	0.22	0.18	0.04	2.45
	Total shoot length	2.43	0.23	0.21	0.06	2.30

Table 3.13

1989 Growth Measurements

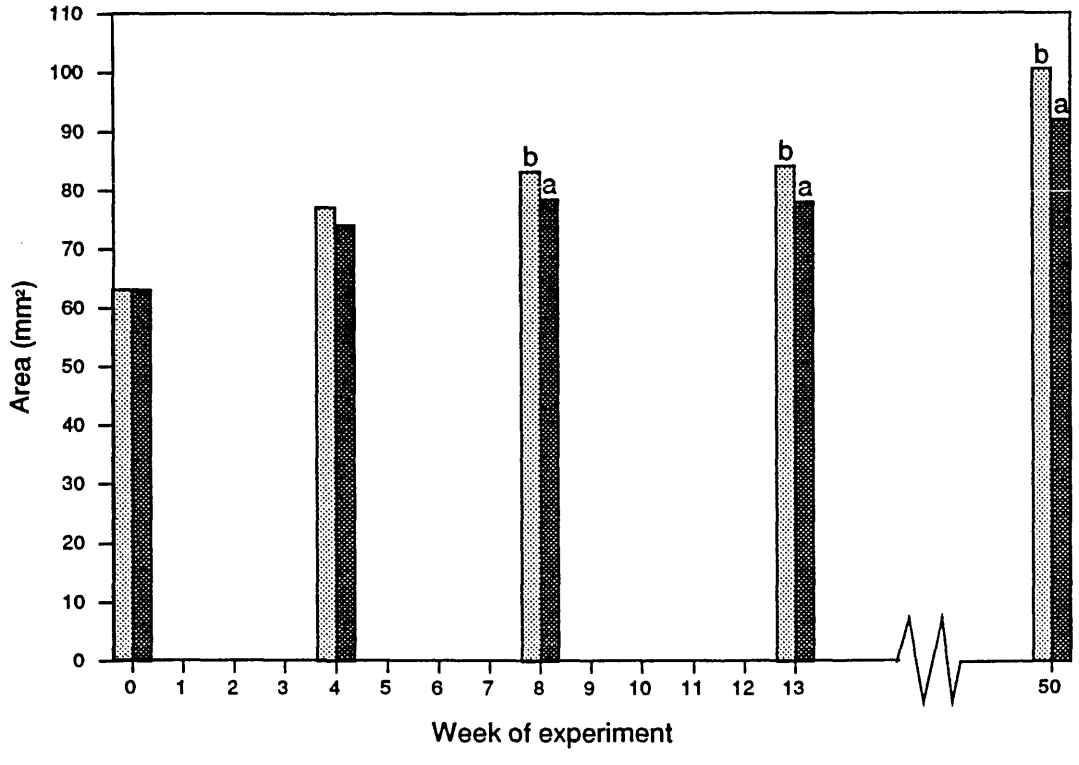
Summary of Analysis of Variance

Parameter	WS	O_3		O_3*WS	
		LIN	QUAD	LIN	QUAD
Basal area	**15.05** -	0.10	0.01	0.12	0.04
Total leaf number	**9.38** -	0.70	0.43	1.67	0.93
'0'year (1988) shoot length	**4.85** -	0.01	0.01	1.80	1.79
Total shoot length	**4.32** -	0.01	0.10	1.93	0.82

Key as for Table 3.6.

The covariates used in the analysis were the initial non destructive measurements, made in 1988 before the treatments began.

Figure 3.18. The effect of water stress on basal area.



Basal area is plotted against week of experiment. Plotted values are means for well watered (▨) and water stressed (▩) plants, averaged across all ozone treatments. Within each week entries not headed by the same letter are significantly different at $p<0.05$.

shoot length of these plants (Figure 3.19). This effect on shoot growth was not seen at the end of the growing season, in the September measurements. In contrast, the longer term effect of water stress was a reduction in shoot extension in the following seasons growth.

In the 1989 measurements, the current year shoot length of plants that had been water stressed during 1988 was significantly reduced (by 13%) compared to plants that had not been water stressed. This resulted in a significant reduction in the total shoot length of plants that had been water stressed. These plants also produced significantly fewer leaves in 1989, compared to plants that had been fully watered for the duration of the experiment (Figure 3.20).

The effect of water stress on lammas growth

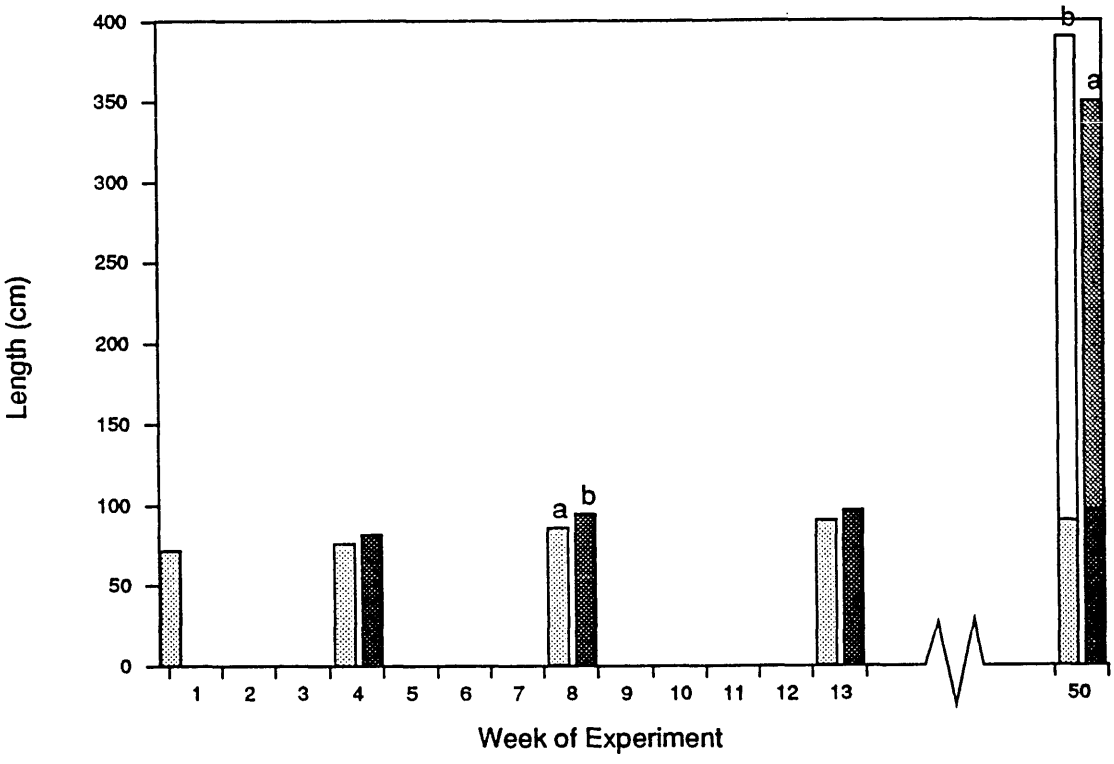
The analysis of variance revealed no significant effects of water stress on the number of lammas leaves or the lammas shoot length. However, the chi^2 analysis of the number of plants with lammas leaves revealed that this parameter was significantly (at $p<0.05$) increased by water stress in the fourth ($chi^2 = 5.23$) and eighth ($chi^2 = 4.91$) weeks of the experiment (Figure 3.20). This effect was not significant by the end of the experiment. When the analysis was repeated for the number of trees with lammas shoots, no effects of water stress were seen.

4.5.1.2 The effect of ozone and ozone/water stress interactions

The only significant effect of ozone alone was on the number of lammas leaves counted at the end of the growing season, in the September measurements. The significant relationship between ozone concentration and number of lammas leaves was a quadratic one, and is illustrated in Figure 3.22. The number of lammas leaves increased in the intermediate ozone concentrations, but decreased again in the highest ozone treatment.

A significant ozone/water stress interaction on total leaf number was seen in the eighth week of the experiment, and on lammas leaf number in the eighth and thirteenth weeks. The nature of this interaction was the same in all three cases; a quadratic relationship between leaf number and ozone and water stress (Figure 3.21). The number of leaves on the fully watered trees was increased in the intermediate ozone treatments, but not in the highest ozone treatment; trees that had been water stressed showed little response to ozone.

Figure 3.19. The effect of water stress on shoot length.



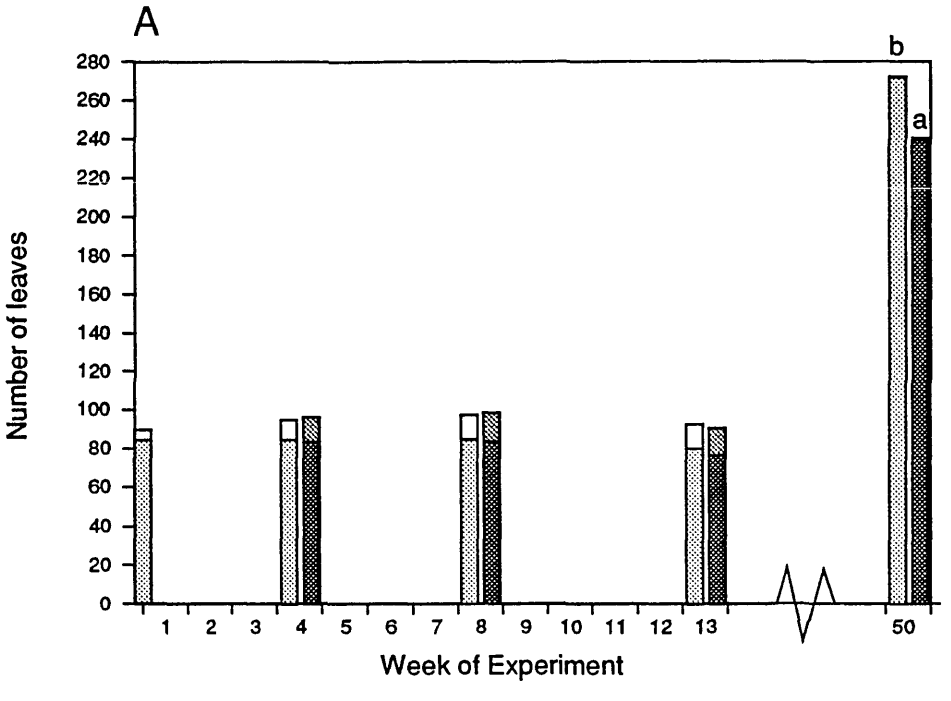
Shoot length is plotted against week of experiment. Plotted values represent the 1988 and 1989 shoot length of well watered (and respectively) and water stressed (and respectively) plants. Within each week entries not headed by the same letter are significantly different at $p<0.05$.

Figure 3.20. The effect of water stress on leaf number.

The number of leaves per plant (A) and the number of plants with lammas leaves (B) is plotted against week of experiment.

A. Plotted values represent the mean number of first flush and lammas leaves on well watered (▒ and □ respectively) and water stressed (▓ and ▨ respectively) plants. Within each week entries not headed by the same letter are significantly different at $p<0.05$.

B. Plotted values represent the mean number of well watered (−■−) and water stressed (−+−) plants with lammas leaves. Within each week entries not headed by the same letter are significantly different at $p<0.05$.


A
b
a
Number of leaves
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
1 2 3 4 5 6 7 8 9 10 11 12 13 50
Week of Experiment

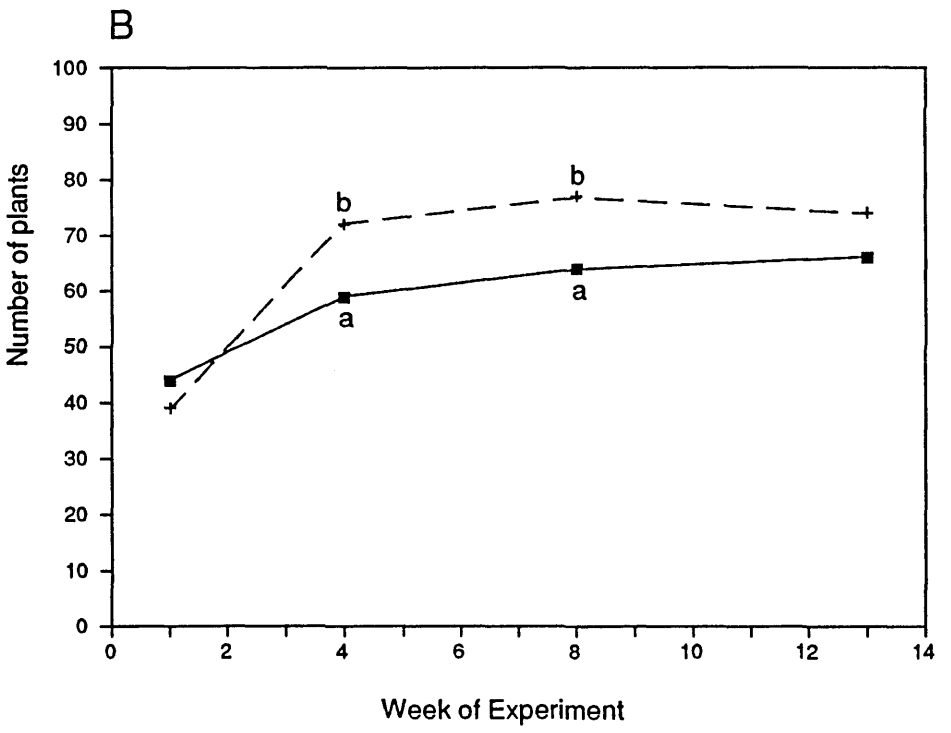

B
Number of plants
100
90
80
70
60
50
40
30
20
10
0
b
b
a
a
0 2 4 6 8 10 12 14
Week of Experiment

Figure 3.21. Ozone/water stress interactions on plant growth (week 8).

The number of lammas leaves (A), the total number of leaves (B), and the total shoot length (C) is plotted against mean cumulative ozone concentration. Plotted values represent treatment means for well watered (■), and water stressed (+) plants. The solid lines represent the fitted relationship between leaf number/shoot length and ozone concentration for well watered plants; the broken lines represent the fitted relationship for water stressed plants.

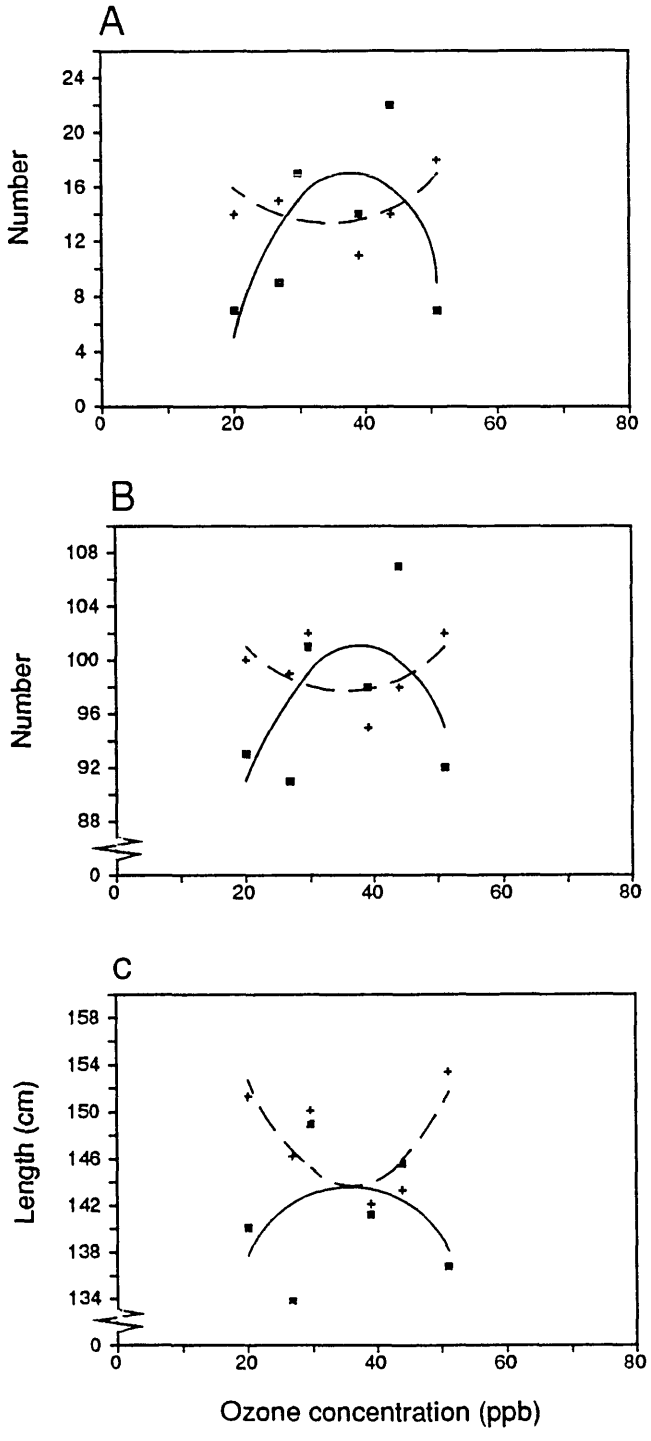
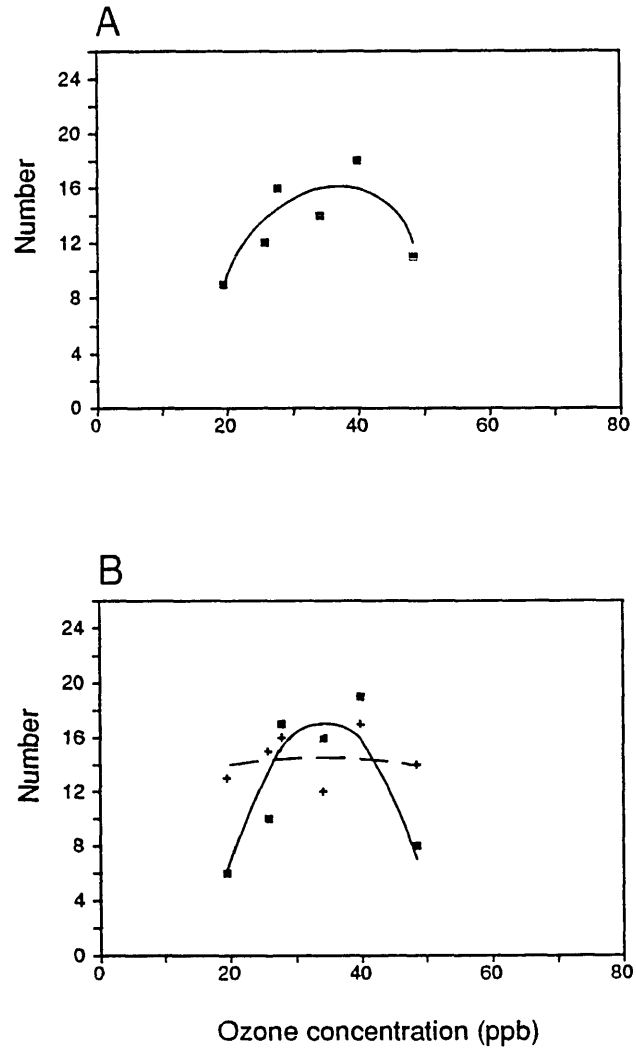

A
Number
24
20
16
12
8
4
0
0
20
40
60
80
B
Number
108
104
100
96
92
88
0
0
20
40
60
80
C
Length (cm)
158
154
150
146
142
138
134
0
0
20
40
60
80
Ozone concentration (ppb)

Figure 3.22. The effect of ozone and ozone/water stress interactions on lammas leaf number (week 13).



The mean number of leaves is plotted against mean cumulative ozone concentration.

A. Plotted values (■) represent treatment means averaged across both water stress treatments. The solid lines represent the fitted relationship between leaf number and ozone concentration.

B. Plotted values represent treatment means for well watered (■), and water stressed (+) plants. The solid lines represent the fitted relationship between leaf number and ozone concentration for well watered plants; the broken lines represent the fitted relationship for water stressed plants.

In the mid August measurements (week 8), there was also a significant ozone/water stress interaction on total shoot length (Figure 3.21). At this stage in the experiment the total shoot length of plants that had been water stressed was significantly greater than well watered plants (Figure 3.20). Figure 3.21 illustrates that the nature of the ozone/water stress interaction on fully watered plants was the same as on leaf number, that is, an increase in total shoot length at intermediate ozone concentrations but not in the highest ozone treatment. However, the total shoot length of plants that had been water stressed showed the opposite relationship with ozone.

The chi^2 analysis of the number of trees with lammas leaves or shoots revealed no significant effects of ozone.

4.5.2 Specific leaf area ratio (SLAR)

One first flush leaf from four trees per treatment was sampled at the end of each episode and each recovery period for plant water stress measurements. The SLAR of these leaves was also calculated, and the effects of ozone and water stress on SLAR are summarised in Table 3.14.

Both water stress and ozone alone had no effect on SLAR. There was a significant ozone/water stress interaction on SLAR at the end of the experiment, after the third episode (Figure 3.23). The SLAR of fully watered plants was increased at intermediate ozone concentrations, but not in the highest ozone treatment. The SLAR of water stressed plants showed the opposite response to ozone, SLAR was decreased at intermediate ozone concentrations, but was unaltered in the highest ozone treatment.

4.5.3 Destructive harvests

The final harvest was made in June 1989. The effects of ozone and water stress on plant growth at the final harvest are summarised in Table 3.15.

4.5.3.1 The effect of water stress

The impact of water stress applied to the plants during the 1988 growing season was to reduce many plant parameters measured in the 1989 final harvest.

The effect of water stress on shoot growth

There was no adverse effect of water stress applied to the plants in 1988 on 1988 stem dry weight, but shoot growth in the following season was reduced.

Table 3.14

The Effects of Ozone and Water Stress on Specific Leaf Area Ratio

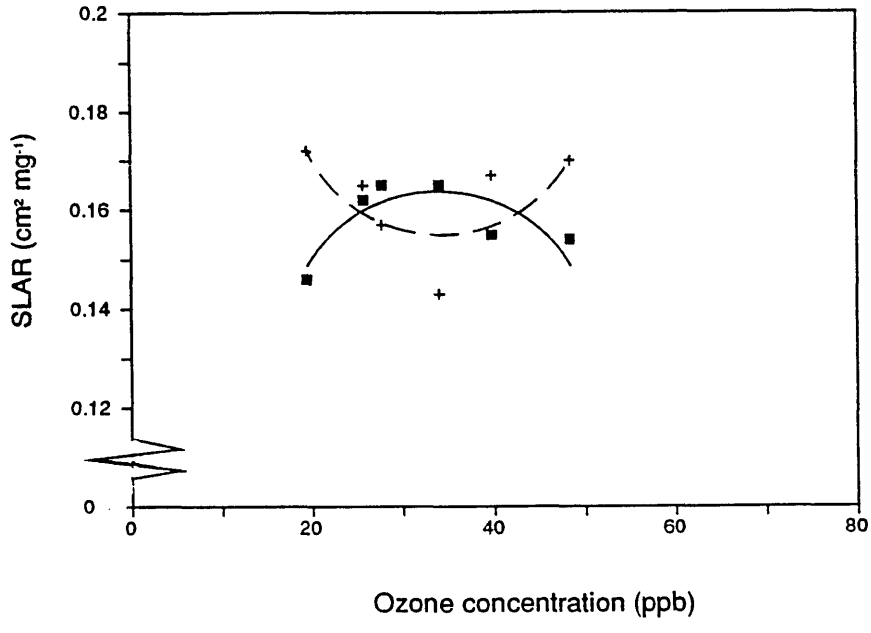
Summary of Analysis of Variance

	WS	O_3		O_3*WS	
		LIN	QUAD	LIN	QUAD
Episode 1 (Week 2)	0.02	1.77	0.61	0.65	0.26
'Recovery' 1 (Week 4)	0.48	0.31	0.01	0.21	0.01
Episode 2 (Week 6)	0.65	0.49	0.07	1.66	0.50
'Recovery' 2 (Week 8)	0.57	2.09	0.37	2.73	0
Episode 3 (Week 10)	1.02	0.01	0.15	0.01	**8.37** *

Measurements were made at the end of each episode (weeks 2, 6 and 10) and each 'recovery' period (weeks 4 and 8). Tabulated values are means of measurements made at dawn and at mid day.

Key as for Table 3.6.

Figure 3.23. Ozone/water stress interaction on specific leaf area ratio (week 10).



Specific leaf area ratio (SLAR) is plotted against mean cumulative ozone concentration. Plotted values represent treatment means for well watered (■), and water stressed (+) plants. The solid lines represent the fitted relationship between SLAR and ozone concentration for well watered plants; the broken lines represent the fitted relationship for water stressed plants.

Table 3.15

Final Destructive Harvest

Summary of Analysis of Variance

Parameter	WS	O_3		O_3*WS	
		LIN	QUAD	LIN	QUAD
Total plant dry weight	4.63 -	0.06	0.46	3.23	0.49
Above ground dry weight	5.28 -	0.04	0.71	1.87	0.09
Root dry weight	2.27	0.30	0.03	4.67 *	0.73
Root:shoot ratio	0.06	0.89	0.36	2.58	0.78
Total stem dry weight	3.88 -	0.05	0.67	1.61	0.40
'0 year' (1989) stem dry weight	5.88 -	0.01	0.03	2.62	0.53
Previous year (1988) stem dry weight	0.04	0.08	0.29	0.88	0.02
Pre 1988 stem dry weight	3.87 -	0.19	1.55	1.07	0.56
Leaf dry weight	7.15 -	0.91	0.57	2.14	0.08
Leaf area	9.35 -	0.29	0.28	1.18	0.26
Leaf area ratio	1.39	1.49	0.09	1.62	0.13
Specific leaf area ratio	0.22	1.45	0.49	1.42	4.12

Key as for Table 3.6. The covariate used in the analysis was the initial total shoot length, measured in 1988 before the treatments began.

The dry weight of 1989 stems was reduced by 15% in trees that had been water stressed, compared to those which had not. Water stress during the 1988 growing season also resulted in a 10% reduction in the dry weight of older shoots (those produced in the two years before the treatments began). The result of these reductions in current year and pre 1988 stem dry weight was a significant reduction in the total stem dry weight of plants that had experienced water stress (Figure 3.24).

As described above, plants that had been water stressed had significantly fewer leaves in 1989 than those that had been fully watered. This effect on leaf number was reflected in the destructive harvest by a 9% reduction in both leaf dry weight and leaf area (Figure 3.24). There were no significant effects of water stress on LAR or SLAR.

The effect of water stress on root growth and total plant size

There was no significant effect of water stress on root growth. The reductions in stem and leaf dry weight in water stressed plants described above resulted in a reduction in the above ground dry weight of these plants. The percentage reductions in root and above ground dry weight were very similar (9 and 8% respectively), resulting in a significant reduction in the total dry weight of plants that had been water stressed, but no significant effect on the root:shoot ratio (Figure 3.24).

4.5.3.2 Ozone/water stress interactions

There were no significant effects of ozone alone on any plant parameters measured in the 1989 final harvest. There was a significant ozone/water stress interaction on root dry weight.

The nature of the interaction between ozone and water stress on root growth is illustrated in Figure 3.25. Plants that had not experienced water stress showed a linear decrease in root dry weight as ozone dose increased. The mean root dry weight of plants which had received the highest ozone dose in 1988, was reduced by 10% compared to those plants in the control treatment. However, the opposite response to ozone was seen in plants that had been water stressed; root dry weight increased with increasing ozone dose. The root dry weight of plants in the highest ozone treatment was 14 % greater than that of those in the control treatment.

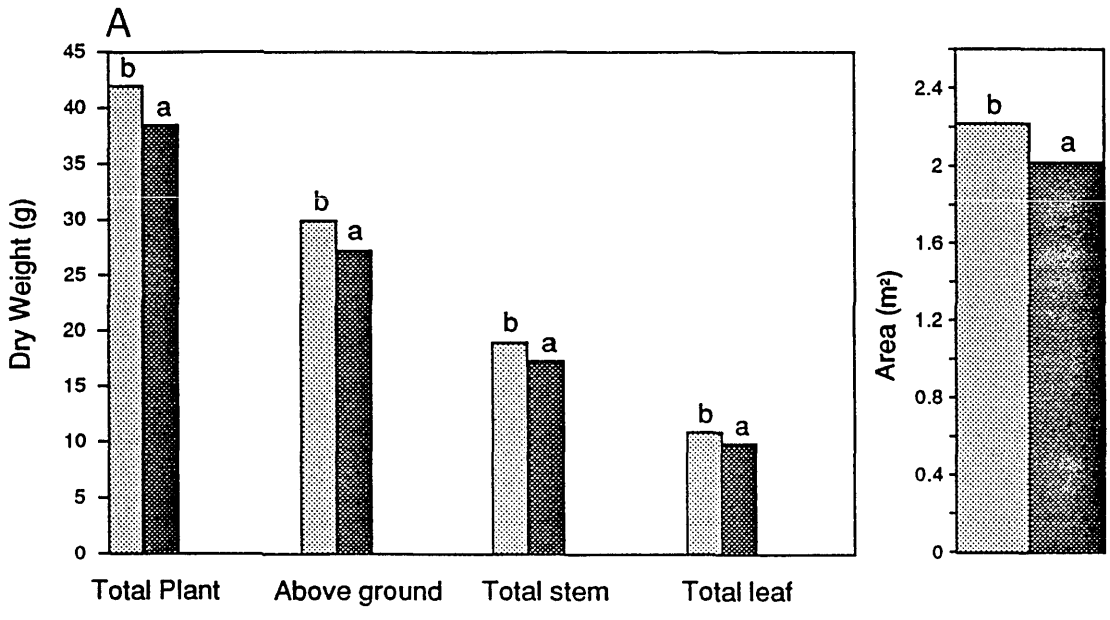
At mean cumulative ozone concentrations below 40 ppb the root dry weight of plants that had experienced water stress was less than that of plants that had not been water stressed. Above 40 ppb the trend was reversed, and the root dry weight of plants that had been

Figure 3.24. The effect of water stress on plant growth (final harvest).

A. Plotted values represent the mean total plant, above-ground, stem and leaf dry weight, and total leaf area of well watered (▒) and water stressed (▓) plants, averaged across all ozone treatments.

B. Plotted values represent the 'O' year (1989) stem dry weight (▓), 1988 stem dry weight (▒) and *pre* 1988 (□) stem dry weight of well watered and water stressed plants, averaged across all ozone treatments.

In each pair entries not headed by the same letter are significantly different at $p<0.05$.


A
Dry Weight (g)
45
40
35
30
25
20
15
10
5
0
b
a
b
a
b
a
b
a
Total Plant
Above ground
Total stem
Total leaf
Area (m²)
2.4
2
1.6
1.2
0.8
0.4
0
b
a

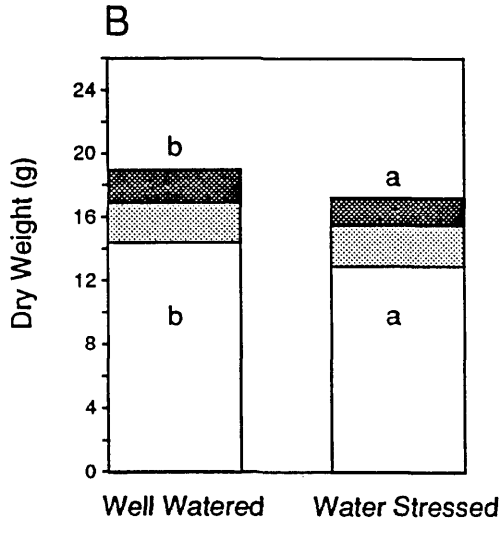
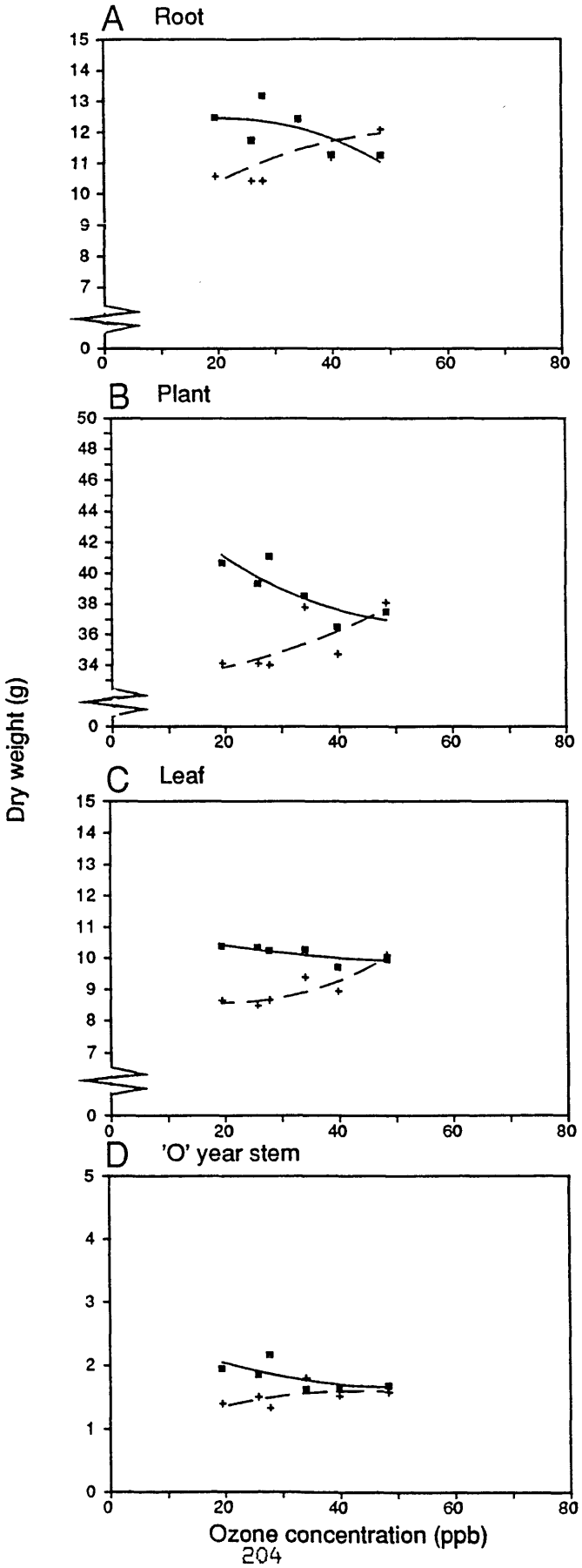

B
Dry Weight (g)
24
20
16
12
8
4
0
b
b
a
a
Well Watered
Water Stressed

Figure 3.25. Ozone/water stress interactions on plant growth (final harvest).

Root (A), total plant (B), leaf (C) and 'O' year stem (D) dry weight are plotted against mean cumulative ozone concentration. Plotted values represent treatment means for well watered (■), and water stressed (+) plants. The solid lines represent the fitted relationship between dry weight and ozone concentration for well watered plants; the broken lines represent the fitted relationship for water stressed plants.


A Root
B Plant
C Leaf
D 'O' year stem
Dry weight (g)
Ozone concentration (ppb)

water stressed exceeded that of plants that had not been.

Similar, non significant ozone/water stress interactions were seen on 'O' year shoot dry weight and leaf dry weight, and the effects of ozone and water stress on root, shoot and leaf dry weight resulted in an ozone/water stress interaction on total plant dry weight, which was significant at $p<0.10$ (Figure 3.25).

4.6 Visual assessments

4.6.1 Characterisation of damage symptoms

In mid July 1988, it was noted that chlorotic symptoms were developing on the leaves of many of the trees. These symptoms fell into two main categories. Firstly (Type 1 damage) an interveinal chlorotic striping of the leaves, and secondly (Type 2 damage) chlorotic patches or spots on the leaves (Plate 3.1).

In addition to these damage types, necrotic marks resulting from mechanical damage to the leaves were observed. This mechanical damage resulted mainly from sealing the leaves into the cuvette of the LCA-2, and from handling and moving the plants within and between the chambers.

Samples of damaged trees were taken to the Forestry Commission Pathology Advisory Service. The Service were unable to identify the Type 1 damage, but were able to confirm that it was not the result of a fungal infection, and speculated that it was caused by a viral infection. They identified the Type 2 damage as the result of infection by the fungus *Gloeosporium fagi*, the imperfect stage of *Apiognomonia errabunda*. It was observed that damage resulting from woolly aphid attacks accounted for some of the chlorotic and necrotic spots and speckles on the leaves.

4.6.2 Assessment of tree damage

Assessments of tree damage were made on six occasions, from mid July to the beginning of October. On the first occasion (22.7.88) only chlorosis was scored as damage had not yet developed into necrosis; on all other occasions both chlorosis and necrosis were scored. At the final assessment (3.10.88) there was a clear difference between the health of first flush and lammas leaves. Therefore, separate scores for first flush and lammas leaves were made for each tree.

Plate 3.1
Tree Health

Type 1 Damage

Type 2 Damage

The deterioration in tree health from week 4 to 14 is illustrated in figure 3.26. The more detailed assessment in week 14 showed that the lammas leaves were relatively healthy compared to the older leaves. It was observed that the lammas leaves looked healthier throughout the experiment, but this was not quantified.

4.6.2.1 The effect of water stress

Table 3.16 summarises the effect of water stress on tree chlorosis and necrosis. From week 6 of the experiment onwards, i.e., from the end of the second stress episode, water stressed plants had significantly higher scores of tree necrosis than fully watered plants (Figure 3.27).

In the fourteenth week of the experiment the level of necrosis of lammas leaves was much less than that of first flush leaves (Figure 3.27), but the adverse effect of water stress in increasing leaf necrosis was relatively greater in lammas leaves compared to the first flush leaves. On this day, the percentage increase in necrosis in water stressed trees (compared to fully watered trees) was 11% in first flush leaves, and 31% in lammas leaves.

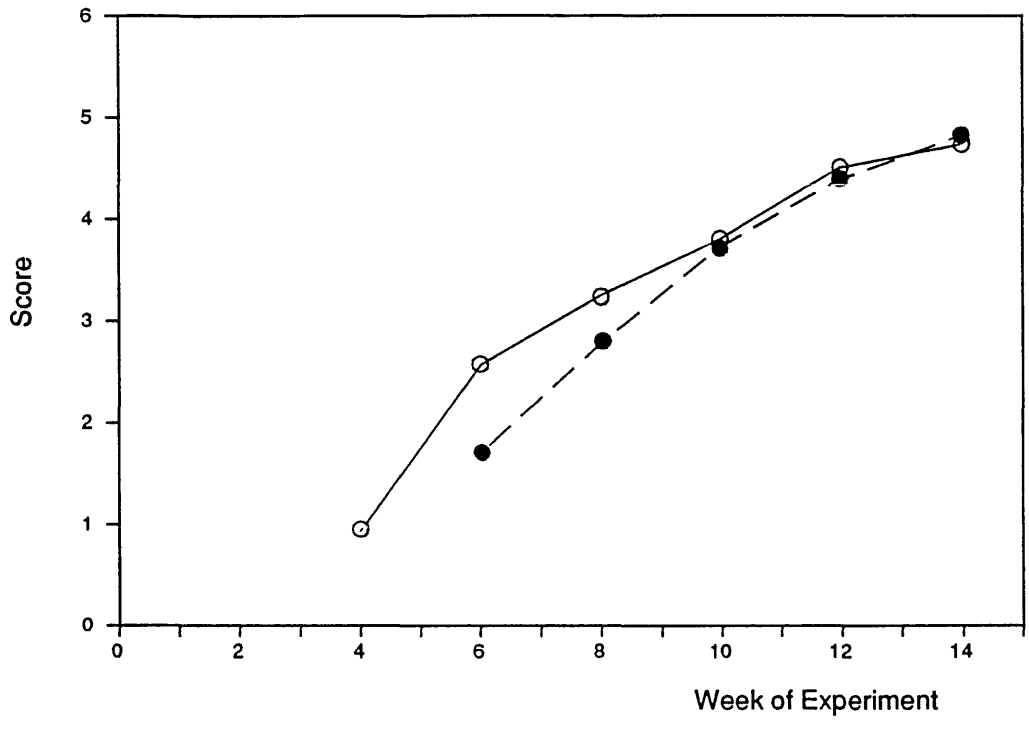
The only significant effect of water stress on tree chlorosis was seen in the last assessment, but was restricted to the lammas leaves. The chlorosis scores for plants that had been water stressed were 34% lower than those for plants that had not been water stressed.

4.6.2.2 The effect of ozone and ozone/water stress interactions

The effect of ozone on tree health is summarised in Table 3.17. Significant relationships between ozone and tree chlorosis were seen in weeks 6 and 12, and between ozone and tree necrosis in weeks 12 and 14. These relationships are illustrated in Figure 3.28. In week 6, the relationship between ozone and tree damage was a linear one, whilst in weeks 12 and 14 the relationships were quadratic.

The assessment made in week 6 of the experiment was made during the second ozone and water stress episode. At this stage there was a significant linear increase in overall tree chlorosis as ozone dose increased. Thus ozone appeared to have an adverse effect on tree health in the early stages of damage development.

Figure 3.26. Tree chlorosis and necrosis.



Chlorosis/necrosis score is plotted against week of experiment. Plotted values represent the mean score of chlorosis (○) and necrosis (●) averaged across all treatments.

Table 3.16

The effect of water stress on tree health

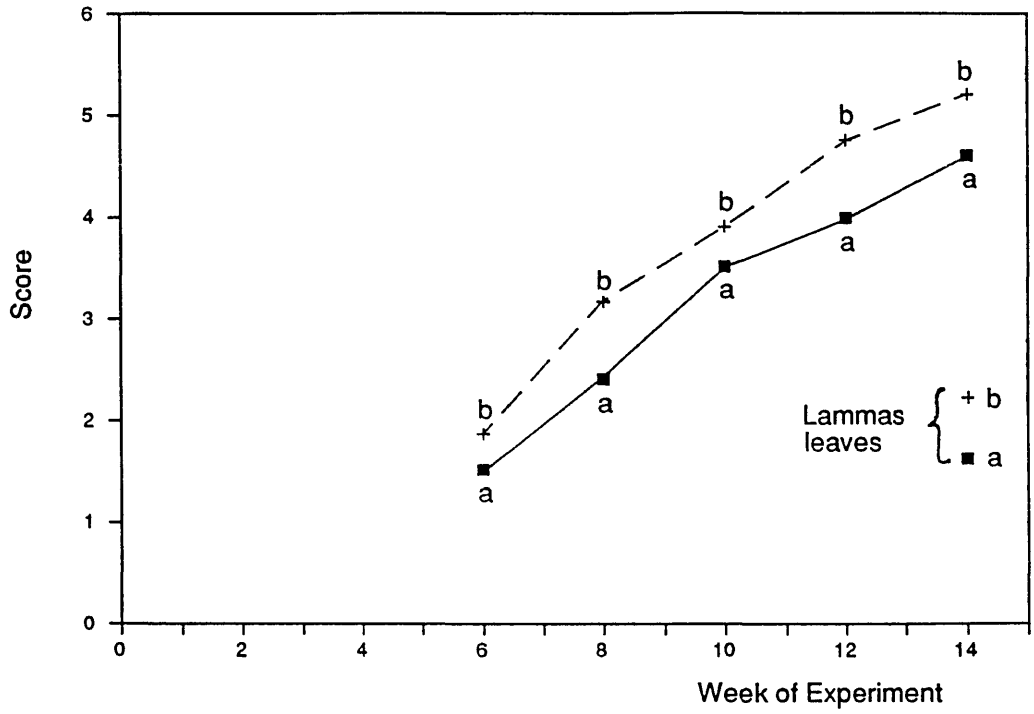
Summary of analysis of variance

Assessment Date (Week of experiment)		Tree Chlorosis	Tree Necrosis
22.7.88 (Week 4)		0.13	---
5.8.88 (Week 6)		0.24	**4.80** +
23.8.88 (Week 8)		2.34	**17.82** +
1.9.88 (Week 10)		0.03	**4.42** +
16.9.88 (Week 12)		0.09	**15.42** +
3.10.88 (Week 14)	**First flush leaves**	3.86	**14.01** +
	Lammas leaves	**10.92** -	**18.38** +

Tabulated values represent variance (F) ratios. F ratios in bold type are significant at $p<0.05$. + represents a significant increase in water stressed plants, - represents a significant decrease in water stressed plants.

Tree necrosis was not assessed in week 4.

Figure 3.27. The effect of water stress on tree necrosis.



Necrosis score is plotted against week of experiment. Plotted values represent the mean score for first flush leaves (with the exception of those points labelled 'lammas leaves') of well watered (– ■ –) and water stressed (– + –) plants, averaged across all ozone treatments. Within each week entries not headed by the same letter are significantly different at $p<0.05$.

Table 3.17

The effect of ozone on tree health

Summary of analysis of variance

Assessment Date (Week of experiment)		Tree Chlorosis		Tree Necrosis	
		LIN	QUAD	LIN	QUAD
22.7.88 (Week 4)		0.57	3.03	----	----
5.8.88 (Week 6)		**12.92** *	0.16	1.43	0.01
23.8.88 (Week 8)		0.30	0.11	0.02	0.82
1.9.88 (Week 10)		1.83	0.11	1.27	0.27
16.9.88 (Week 12)		2.05	**4.28** *	0.01	**6.56** *
3.10.88 (Week 14)	First flush leaves	3.73	1.53	0.01	**14.97** *
	Lammas leaves	1.57	0.62	1.60	0.01

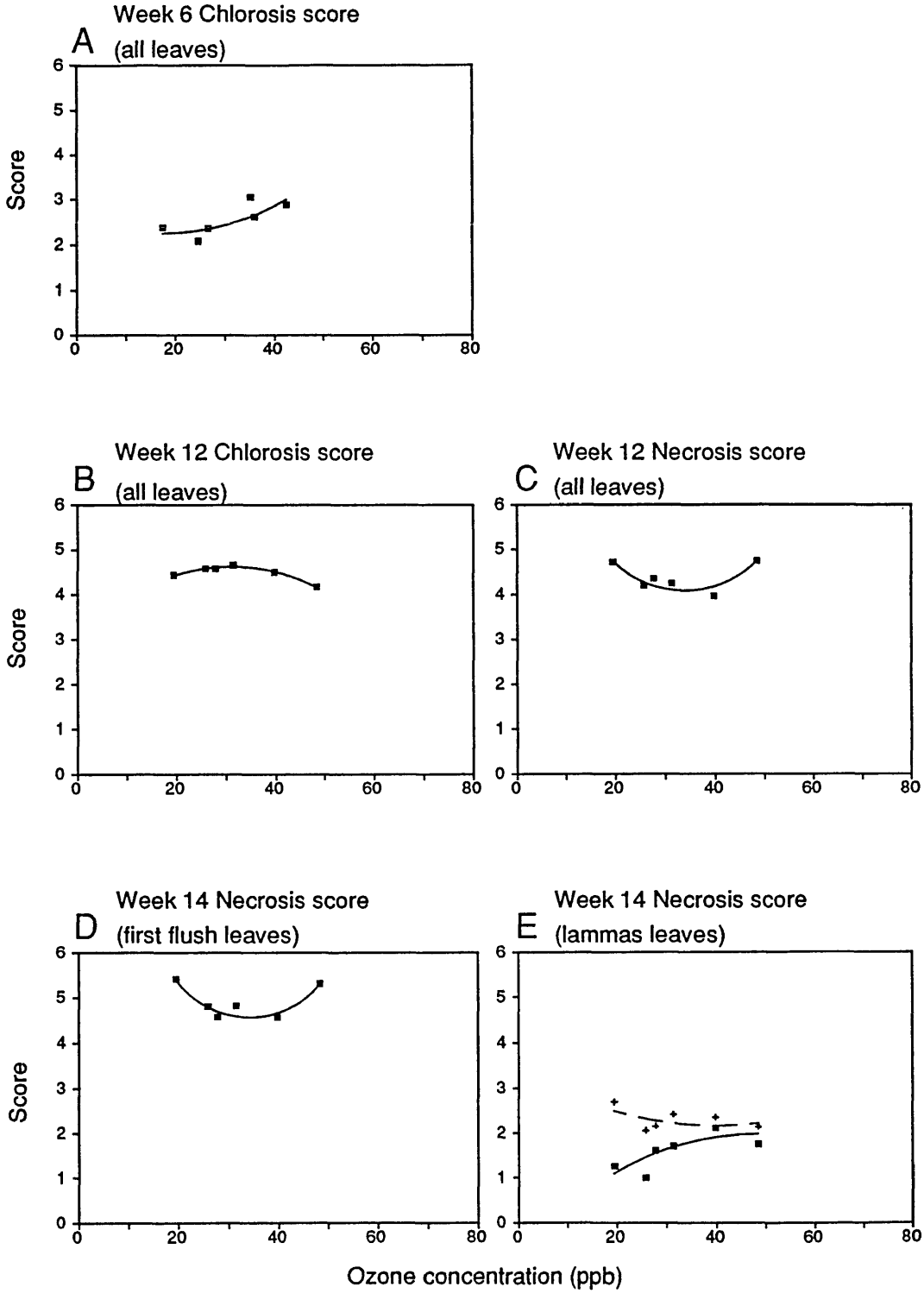
Tabulated values represent variance (F) ratios. LIN indicates a linear relationship with ozone (O_3) and QUAD indicates a quadratic relationship with ozone. F ratios in bold type are significant at $p<0.05$ and * represents a significant relationship with ozone.

Figure 3.28. The effect of ozone and ozone/water stress interactions on tree chlorosis and necrosis.

Chlorosis or necrosis score is plotted against mean cumulative ozone concentration.

A, B, C and D. Plotted values (▪) represent treatment means averaged across both water stress treatments. The solid lines represent the fitted relationship between score and ozone concentration.

E. Plotted values represent treatment means for well watered (▪), and water stressed (+) plants. The solid lines represent the fitted relationship between score and ozone concentration for well watered plants; the broken lines represent the fitted relationship for water stressed plants.


A
Week 6 Chlorosis score
(all leaves)
B
Week 12 Chlorosis score
(all leaves)
C
Week 12 Necrosis score
(all leaves)
D
Week 14 Necrosis score
(first flush leaves)
E
Week 14 Necrosis score
(lammas leaves)
Score
Ozone concentration (ppb)
0
1
2
3
4
5
6
0
20
40
60
80

Later on in the experiment (weeks 12 and 14), when the damage development was advanced, tree necrosis was decreased in plants that had been exposed to the intermediate ozone doses, but not in those plants in the highest ozone treatment. Necrosis scores for plants in the control and the highest ozone treatment were very similar.

There was a significant interaction between ozone and water stress on necrosis of lammas leaves at the final assessment ($F=5.34$, $p<0.05$). Unlike the older, first flush leaves, the majority of the lammas leaves grew during the experimental period. The lammas leaf necrosis scores for trees that had not experienced water stress increased linearly as ozone dose increased. The necrosis score of leaves of trees that had been water stressed decreased with increasing ozone dose (Figure 3.28).

4.7 Autumn leaf loss

Leaf loss ceased when, on average, 80 % of the leaves had fallen off the trees. This behaviour, of retaining some dead leaves on the tree over winter, is common in beech saplings.

4.7.1 The effect of water stress

The effect of water stress on leaf loss is summarised in Table 3.18. The first two weeks of the leaf collection coincided with the third ozone and water stress episode. There was no significant effect of water stress on the rate of leaf loss during this stress episode. However, in each week of the collection the percentage of leaves which fell off the trees that had been water stressed was greater than the percentage which fell off the trees that had not been water stressed (Figure 3.29). This difference was significant (in terms of percentage leaf dry weight and percentage leaf number) over two monthly periods; one soon after the onset of leaf fall, and the other over the last month of leaf fall.

The rate of leaf fall was increased in trees that had been water stressed, and the percentage of leaves remaining on the trees at the end of the Autumn leaf fall was reduced.

4.7.2 The effect of ozone

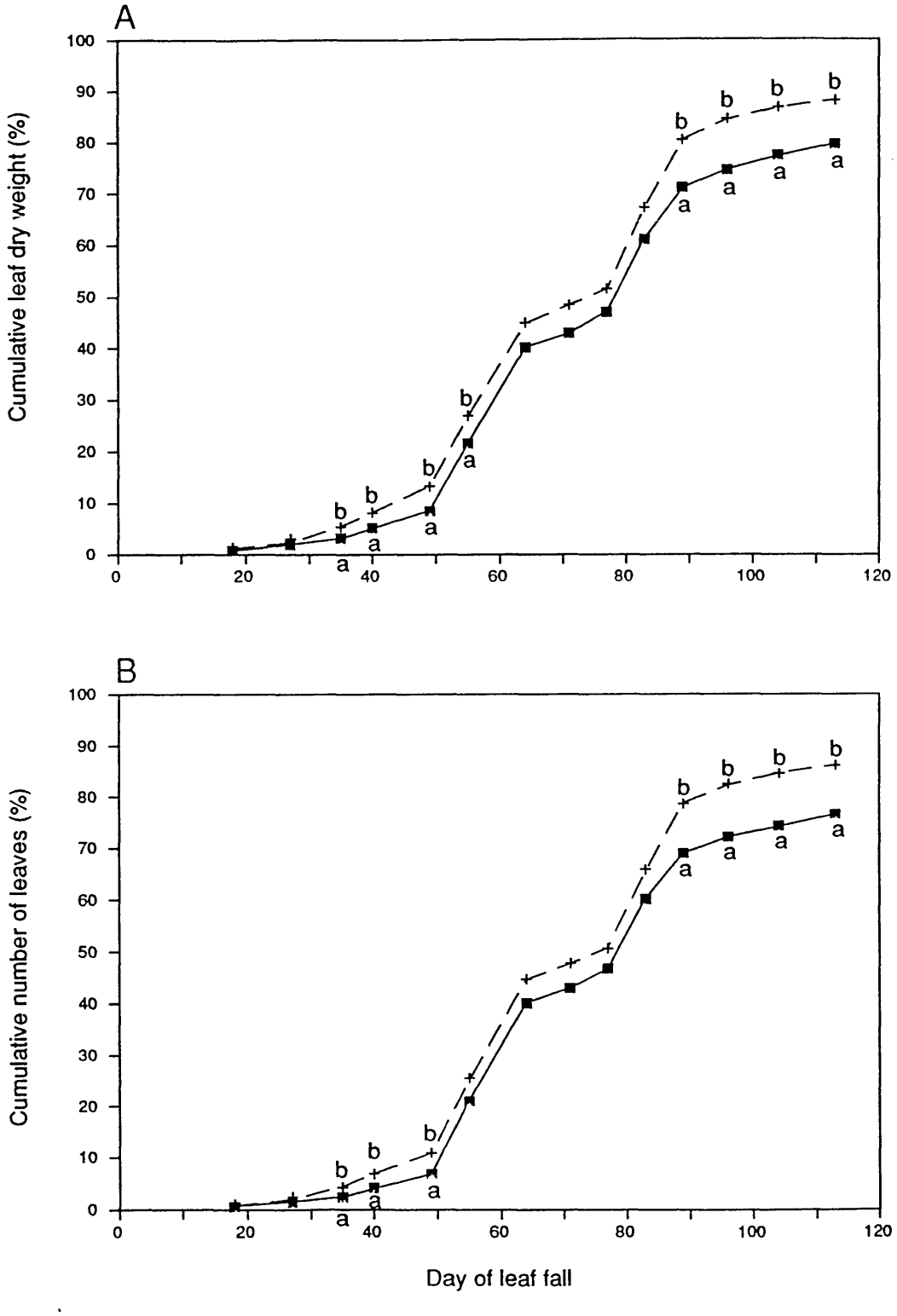
Exposure to ozone had no significant effect on the rate of Autumn leaf loss.

Table 3.18
The effect of water stress on autumn leaf fall
Summary of analysis of variance

Tabulated values represent variance (F) ratios. F ratios in bold type are significant at $p<0.05$. + represents a significant increase in water stressed plants and - represents a significant decrease in water stressed plants.

Leaf Collection		Cumulative percentage	
Week of collection	Day of collection	Leaf dry weight	Leaf number
2	18	1.71	2.07
3	27	2.99	3.23
4	35	**4.64** +	**5.33** +
5	40	**4.98** +	**5.14** +
6	49	**6.22** +	**6.20** +
7	55	**3.91** +	3.04
8	64	2.09	1.80
9	71	2.59	2.01
10	77	1.60	1.16
11	83	2.48	2.08
12	89	**5.45** +	**5.25** +
13	96	**6.54** +	**6.05** +
14	104	**6.60** +	**6.60** +
15	113	**6.00** +	**6.34** +

Figure 3.29. The effect of water stress on autumn leaf fall.



Cumulative percentage leaf dry weight (A) and number of leaves (B) are plotted against day of leaf fall. Plotted values represent means for well watered (– ■ –) and water stressed (– + –) plants, averaged across all ozone treatments. Within each day entries not headed by the same letter are significantly different at $p<0.05$.

4.8 Spring bud burst

4.8.1 The effect of water stress

This effect of water stress during 1988 on the rate of bud burst in the following spring is illustrated in Figure 3.30.

There was a significant effect of water stress on the rate of bud burst from the 8th to the 19th of May inclusive (Table 3.19). On these four assessment days, the number of trees at or above the mean bud burst stage was significantly less in the treatment group that had been water stressed, compared to the group that had been fully watered for the duration of the experiment. At the final bud burst assessment there was no significant effect of water stress.

4.8.2 The effect of ozone

The ozone dose experienced by the trees in 1988 had no significant effect on the rate of bud burst in Spring 1989.

Table 3.19

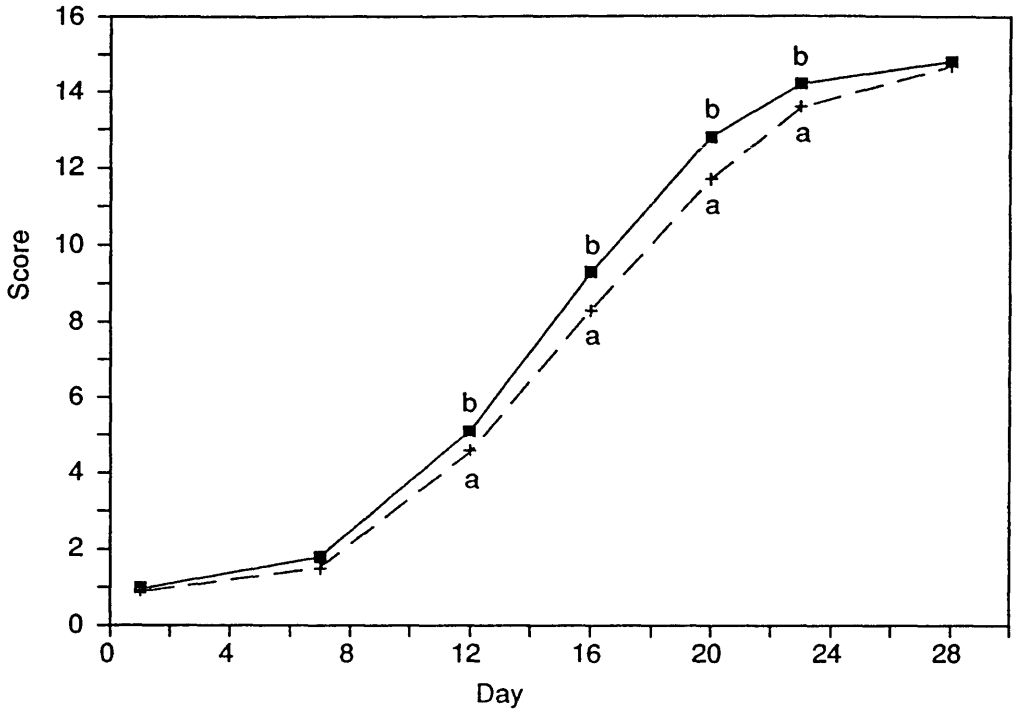
The Effect of Water Stress on Spring Bud Burst Score

Summary of Chi^2 analysis

Date	Day of bud burst	Value of Chi^2
27.4.89	1	0.41
3.5.89	7	1.36
8.5.89	12	**4.70** -
12.5.89	16	**5.09** -
16.5.89	20	**8.83** -
19.5.89	23	**7.65** -
24.5.89	28	0.57

Values of Chi^2 in bold type are significant at $p<0.05$. - represents a significant decrease in bud burst score in water stressed plants.

Figure 3.30. The effect of water stress on spring bud burst.



Bud burst score is plotted against day from beginning of bud burst. Plotted values represent means for well watered (— ■ —) and water stressed (— + —) plants, averaged across all ozone treatments. Within each day entries not headed by the same letter are significantly different at $p<0.05$.

5 Discussion

5.1 Ozone Fumigation

The ozone exposure characteristics for treatments 1-6 are summarised in Table 3.20, for comparison with equivalent data collected at rural monitoring stations in the U.K., which are summarised in Table 3.21.

The maximum, mean, and range of ozone concentrations in each treatment were typical of the range of concentrations monitored at rural sites in the U.K. The number of hours for which the plants were exposed to ozone concentrations exceeding 60, 100 and 120 ppb, were typical of field conditions; the number of hours above 60 ppb ranged from 0 to 191 in treatments 1 to 6, and from 2 to 658 (at a number of sites in a number of years) in the field. The maximum hourly ozone concentrations administered were also highly representative of rural U.K. conditions. They ranged from 58 ppb in treatment 1, to 198 ppb in treatment 6; for comparison, the equivalent data monitored at 6 rural sites ranged from 63 to 258 ppb. The cumulative 24 hour mean ozone concentrations for the whole experiment ranged from 13 ppb in treatment 1 to 26 ppb in treatment 6, and therefore the mean for all treatments was below the rural U.K. average of 27 ppb. The low 24 hour means experienced by plants in all treatments were probably the consequence of the relatively low night time concentrations administered.

5.2 Water Stress

The development of soil water stress was steady, and soil water deficits did not exceed 45 %. This level of soil water stress did not result in a significant reduction in the dawn or mid day relative water content of leaves of water stressed plants, but did result in reduced leaf conductance and an increase in leaf necrosis.

There was no effect of water stress on the pre dawn relative leaf water content (RLWC). At the end of each episode the mid day RLWC of water stressed plants was less than that of well watered plants, but not significantly so. The small effect of soil water stress on RLWC was the consequence of plant water stress developing in well watered as well as water stressed plants; at the end of each episode the fall in RLWC between dawn and midday was very similar in well watered and water stressed plants (Table 3.22).

After each recovery period, in the absence of water stress, the mid day RLWC of plants that had been water stressed was greater than that of plants that had not been water stressed. After the second 'recovery' period this increase was significant, and was dependant on ozone dose; the increase was restricted to water stressed plants in the low

Tables 3.20 and 3.21
Comparison of Experimental Ozone Treatments and Ozone Data from Rural Monitoring Stations in the U.K. (1972-1985)

Table 3.20
Summary of Experimental Ozone Treatments

Table 3.21
Ozone Data from Rural Monitoring Stations in the U.K. (1972-1985)

Number of Hours per Year Exceeding 60, 100 and 120 ppb Ozone, Maximum Hourly Mean Ozone Concentration and Summertime Means (April to September). From UK PORG (1987)

Table 3.20

Treatment	No. of hours exceeding			Max. hourly mean conc.	Cumulative mean	
	60 ppb	100 ppb	120 ppb		8 hour	24 hour
1	0	0	0	58	19.5	13.1
2	24	0	0	98	25.8	16.3
3	52	5	0	120	27.8	16.7
4	83	31	18	138	34.1	20.3
5	127	56	26	192	39.8	21.9
6	191	79	42	198	48.4	25.5

Table 3.21

Site	Number of years data	Number of hours per year exceeding			Max. hourly mean concentration (ppb)	Summertime 24 hour mean concentration (ppb)
		60 ppb	100 ppb	120 ppb		
Devilla (Fife)	3	3-24	0	0	74-99	21-23
Bottesford (Leics.)	8	54-658	0-39	0-14	79-144	22-34
Sibton (Suffolk)	9	2-358	0-95	0-47	63-207	19-37
Stevenage (Herts.)	10	7-324	0-115	0-60	68-207	8-28
Harwell (Oxon.)	4	70-261	1-154	0-111	102-258	33-36
Ascot (Berks.)	9	39-210	0-20	0-5	82-137	---
All sites	43	2-658	0-115	0-111	63-258	8-37

Table 3.22
The difference between dawn and mid day relative leaf water content at the end of each episode and each 'recovery' period

	Well watered plants	Water stressed plants
End of episode 1	-11.9	-11.4
End of 'recovery' period 1	-29.4	-17.8
End of episode 2	-14.6	-14.0
End of 'recovery' period 2	-21.3	+11.5
End of episode 3	-3.6	-5.1

Tabulated values are averaged across all ozone treatments. A negative value indicates a decline in relative leaf water content from dawn to mid day, while a positive value indicates an increase.

ozone treatments. These results suggest that in the absence of ozone some adaptation to water stress occurred. The possible mechanisms of such an adaption are numerous, but one possible explanation is provided by the gas exchange data from the end of the second 'recovery' period. On this day, in the absence of water stress, reduced leaf conductance was observed in plants that had been water stressed, but only in the control ozone treatment. RLWC (averaged over all treatments) did fall over the course of this day, and so it appears that in the absence of ozone the stomata of plants that had been water stressed closed more rapidly in response to increased evaporative demand than those of plants that had not been water stressed. Such stomatal closure in the absence of soil water stress could account for the increase in mid day RLWC in plants that had been water stressed on this day (Table 3.22).

5.3 Gas Exchange

At the end of the first ozone/water stress episode significant increases with ozone concentration in g_l and P were seen, which were linear with respect to ozone concentration. At 1000 there was a linear decline in Ci with increasing ozone concentration. Increases in g_l and P were more frequently seen in water stressed plants, at least in the morning measurements. In the control ozone treatment water stress resulted in reductions in g_l and P. This was not the case in all the other ozone treatments.

Two weeks later, at the end of the first 'recovery' period, a linear decline in g_l, and increase in P and Ci, with increasing ozone concentration was seen, and there were no ozone/water stress interactions.

At the end of the second episode significant ozone/water stress interactions on g_l and P were seen in the morning measurements; water stress resulted in reductions in g_l and P in the low and high ozone treatments, but not in the intermediate ozone treatments. At one time in the morning there was a linear increase in Ci with increasing ozone concentration, and at one time in the afternoon there was a linear decline in P with increasing ozone concentration.

Significant interactions between ozone and water stress on g_l, p and Ci were seen at the end of the second 'recovery' period, indicating that differential gas exchange responses to ozone in plants which have, and have not, experienced water stress are still found in the absence of water stress two weeks after the end of the ozone episode. Significant ozone/water stress interactions on g_l were seen throughout the day; in the control ozone

treatment the g_l of plants that had been water stressed was much less than those plants that had not been, and in plants that had not been water stressed there was some evidence of stomatal closure in the intermediate ozone treatments.

After the third ozone/water stress episode g_l and P were increased in intermediate ozone treatments, but not in the highest treatment. In agreement with measurements made at the end of the first episode, significant ozone/water stress interactions on g_l and Ci provided some evidence that effects of ozone on gas exchange were dependant on water stress.

Increases in conductance and photosynthesis in response to ozone are not widely reported in the literature. However in other experiments with *F. sylvatica*, exposure to low concentrations of ozone has been reported to cause both increases and decreases in leaf conductance and photosynthesis. Leonardi (pers. comm.) exposed seedlings of *F. sylvatica* to 110 ppb ozone for five days per week, for ten weeks. He observed a significant increase in the stomatal conductance of fumigated plants in weeks 1-4, and a significant decrease from week 5 onwards. This is a very similar response to ozone to that observed in the highest ozone treatment in the present experiment, despite the fact that in this experiment measurements were made on well watered, and not water stressed trees. In contrast, Leonardi reports that P was significantly inhibited throughout the experiment, with the magnitude of inhibition increasing with increasing duration of exposure.

In a filtration experiment in Southern Britain, Taylor & Dobson (1989) observed significant effects of air quality on the gas exchange of *F. sylvatica* saplings. The leaf conductance of first flush leaves was significantly decreased (at $p<0.05$), and of lammas leaves was significantly increased (at $p<0.10$) in non filtered compared to filtered air. A/Ci analysis of lammas leaves in the laboratory showed increases in the maximum rates of photosynthesis in polluted air. In this study, significant effects on gas exchange were observed in well watered but not water stressed plants. This is in contrast to the results from the present work, in which effects of ozone on gas exchange were observed more frequently in water stressed than well watered plants. However, these results, like mine and those of Leonardi, do suggest that ozone at low concentrations increases conductance, and at higher concentrations decreases conductance, since the authors suggest that one explanation for the difference in response of leaves from the first and second flush may be that episodes of ozone occurred primarily during May and June, prior to the onset of lammas growth.

Throughout this experiment in the low ozone treatments, water stress resulted in reductions in g_l, P and Ci. In the higher ozone treatments this was not always the case, and in these treatments gas exchange of water stressed plants frequently exceeded that of well watered plants. These ozone/water stress interactions provide some evidence that plants exposed to ozone have a reduced ability to control foliar water loss in response to water stress.

A loss of control of water loss from foliage to the atmosphere would be expected to increase plant water stress and indeed the effect of ozone on the mid day water status of water stressed plants was closely associated with the effect of ozone on leaf conductance. Reductions in mid day RLWC or leaf water potential in response to water stress in the highest ozone treatments at the ends of weeks 2 and 8, and in intermediate ozone treatments at the end of week 6, coincided with increases in g_l in water stressed plants in these same ozone treatments on these days.

Increased leaf conductance is unlikely to have resulted from treatment effects on cuticular transpiration, since Kerstiens & Lendzian (1989) report that exposure of leaves of *F. sylvatica* to 130 ppb ozone over a period of five months had no effect on the water permeability of the cuticle. Changes in stomatal density are unlikely to have increased leaf conductance, since measurements were made on fully expanded leaves. Increased transpiration would be expected from senescent leaves, and while there was evidence that water stress increased leaf necrosis and accelerated senescence, there were no ozone/water stress interactions on the health of first flush leaves, and no evidence that ozone accelerated leaf senescence. When leaves were selected for gas exchange measurements attempts were made to select healthy leaves, and these attempts were successful; analysis of the chlorosis and necrosis scores made for the individual leaves on which gas exchange measurements were made revealed that there were no significant effects of ozone or water stress on leaf health.
It appears most likely that increases in gas exchange were the result of direct effects of ozone and water stress on the photosynthetic or stomatal apparatus.

Water stress or ozone may initially alter mesophyll activity resulting in an increase or decrease in Ci and the closure or opening of stomata, respectively. Conversely, the stomata themselves may also be directly altered by exposure to ozone or water stress. The measurements of Ci enable some interpretation of the underlying mechanisms of effects of ozone and water stress on gas exchange, but in this experiment there are difficulties in interpretation since the gas exchange measurements were not made under constant light conditions, and very few measurements were made at light saturation (Figure 3.31).

Figure 3.31. Photosynthesis plotted against light intensity.

Net photosynthesis (ordinate) in umol m^{-2} s^{-1} is plotted against photon flux density (abscissa) in umol m^{-2} s^{-1}. Values for individual plants are plotted. Measurements were made at 0700, 1000, 1300, 1600 and 1900 at the end of week 2, 4 and 8, and at 0700, 1000, 1300 and 1600 at the end of week 6 and 10.

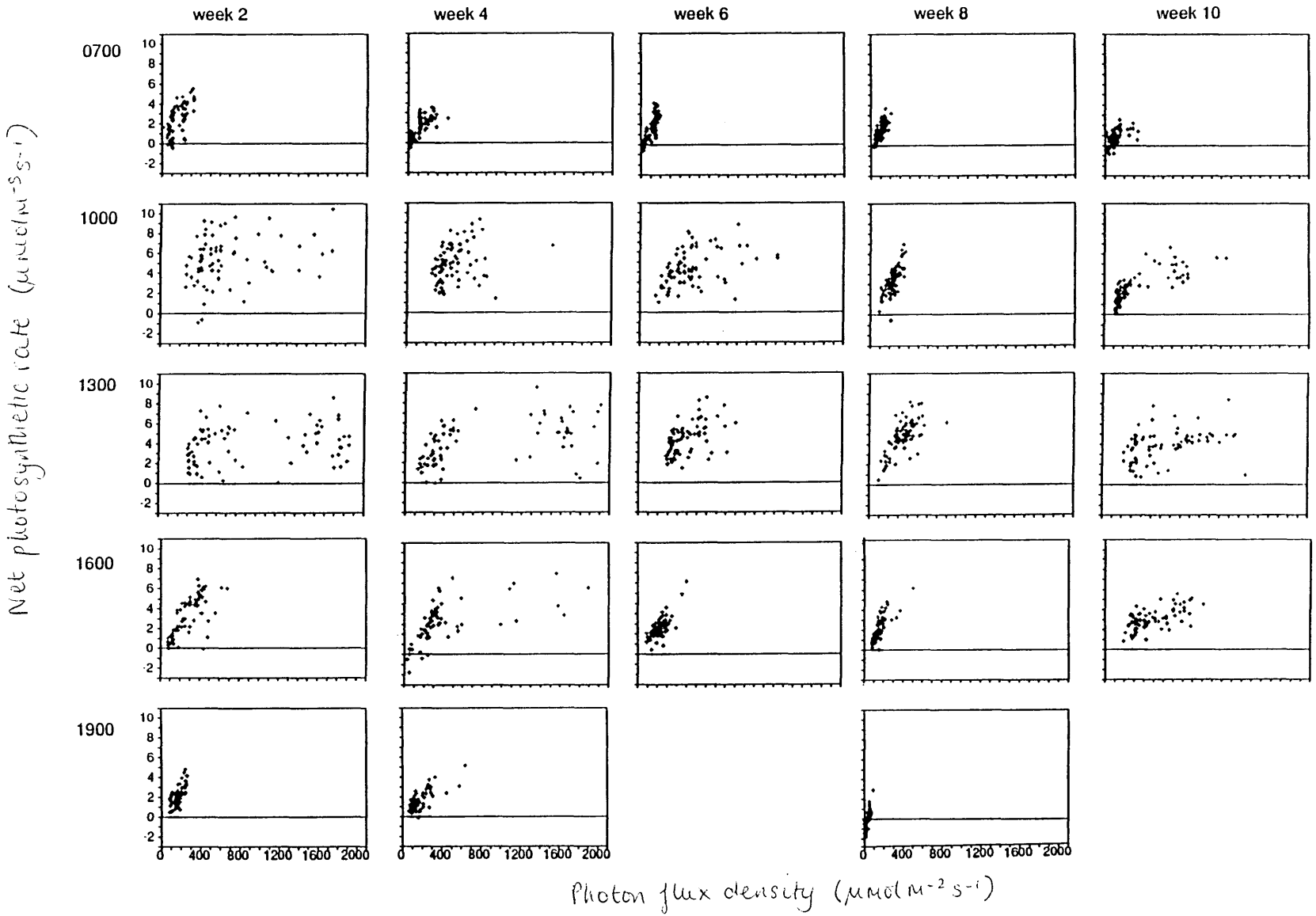
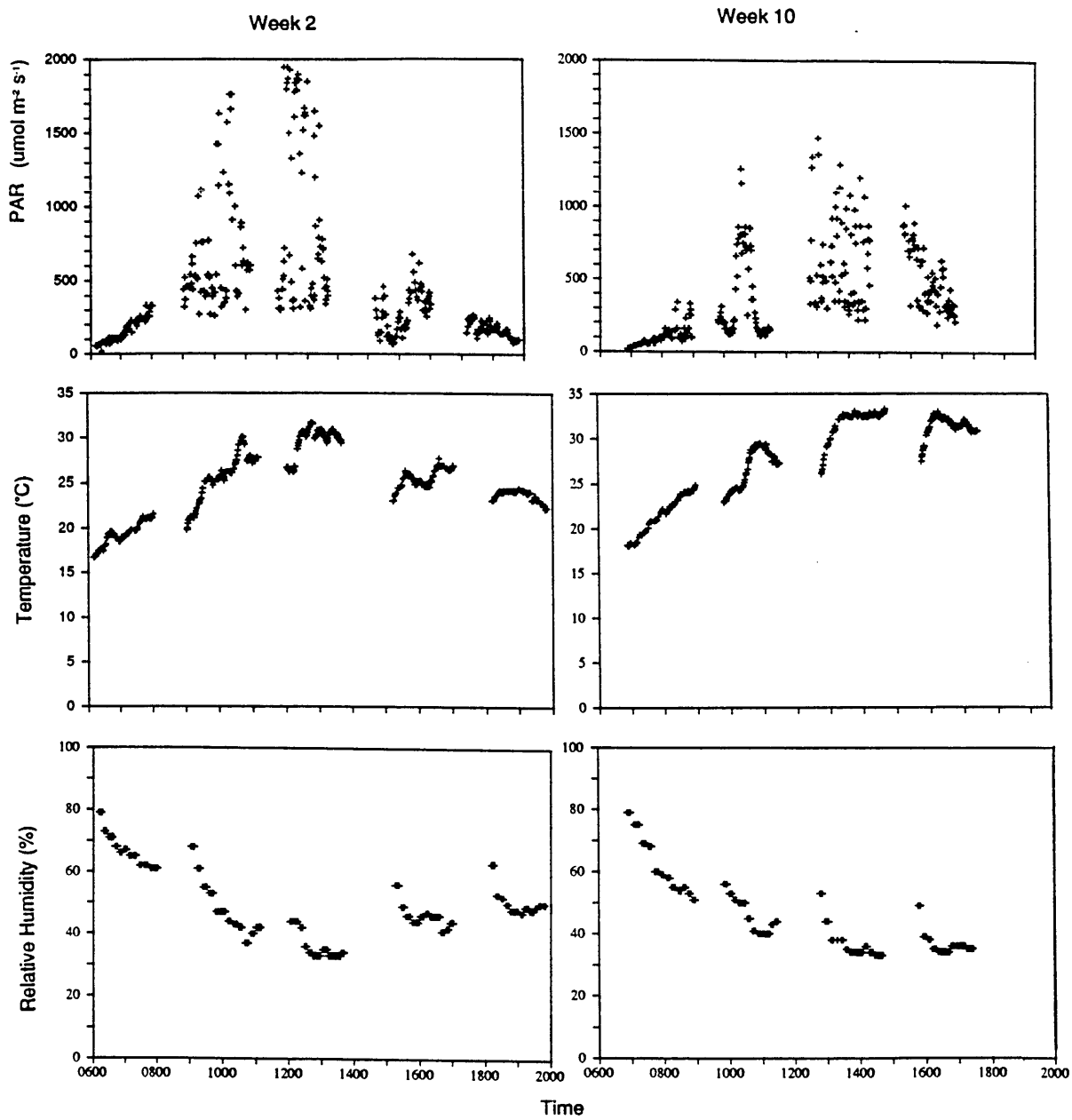

week 2
week 4
week 6
week 8
week 10
0700
1000
1300
1600
1900
Net photosynthetic rate (μmol m⁻² s⁻¹)
Photon flux density (μmol m⁻² s⁻¹)
10
8
6
4
2
0
-2
0
400
800
1200
1600
2000

Figure 3.32. The daily time course of light intensity, air temperature and relative humidity in the fumigation chambers (weeks 2 and 10).



Photon flux density (PAR), air temperature, and relative humidity are plotted against time of day. Values for individual plants are plotted. Measurements were made with the LCA-2 at plant height in the fumigation chambers.

The fact that in the control ozone treatment Ci was decreased in water stressed plants suggests that stomatal responses were dominant in the inhibition of P by water stress, and that water stress caused stomatal closure, a fall in Ci, and thus an inhibition of P.

The linear increase in g_l and P with increasing ozone concentration in water stressed plants at the end of week 2 was accompanied by a linear decrease in Ci at the same time. These results suggest that at this time increases in gas exchange were in least part due to direct effects of ozone and water stress on photosynthetic processes, and that increased rates of P led to a depletion of Ci, and stomatal opening. Later on in the experiment it appeared that stomatal responses were dominant; in weeks 8 and 10 effects of ozone and ozone/water stress interactions on g_l were more frequent than effects on P, and values of Ci in both well watered and water stressed plants showed the same direction of response to ozone as values of g_l.

Temporal changes in the effect of ozone on P may have been due to increasing leaf age, as gas exchange measurements were always made on first flush leaves and P rates in all species change as leaves age (Reich 1983). Reich (1983) observed that effects of ozone on the photosynthetic rate of poplar leaves were greatest in older leaves.

It is not clear from these results why plants respond differently to ozone if water stressed, and why exposure to both ozone and water stress resulted in reduced control of stomatal water loss. Significant ozone/water stress interactions on g_l and P were seen in the morning measurements and throughout the day at the end of week 8, and at all these times incident levels of PAR were relatively low (Figure 3.31). Therefore, it appears that water stressed plants responded differently to ozone only in low light conditions.

A possible explanation for this observation is that on bright sunny days, or during the afternoon, well watered plants experienced temporary water stress, due to increased evaporative demand resulting from relatively high mid day temperatures and low relative humidities. This might account for 'well watered' and water stressed plants responding in the same way to ozone, and might explain the timing of the ozone/water stress interactions. Figure 3.32 summarises the diurnal changes in PAR, air temperature and relative humidity in the fumigation chambers at the ends of weeks 2 and 10. On these days ozone/water stress interactions were seen on P and g_l in the morning measurements (0700 or 1000) while no interactions, but only effects of ozone were seen at various times from 1300 onwards. On both these days midday chamber temperatures exceeded 30 °C, and the difference between dawn and midday RLWC was very similar in well watered and water stressed plants (Table 3.22).

5.4 Tree Health

Fumigation with ozone had a significant effect on tree health, and resulted in both increases and decreases in leaf chlorosis and necrosis. The nature of the relationship between ozone dose and tree damage is similar to that for ozone dose and plant gas exchange. In the plant gas exchange measurements, the relationship between ozone dose and leaf conductance or photosynthetic rate changed from a linear relationship, at the beginning of the set of measurements, to a quadratic relationship at the end. The same is true for the scores of tree damage.

Linear increases in tree chlorosis with increasing ozone concentration were seen at the end of the second episode, while later in the experiment (week 12) chlorosis was increased in the intermediate ozone treatments but not in the highest treatment. In weeks 12 and 14 necrosis scores were reduced in intermediate ozone treatments, but not in the highest ozone treatment. In general lammas leaves were healthier than first flush leaves, and in week 14, water stress resulted in increased necrosis of lammas leaves in the low ozone treatments, but in the high ozone treatments necrosis scores were not affected by water stress; exposure to ozone appeared to reduce water stress-induced leaf injury.

The scores of tree chlorosis and necrosis were scores of an overall disease complex, identified by the Forestry Commission Pathology Advisory Service to consist of fungal (*Apiognomonia errabunda*), insect (*Phyllaphis fagi*), and possibly viral components. It is likely that the dominant component of this complex changed as the experiment progressed (perhaps due to climatic factors, leaf age, or intermittent aphid control) and these changes might explain differences in the effect of ozone treatment on tree health.

In general, populations of many insect herbivores are increased in the presence of moderate levels of pollutants (McNeill & Whittaker, 1990). However, aphids have shown contradictory responses to ozone fumigation (Whittaker & Warrington, 1990). Fluckiger & Braun (1986) found that the growth rate of *Phyllaphis fagi* on *F. sylvatica* was stimulated by exposure to ambient air on a motorway verge. Although ozone was a component of the motorway pollution, it was in combination with other gases, and the separate effects of individual gases could not be detected. In several fumigation studies of aphids on broadleaved trees ozone has not had a significant effect on aphid performance (e.g. Coleman & Jones, 1988).

Aphids are thought to be important in the spread of viral infections, and in a number of studies plant viruses have been shown to protect, partially or completely, a range of plants against ozone injury (eg. Vargo *et al.* 1978). On the other hand, tobacco streak virus

infection increased the sensitivity of tobacco plants to ozone (Reinert & Gooding, 1978).

Ozone displays fungicidal and fungistatic activity (Guderian, 1985). In controlled exposure experiments ozone has been demonstrated to reduce the performance of biotrophic fungal pathogens (Hibben & Taylor, 1975), and to have both inhibitory and stimulatory effects on non biotrophic fungi, depending upon the ozone dose (James *et al.*, 1980) and on the timing of fumigation in relation to innoculation (Heagle, 1977).

Water stress increased the amount of tree necrosis from the end of the second stress episode onwards. Infection with the fungus *Apiognomonia errabunda* resulted in large necrotic patches on the leaves, and this fungus has been reported to account for a large proportion of green leaf fall in declining beech trees (Roloff 1985). It is possible therefore, that the acceleration of leaf fall seen in water stressed trees in this experiment, was due in part to an indirect effect of water stress on the growth of this pathogen.

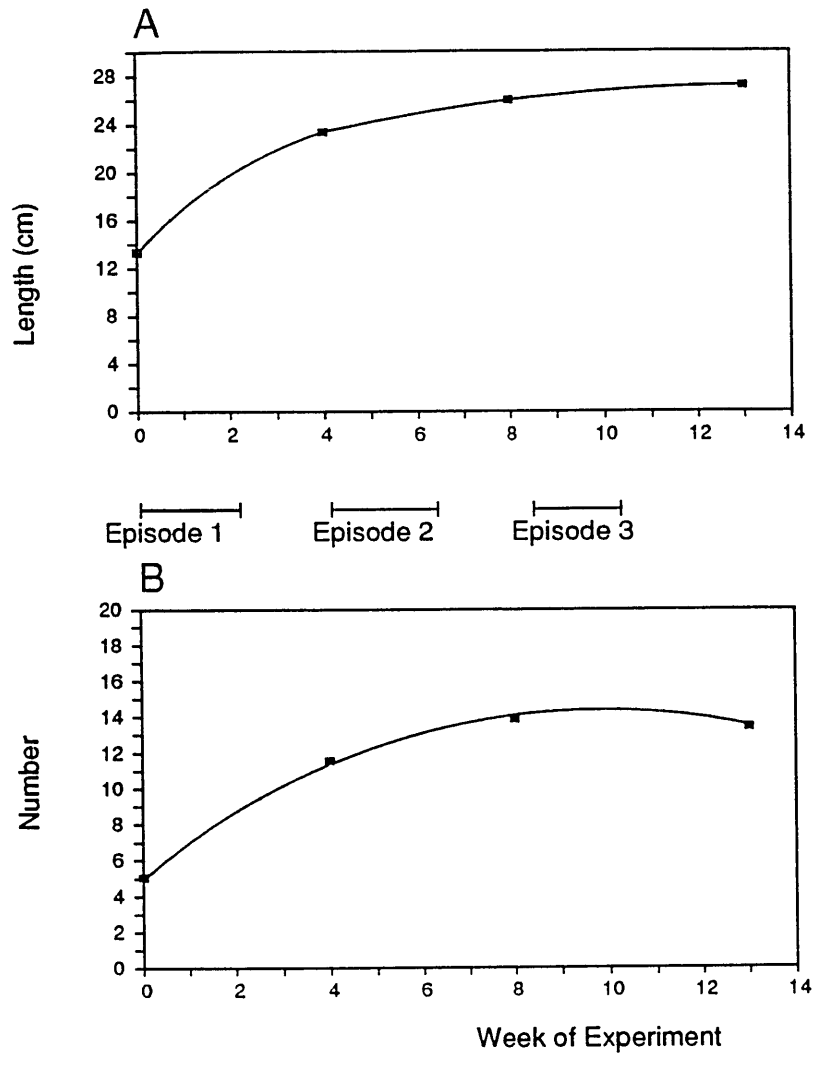
5.5 Growth

Exposure to ozone and water stress began in late June, after the spring flush of growth was complete. Therefore, it is not surprising that the majority of the effects of ozone and water stress on plant growth during the 1988 season were on lammas growth and secondary thickening.

The effect of water stress on lammas growth was to increase lammas leaf number and shoot length in weeks 4 and 8 of the experiment. Significant interactions between ozone and water stress were seen on the number of lammas leaves from week 8 of the experiment onwards, on lammas shoot length, and therefore total shoot length, in week 8, and on the SLAR of first flush leaves after the final episode. These interactions were all quadratic, and in all cases growth was greater in water stressed plants than in well watered plants in the control and highest ozone treatments.

The majority of lammas growth was complete by the fourth week of the experiment (Figure 3.33). Ozone and water stress effects on P seen at the end of the first episode provide one possible explanation for the increases in lammas growth in water stressed plants, since at this time P was increased in water stressed plants in all treatments except the control ozone treatment (Figure 3.34). These increases in P were driven by differential effects of ozone on the gas exchange of water stressed plants. However, interactions between ozone and water stress on gas exchange in week 2 do not explain the observed

Figure 3.33. The timing of lammas growth in relation to ozone and water stress episodes.

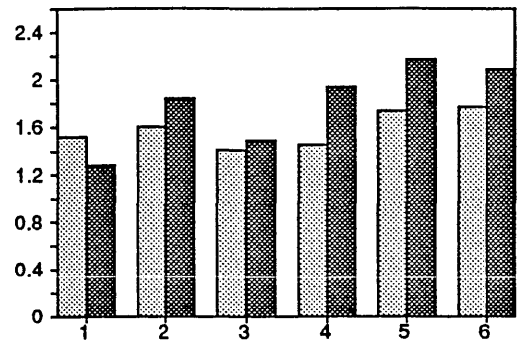


Lammas shoot length (A) and the number of lammas leaves (B) are plotted against week of experiment. Plotted values represent means averages across all treatments.

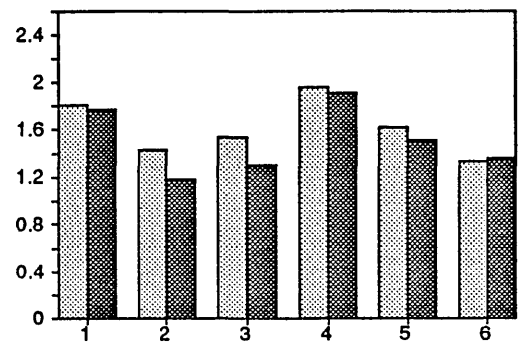
Figure 3.34. Summary of daily mean net photosynthetic rate.

Plotted values represent the treatment means for well watered () and water stressed () plants, averaged across all measurement times during the day.

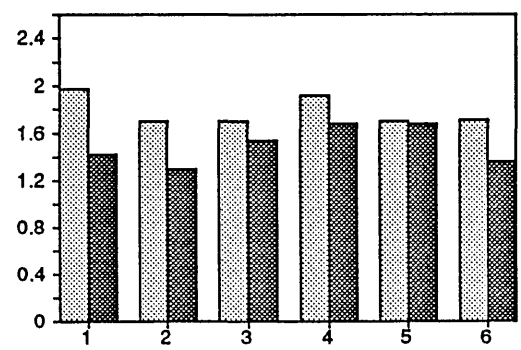
End of Episode 1



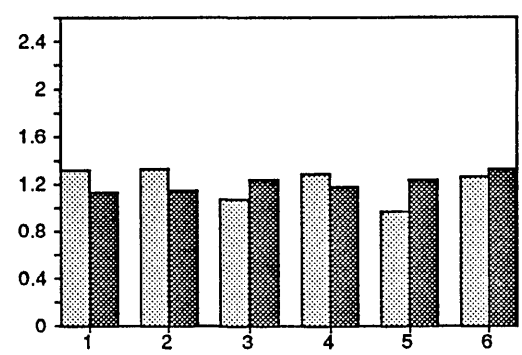
End of First 'Recovery'Period



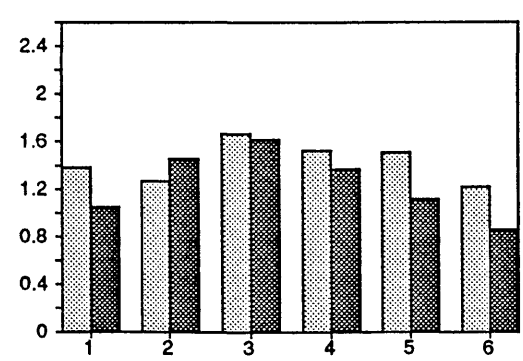
End of Episode 2



End of Second 'Recovery' Period



End of Episode 3



net photosynthetic rate ($\mu mol\ m^{-2}s^{-1}$)

Ozone treatment

ozone/water stress interactions on lammas growth, since interactions on gas exchange were linear, while those on growth were quadratic, and the nature of the interaction on P was very different to that on lammas growth.

Effects on ozone and water stress on lammas growth were unlikely to be mediated via effects on tree health, since significant treatment effects on health scores were not evident until week 6 of the experiment, after the majority of lammas growth was complete.

These data provide no clear explanation for the effects of ozone and water stress on lammas growth, although it should be noted that no measurements of gas exchange were made on the lammas leaves themselves. It is possible that changes in lammas growth resulted from effects of ozone and water stress on biomass partitioning which were not detected by the non destructive growth measurements, and were no longer present in the following spring.

The basal area of water stressed trees was reduced from week 8 of the experiment onwards. This reduction in radial thickening was apparent in the final harvest as a significant reduction in the pre 1988 stem dry weight.

Intermittent exposure to moderate water stress in 1988 resulted in considerable growth reductions at the final harvest. Plants that had been water stressed had significantly reduced total plant dry weights, this was the result of a reduction in above ground growth; leaf and stem dry weight were reduced in plants that had been water stressed. Leaf production in 1989 was also reduced by exposure to water stress, leaf number and area were significantly reduced.

Exposure to ozone alone had no significant effect on any plant parameters in 1989, but there were ozone/water stress interactions on root dry weight ($p<0.05$) and total plant dry weight ($p<0.10$). There was no effect of ozone or water stress on root:shoot partitioning. The total and root dry weight of plants that had not been water stressed declined with increasing ozone concentration. The total and root dry weight of plants that had been water stressed increased with increasing ozone concentration. At ozone concentrations less than 40-45 ppb, the growth of water stressed plants was less than that of well watered plants; above 40-45 ppb the root and total dry weight of water stressed plants exceeded that of well watered plants, ie. exposure to ozone at these concentrations appeared to 'protect' plants from water stress.

Carbon gain integrated through time for a whole plant is dependant not only on the rate of carbon acquisition per unit leaf tissue but also on the amount of photosynthetic tissue present, as well as its residence time on the plant (Mooney & Winner, 1988). Therefore, effects of ozone and water stress at the final harvest may have been due to treatment effects on P, leaf production, tree health (chlorosis and necrosis), autumn leaf fall, or spring bud burst, or to a combination of these factors.

Growth reductions in water stressed plants could have been the consequence of a combination of all of these factors, since water stress resulted in significant inhibition of photosynthesis, increased leaf necrosis, accelerated autumn leaf fall, and delayed bud burst in the following spring.
The ozone/water stress interaction on root growth at the final harvest was unlikely to be the result of treatment effects on leaf fall, bud burst, or the health of first flush leaves, since there were no significant interactions on any of these parameters.

The significant interactions on lammas growth in 1988 were quadratic and do not explain the linear interactions seen at the final harvest. It is perhaps not surprising that treatment effects on lammas growth were not related to longer term growth effects since the proportion of lammas leaves was small (maximally 14 % of the total leaf number).

Ozone/water stress interactions on gas exchange were seen throughout the exposure period, and it is likely that treatment effects on conductance and P were important in determining effects on growth at the final harvest. Effects on root growth were linear with respect to ozone dose, and this suggests that interactions on P at the beginning of the experiment may have been of greater importance in terms of long term growth effects than those quadratic interactions on P seen later in the experiment. At the end of the first episode P was reduced by water stress in the control ozone treatment, but increased in water stressed plants in all the other ozone treatments (Figure 3.34). Two weeks later, at the end of the first recovery period, water stress resulted in reductions in P in all ozone treatments except the highest ozone treatment, in which there was a slight increase in P in the water stressed plants. It appears that increased rates of P in plants exposed to both ozone and water stress result in growth stimulations in these plants.

It is perhaps not surprising that the greatest effect of ozone at the final harvest in this experiment was on root growth, since the experimental treatments did not begin until late June. In temperate latitudes the root and shoot growth of trees follow distinct seasonal patterns (Morey 1978). In some species, the first surge of root growth coincides with that of shoot growth in the spring, and then only a small amount of shoot growth occurs in late

summer, while at this time and later in the autumn roots show intense elongation. This might explain why the effect of water stress on shoot growth at the final harvest was independent of ozone treatment, but the effect of water stress on root growth was not.

To summarise, intermittent exposure to moderate water stress and low concentrations of ozone resulted in significant ozone/water stress interactions on both plant growth and gas exchange during the exposure period. The plants did not appear to recover between episodes, since treatment effects on gas exchange and plant water stress were seen in the absence of water stress, two weeks after exposure to ozone. Measurement of root dry weight in the following spring revealed that growth reductions due to water stress decreased with increasing ozone dose, and in the highest ozone treatment the root growth of water stressed plants exceeded that of plants that had been well watered for the entire experiment. This effect of ozone in 'protecting' plants against water stress was not the consequence of reduced ozone flux into water stressed leaves since ozone-induced increases in leaf conductance were seen more frequently in water stressed plants than well watered ones.

Chapter 4

Discussion

1 The effect of ozone on gas exchange and growth

Exposure to low concentrations of ozone resulted in significant increases in leaf conductance in both *Vicia faba* and *Fagus sylvatica*. This observation contradicts much of the published literature which reports that ozone causes a decrease in leaf conductance, although it is now recognized that the effects of low concentrations (<200 ppb) of ozone on stomata are somewhat unpredictable (Darrall, 1989). There have been a few isolated reports of exposure to ozone resulting in increased leaf conductance (Olszyk & Tibbitts, 1981a; Freer-Smith & Dobson, 1989), but very few experiments have been published in which the impact of realistic exposure to low concentrations of ozone on plant gas exchange has been investigated.

In the one experiment with *V. faba* in which photosynthesis was measured, ozone treated plants exhibited both increased and reduced photosynthesis compared to the controls. Likewise, fumigation of *F. sylvatica* with ozone resulted in both increased and decreased rates of photosynthesis, depending on the ozone concentration and duration of exposure.

Low concentrations of ozone (50-85 ppb) are reported to inhibit photosynthesis in a variety of species, for example in *Glycine max* (Reich, Schoettle *et al.*, 1986), *Avena sativa* (Myhre *et al.*, 1988), *Triticum aestivum* (Amundson *et al.*, 1987) and *Quercus rubra* (Reich, Schoettle & Amundson, 1986). However, there have also been three reports of exposure to ozone at concentrations below or within this range resulting in increased photosynthesis (Sutinen *et al.*, 1988; Freer-Smith & Dobson, 1989; Eamus *et al.*, 1990).

In these experiments with *F. sylvatica* and *V. faba* ozone was consistently found to have a persistent impact on plant gas exchange. Increases in conductance and photosynthesis of *F. sylvatica* were observed one day (13 hours or more) and two weeks after fumigation, and in *V. faba* increases in conductance were not usually seen during the fumigation but (with the exception of one experiment) were observed between 4 and 13 days after fumigation. This delayed impact of ozone on plant gas exchange is not generally reported in the literature, but almost without exception in published experiments plant gas exchange was not measured after the ozone fumigation. With the exception of Eamus *et al.* (1990), in all the examples referred to here gas exchange was measured either during the ozone fumigation (Olszyk & Tibbitts, 1981a; Reich, Schoettle *et al.*, 1986; Amundson *et al.*, 1987; Reich, Schoettle & Amundson, 1986 and Sutinen *et al.*, 1988) or in the absence of ozone but within one hour of fumigation (Freer-Smith & Dobson, 1989; Myhre *et al.*, 1988).

There is only one example of gas exchange measurements being made one day after ozone fumigation; Black *et al.* (1982) measured net photosynthesis rates of *V. faba* 20 hours after a 4 hour ozone fumigation, but found that at low ozone concentrations (<90 ppb) plants recovered from ozone-induced inhibition of photosynthesis within 20 hours, and no increases in photosynthesis were observed. However, in two experiments in which gas exchange was measured a number of weeks (Wallin *et al.*, 1986), and a number of months (Eamus *et al.*, 1990) after ozone fumigation persistent effects of ozone on plant gas exchange were observed. Wallin *et al.* (1986) observed a significant effect of ozone in decreasing photosynthesis and transpiration of *Pinus silvestris* in measurements made 2 weeks after an 11 week exposure to approximately 40 ppb ozone. Eamus *et al.* (1990) exposed four-year old *Picea abies* to filtered air or ambient air enriched with 40-50 ppb ozone (8 h daily mean 27 ppb) in open-top chambers over three consecutive summers (1986-1988). Gas exchange measurements were made in May 1988, before the third season of fumigation and more than 7 months after the end of ozone exposure. Photosynthesis, leaf conductance and daily transpiration rate (Barnes *et al.*, 1990) were consistently increased in plants previously exposed to ozone compared to the controls, but the effect of ozone on leaf conductance was only marginally significant ($p<0.1$). The rate of water loss from excised needles, and the chlorophyll content and wettability of needles exposed to ozone was also increased, but the effect on chlorophyll content was not significant (Barnes *et al.*, 1990; Eamus *et al.*, 1990).

Persistent effects of ozone in increasing leaf conductance may increase plant sensitivity to drought many weeks, or even months after exposure to ozone has terminated, and may have far reaching physiological consequences for trees in the field. On the other hand, in the absence of water stress, persistent effects of ozone in increasing photosynthesis may have a positive effect on plant growth.

Stimulated shoot growth was seen in both species following fumigation with ozone. There was no evidence of an effect of ozone on root:shoot partitioning. Significant increases in shoot growth following fumigation with low concentrations (<100 ppb) of ozone have been reported for a few crop species (Ormerod, 1973; Bennett *et al.*, 1974), and for several tree species. Taylor *et al.*, (1989) observed a significant increase in the total leaf area of *F. sylvatica* saplings exposed to low levels of ambient air pollution, compared to those in filtered air, and Kress & Skelly (1982) observed significant increases in above ground biomass of white ash (*Fraxinus americana*) and yellow poplar (*Liriodendron tulipifera*) following fumigation with 50 ppb ozone for 28 days. In summarising a series of fumigation studies on tree seedlings, Peterson *et al.* (1989) concluded that it was not uncommon for growth rate of above ground parts to be greater

at intermediate ozone concentrations (40-80 ppb) than in carbon filtered air. A carbon filtered air treatment was not included in this experiment with *F. sylvatica*, but in keeping with the results of Peterson *et al.* increases in lammas growth (number of leaves and shoot length) were observed in treatments in which the mean ozone concentration during lammas growth was approximately 40-60 ppb, but not in the control treatment.

These results clearly demonstrate that realistic exposure to low concentrations of ozone results in increases in leaf conductance (and in some cases net photosynthesis), and in increases in above ground growth. In the absence of water stress these positive effects of ozone on photosynthesis and growth may be advantageous, but on the other hand, increased conductance would be expected to increase plant sensitivity to water stress, especially as such increases persisted after the ozone fumigation. Ozone did result in increased plant water stress in *F. sylvatica*. In the published literature ozone is not generally reported to increase plant sensitivity to water stress, but very little work has been done at ozone concentrations typical of the U.K., and using the realistic ozone exposures adopted in this experiment with *F. sylvatica*.

2 The effect of water stress in modifying plant response to ozone

In the experiment with *F. sylvatica* exposure to ozone and water stress was simultaneous, while in the experiments with *V. faba*, exposure to ozone either preceded or coincided with the first week of water stress.

Significant ozone/water stress interactions on the total dry weight of both *V. faba* and *F. sylvatica* were observed, despite the low concentrations of ozone used, and the relatively short duration of water stress. In beech this interaction was largely the result of treatment effects on root growth, while in *V. faba* the significant interaction was the result of treatment effects on above-ground growth, not on root growth.

The effect of water stress in modifying plant growth and gas exchange responses to ozone was extremely variable. In the experiments with *V. faba*, water stress either had no effect on plant responses to ozone (for example on growth in experiment 2b, and on leaf conductance in experiment 1), or water stress reduced the positive impact of ozone (for example on the growth and gas exchange in experiment 4, and on leaf conductance in experiments 2b and 3).

The effects of ozone and water stress on *V. faba* are similar to the published ideas of how water stress modifies plant response to ozone. In the literature water stress is frequently reported to have no effect on plant responses to ozone, for example on gas exchange (Tseng *et al.*, 1988), and on growth (Temple *et al.*, 1985b), or to reduce the impact of low concentrations of ozone on both growth (e.g. Amundson *et al.*, 1986 and Temple, Kupper *et al.*, 1988) and gas exchange (e.g. Bucher *et al.*, 1988). However, in these examples, unlike the present experiments with *V. faba*, water stress reduced the negative impact of ozone on plant growth or gas exchange, and not the positive impact of ozone. There is one published example of exposure to low concentrations of ozone increasing yield losses and stomatal closure due to water stress (Heggestad *et al.*, 1985), and while no such effect was seen on growth or gas exchange of *V. faba*, in one experiment (experiment 3) exposure to ozone and water stress resulted in greater death of leaves than exposure to either stress alone.

There are many reports in the literature of water stress reducing the impact of ozone during simultaneous exposure to the two stresses. To some extent the experiments with *V. faba* (especially the visible injury and gas exchange data) support this generalisation, since ozone/water stress interactions of this nature were observed more commonly after simultaneous than sequential exposure to ozone and water stress. It is probable that these interactions are the result of reduced ozone flux into water stressed leaves due to stomatal closure in response to water stress.

In contrast, the ozone/water stress interactions on the gas exchange of *F. sylvatica* were of a very different nature, and do not fit in with any of the published ideas of how ozone modifies plant response to water stress.

In *F. sylvatica* ozone-induced increases in conductance and A_{net} photosynthesis were seen only in water stressed plants. In the control ozone treatment water stress always reduced gas exchange, but in the higher ozone treatments conductance and A_{net} photosynthesis of water stressed plants frequently exceeded that of the well watered plants. These strong stomatal responses to ozone in water stressed plants have not been reported in the literature, and there are no published examples of effects of ozone being restricted to water stressed plants. However, there is no information in the literature of the effect of water stress on positive effects of ozone, all published examples of ozone/water stress interactions are on negative effects of ozone.

At the final harvest the greatest impact of ozone on *F. sylvatica* was on root growth; in well watered *F. sylvatica* root dry weight decreased with increasing ozone dose, while the opposite response to ozone was observed in water stressed plants. In the low ozone treatments the root dry weight of water stressed plants was less than that of well watered plants, but in the high ozone treatments this was not the case, ie. exposure to elevated concentrations of ozone reduced the impact of water stress on root growth.

There is very little information on the effect of pollutants on the root growth of water stressed trees, or on the effects of pollution on root:shoot partitioning in trees (Mansfield, 1988). In other ozone fumigation studies with well watered tree seedlings, reductions in root growth have exceeded reductions in shoot growth following exposure to ozone (for example, Kress & Skelly, 1982; Hogsett *et al.*, 1985). In a labelled carbon study of effects of ambient ozone on 25 year old white pine (*Pinus strobus*) McLaughlin *et al.*, (1982) observed that in sensitive (declining) trees a higher proportion of photosynthate was retained by the foliage and branches, and less was exported to the bole and roots. These authors hypothesized that this partitioning change, rather than photosynthesis reductions *per se*, would be more damaging to the long term health of the trees.

If McLaughlin and colleagues are correct, then the observed impact of ozone on the root growth of well watered *F. sylvatica* suggests that ozone episodes in the U.K. might have a long term damaging impact on tree health, if trees are well watered during ozone episodes. However, if plant water stress coincides with periods of elevated ozone, then the results for water stressed *F. sylvatica* suggest that the impact of ozone on root growth will be reduced.

It appears that in this experiment with *F. sylvatica* stomatal opening in response to ozone in water stressed plants permitted increased photosynthesis, and therefore increased growth. However, if water stress had been more severe, or continuous rather than intermittent (a situation that does arise in some British summers), then increases in leaf conductance in water stressed plants could have serious long term implications in terms of increasing plant susceptibility to drought, especially as effects of ozone on conductance persisted in the absence of ozone and water stress. It seems likely that whether increases in conductance result in increased growth, or increased susceptibility to drought will depend on the severity of water stress to which the plants are exposed, and differences in water stress severity might explain the variation between species (*V. faba* and *F. sylvatica*) and within the experiments with *V. faba*, in the ozone/water stress

interactions on plant growth. As discussed in Chapter 2, the experiments with *V. faba* provide some evidence that the severity of water stress is important in determining the occurence of ozone/water stress interactions on plant growth.

In *F. sylvatica*, and in some experiments with *V. faba*, ozone-induced increases in growth can be explained by stomatal opening. However, these results provide no clear explanation of the mechanism of ozone-induced stomatal opening, or of the fundamental difference between species in that increased leaf conductance was generally observed in well watered *V. faba*, but water stressed *F. sylvatica*.

It has been suggested that stomatal responses are mediated through changes in intercellular CO_2 concentration, or leaf water potential. However, the nature of effects of ozone on leaf water stress, and to some extent Ci, of *F. sylvatica* suggests that these effects were the consequence of increases in conductance in this species, rather than the cause.

Gaseous pollutants are reported to increase synthesis of plant hormones, including ethylene and ABA (Fink, 1988), and following the discovery that stomatal closure can be induced by ABA, considerable effort has been directed towards elucidating the relationship between the increase in ABA levels and stomatal behaviour during water stress (Bradford & Hsiao, 1982).

Recent work at the University of Lancaster suggests that exposure to gaseous pollutants can inhibit stomatal closure in response to ABA (Wookey, pers. comm.). Wookey and colleagues exposed barley (*Hordeum vulgare*) to low (<30 ppb) concentrations of SO_2 and NO_2 for 4 to 5 days, and then measured the rate of water loss from excised portions of leaves following treatment with ABA. Leaf drying proceeded more rapidly from polluted leaves than from leaves exposed only to filtered air.

In the light of these results, it is a possibility that the loss of stomatal control in water stressed *F. sylvatica* exposed to ozone was the result of ozone-induced inhibition of stomatal closure in response to ABA. This hypothesis provides one explanation for stomatal reponses to ozone being restricted to water stressed *F. sylvatica,* and an effect of ozone on the hormonal control of stomatal opening might explain the persistent stomatal response to ozone. Barnes *et al.* (1990) observed that needle drying proceeded more rapidly from excised needles of *Picea abies* previously exposed to 78 ppb ozone (8 hr daily mean) than from those exposed to filtered air. This effect of ozone was persistent, and was observed 7 months after summer fumigation with ozone. Furthermore, the

importance of interactions between pollutant exposure and hormone levels in forest trees is suggested by the fact that several symptoms of forest decline, such as loss of apical dominance, premature shedding of leaves, development of epicormic shoots and altered branching patterns, are all under hormonal control (Ainsworth & Ashmore, 1988).

It is more difficult to envisage a role of ABA in ozone-induced stomatal opening in *V. faba*, since in this species stomatal responses to ozone were generally seen in well watered plants. Black & Black (1979) have demonstrated that stomatal opening in well watered *V. faba* in response to low concentrations of SO_2 is associated with extensive destruction of the epidermal cells adjacent to the guard cells. Reduction of turgidity in the adjacent cells caused by death or membrane damage would result in passive stomatal opening, and since there is considerable experimental evidence to suggest that membranes are the primary site of ozone action (Guderian, 1985) microscopic examination of the impact of ozone on the stomatal complex may be a useful line of investigation for this species.

Recent research suggests that ABA originating in the root has an important role in the root to shoot communication of soil drying (Davies *et al.*, 1982) and Atkinson *et al.* (1989) have shown that the restriction of leaf conductance by ABA relies on continuous supply via the xylem stream, rather than on the amount which has already arrived in the leaves. It is possible that effects of ozone on the conductance of water stressed *F. sylvatica* might be mediated via effects on root physiology or xylem transport, although the nature of the ozone/water stress interaction on root dry weight does not support this theory.

However, the dry weight of roots is not necessarily a good measure of root physiological activity. Taylor *et al.* (pers. comm.) observed that exposure of well watered saplings of *F. sylvatica* to ambient air in southern Britain resulted in a reduction in the root dry weight, but in an increase in the root length and specific root length (root length per unit root dry weight) compared to trees in filtered air. The longer thinner roots of polluted trees might increase susceptibility to drought, since thinner roots may be prone to loss of turgor and rapid dehydration (Taylor *et al.*, 1989), or might enable greater water uptake from the soil. These observations of root growth and morphology make interpretation of the present root dry weight data difficult, in terms of the impact of ozone on both root physiology and water uptake.

Field experiments may be more useful than pot ones in determining the combined effects of ozone and water stress on root growth and root:shoot partitioning. In pots plants will tend to suffer rapid fluctuations in soil water as the small volume of soil in the pot dries out between waterings. Some evidence for this was provided by the observation that 'well watered' *V. faba* and *F. sylvatica* appeared to suffer water stress at mid day. Pot-grown plants with restricted root volumes may become stressed too rapidly, thus precluding adaptive responses to soil moisture stress, and effects of ozone and water stress on root growth may be limited by the size of the pot. Characteristically plants under soil moisture stress develop larger root:shoot ratios (Bradford & Hsiao 1982). This may be due to greater impact of soil moisture stress on top growth than on roots, but it may also be due to increases in root growth (Sharp & Davies, 1975).

3 Ozone and crop yield

Much of our knowledge of the impact of ozone on crop yield comes from experiments performed within the NCLAN programme, in which plants were exposed to elevated ozone concentrations daily throughout the growing season. The ozone concentrations used and the continuity of ozone exposure are not typical of conditions in the U.K. These experiments with *V. faba* clearly demonstrate that exposure to ozone at low concentrations can have a fundamentally different impact on plant growth than results from exposure to the higher concentrations used in the NCLAN programme. These were short term experiments, and it is difficult to translate the effects of ozone on vegetative growth to commercial yield. However, the growth stimulations observed do raise the possibility that ozone could have a positive effect on yield, whereas the model used to relate yield to ozone concentration in the NCLAN studies (the Weibull model) assumes inhibition of yield in response to ozone (Heagle *et al.*, 1987).

Ozone-induced increases in shoot growth may increase the number of nodes, and therefore allow greater flower initiation, but increases in vegetative shoot growth may represent a reallocation of assimilates within the shoot, away from reproductive organs. McLaughlin & McConathy (1983) report that retention of ^{14}C-labelled photosynthate in leaves of *Phaseolus vulgaris* increased when plants were exposed to ozone. This increased retention of assimilates by leaves was accompanied by decreased allocation to the pods.

In the U.K. *V. faba* is grown commercially for canning and freezing and is harvested at a critical stage of maturity (Gane *et al.*, 1975). Many commercial growers harvest broad beans sequentially after peas, and beans for freezing are harvested at an earlier stage of

maturity than those for canning (Gane *et al.*, 1975). Factors which hasten or delay crop development can disrupt this critical harvesting sequence and so short term effects of ozone on gas exchange, vegetative growth and the rate of crop development may be as important in terms of the commercial value of the crop, as effects on the final yield.

In longer term filtration experiments in the U.K., ambient air pollution has not affected the final seed yield or vegetative dry weight of *V. faba*, but has resulted in significant reductions in the weight of individual seeds (Colls *et al.*, 1988) and has delayed the early vegetative development of the crop in terms of height growth, initiation of racemes, and development of pods (Ashmore *et al.*, 1988). There were no ozone/water stress interactions on the delay in early crop development (Ashmore *et al.*, 1988), but significant reductions in the weight of seeds were seen only during a dry season when soil water was limiting (Colls *et al.*, 1988).

Ashmore *et al.* (1988) investigated the combined effects of ambient air pollution, soil water stress and infestation of black bean aphid (*Aphis fabae*) on the growth and yield of *V. faba* and found consistent effects of ambient air pollution in stimulating aphid performance, and a significant aphid/water stress interaction on final bean yield; aphid infestation resulted in a significant reduction in the yield of water stressed, but not well watered plants. These results suggest that as well as direct effects of ozone on the growth of *V. faba*, the potential exists for indirect effects on crop yield via increased susceptibility to secondary stresses (in this case *Aphis fabae* infestation).

4 Forest Decline

Following an extensive survey of the health of *F. sylvatica* in central and southern Britain, Power *et al.* (1989) conclude that approximately 25 % of all beech trees surveyed were in a moderate to severe state of health as assessed by crown thinness and architecture. Variations in local environmental conditions and soil characteristics, particularly pH and drainage properties, explained much of the variation in levels of beech health, but there was some evidence to suggest that air pollutants were influencing tree health, and that pollutant impact may be dependant on soil conditions. However, the effect of ozone pollution on tree health was not assessed in this survey, due to the absence of data on ozone distribution in the U.K. Soil water status was considered an important factor in determining tree health, and the level of drought in 1976 was the most important meteorological parameter explaining the health of beech.

It is now widely believed that forest decline is a complex disease, involving a number of factors (Blank, 1985), and interactions between pollutant stresses and other stresses may have an important role (UK TERG, 1988). The stresses involved can be categorised into three classes, predisposing, inciting and contributing, though one stress may play more than one of these roles, depending on the situation under which it is acting (Table 4.1).

Table 4.1
A Summary of Factors Influencing Declines of Forest Trees
(from McLaughlin 1985)

	Types of Influencing Factors		
	Predisposing	**Inciting**	**Contributing**
Functional Role	Chronic weakening	Triggering episodes	Accelerators
Stressing Agents	Climate Soil moisture Genotype of host Soil nutrients Air pollutants Competition	Insect defoliation Frost Drought Salt Air pollutants Mechanical Injury	Bark beetles Canker fungi Viruses Root-decay fungi Competition

In this experiment, water stress had a considerable impact on the growth of *F. sylvatica* despite the relatively short length of time to which the plants were exposed to water stress and the intermittent nature of the drought episodes. Beech is considered to be a drought sensitive species (Krause *et al.*, 1986) and these results support this theory.

The survey results of Power *et al.* (1989) and the observation that twig extension growth of *F. sylvatica* has not recovered the rates measured before 1975/1976 at a number of sites in southern Britain (Lonsdale *et al.*, 1989) suggest that in this country drought has an important impact on the growth and health of beech. The observed ozone/water stress interactions on the gas exchange of beech seedlings suggest that ozone may be an important predisposing stress in terms of making the trees more susceptible to drought.

The effects of ozone on the health of beech seedlings suggest that ozone episodes may have a role in altering tree sensitivity to contributing factors, such as biotic stresses, but whether exposure to ozone will favour or discourage such biotic contributing stresses is not clear.

For practical reasons this experiment with *F. sylvatica* was performed on young trees, grown in pots in artificial rooting media, and the duration of the experiment was considerably less than the usual time scale over which forest health declines in the field.

It is likely that the fundamental biological processes by which a pollutant affects the functioning of an individual leaf, stem or root are similar in seedlings and mature trees. In contrast, the complexity and heterogeneity of a mature tree means that the consequences of these effects for the overall cycling of carbon, water and mineral nutrients may be very different from those for a seedling (Ainsworth & Ashmore, 1988), and therefore these results are of limited value in assessing how ozone and water stress combine to affect the mature forest ecosystems in which forest decline is actually occurring.

In their review of differences between seedlings and mature trees, Ainsworth & Ashmore (1988) conclude that the most important differences in terms of responses to pollutants include water relations, the distribution of biomass/allocation of assimilate, and the size and complexity of the root system. The root environment of mature trees is considerable more complex than that of seedlings, and heterogeneity, in terms of soil structure, chemical composition and water content, in the field is certainly greater than in the pot (Ainsworth & Ashmore, 1988). It is difficult to extrapolate water availability in a pot to water availability in an undisturbed forest soil (Havranek & Benecke, 1978), and since ozone and water stress effects on root growth may be affected by the size and artificial rooting medium of the pot, care must be taken in interpreting treatment effects on root growth.

Despite these limitations this experiment with *F. sylvatica* has produced novel and exciting results concerning the impact of ozone and water stress on gas exchange and growth. The approach of exposing trees to low ozone concentrations and cycles of stress events is highly realistic of field conditions within the natural range of *F. sylvatica*, and therefore these results are more relevant than most in predicting how ozone and water stress may affect beech health in the U.K.

Increased leaf conductance in response to ozone appears to be a key factor in controlling both growth and gas exchange responses to ozone, and in future research attention could usefully be paid to investigating the effect of low concentrations of ozone on the hormonal control of stomatal responses to water stress, on non stomatal aspects of leaf conductance, and on plant gas exchange measured under realistic, but controlled conditions.

As discussed above, and in chapters 2 and 3, there are limitations to these experiments, in terms of interpreting the mechanisms of treatment effects, and predicting how ozone and water stress will affect crop yield and tree health. However, the results of these experiments clearly demonstrate that ozone episodes at concentrations typical of British summers can affect the growth and gas exchange of both *F. sylvatica* and *V. faba*. These experiments were unusual in that plants were exposed to low levels of ozone and short periods of water stress, and illustrate that results obtained under ozone and water stress conditions atypical of north-west Europe cannot be used to predict plant responses to ozone and water stress conditions in this country.

Appendix 1
Analysis of Variance

1. Vicia faba

Experiments 1, 2a, 2b and 3:

Treatment	3	Where n is the number of replicates. In experiment 1 n=14 and in experiment 3 n=15. In experiments 2a and 2b n=10 for leaf conductance measurements and n=15 for all harvests.
WS	1	
O_3	1	
O_3*WS	1	
Residual	(4n-1)-3	
Total	(4n-1)	

Experiment 4:

Treatment	5	Where n is the number of repicates. For gas exchange measurements n=8-16; for intermediate harvests n=8; for the final harvest n=20; for leaf injury assessments n=32; for relative leaf water content, leaf water potential and soil water potential measurements n=6.
WS	1	
O_3	2	
O_3*WS	2	
Residual	(6n-1)-5	
Total	(6n-1)	

Soil water deficit measurements: water stressed treatments only

Experiments 1, 2a, 2b and 3:

Treatment	1	Where n is the number of replicates. For experiment 1 n=36; for experiments 2a and 2b n=30; for experiment 3 n=45.
O_3	1	
Residual	(2n-1)-1	
Total	(2n-1)	

Experiment 4:

Treatment	2	Number of replicates =32
O_3	2	
Residual	93	
Total	95	

2. Fagus sylvatica

Treatment	11	Where n is the number of replicates. For relative leaf water content measurements n=4; for gas exchange measurements n=6; for non destructive growth measurements, final harvest, visual assessments, autumn leaf fall and spring bud burst measurements n=16.
WS	1	
O_3	5	
O_3*WS	5	
Residual	(12n-1)-11	
Total	(12n-1)	

Leaf water potential and soil water deficit measurements: water stressed treatments only

Treatment	5	Where n is the number of replicates. For leaf water potential measurements n=4; for soil water deficit measurements n=16.
O_3	5	
Residual	(6n-1)-5	
Total	(6n-1)	

References

Adams, R.M., Glyer, J.D., McCarl, B.A. (1987). The NCLAN economic assessment: approach, findings and implication. In *Assessment of Crop Loss from Air Pollutants.* eds. W.W. Heck, O.C. Taylor & D.T. Tingey. Elsevier Applied Science, London & New York. pp. 473-504.

Ainsworth, N. (pers. comm.). Department of Pure and Applied Biology, Imperial College, Silwood Park, Ascot, Berks. SL5 7PY.

Ainsworth, N. & Ashmore, M.R. (1988). Experimental techniques for the study of pollutant effects on mature trees. Centre for Environmental Technology, London, SW7 2AZ.

Amundson, R.G., Kohut, R.J., Schoettle, A.W., Raba, R.M., Reich, P.B. (1987). Correlative reductions in whole plant photosynthesis and yield of winter wheat caused by ozone. *Phytopathol.* 77, 75-79.

Amundson, R.G., Raba, R.M., Schoettle, A.W., Reich, P.B. (1986). Response of soybean to low concentrations of ozone II: Effects on growth, biomass allocation and flowering. *J. Environ. Qual.*, 15(2), 161-167.

Apling, A.J., Sullivan, E.J., Williams, M.L., Ball, D.J., Bernard, R.E., Derwent, R.G., Eggleton, A.E.J., Hampton, L., Waller, R.E. (1977). Ozone concentrations in south east England during the summer of 1976. *Nature*, 269, 569-573.

Ashmore, M.R. (1984). Effects of ozone on vegetation in the United Kingdom. In *Ozone.* ed. P. Grennfelt. IVL, Goteburg. pp. 92-104.

Ashmore, M.R., Bell, J.N.B., Dalpra, C. (1980). Visible injury to crop species by ozone in the United Kingdom. Environ. Pollut., 21, 209-215.

Ashmore, M.R., Bell, J.N.B., Mimmack, A. (1986). Specifications of open-top chambers. In *Microclimate and Plant Growth in Open-Top Chambers.* COST/CEC Report No. 5, CEC Brussels. pp. 126-135.

Ashmore, M.R., Bell, J.N.B., Reily, C.L. (1978). A survey of ozone levels in the British Isles using indicator plants. *Nature*, 276, 813-815.

Ashmore, M.R., Bell, J.N.B., Rutter, A.J. (1985). The role of ozone in forest damage in West Germany. *Ambio*, 14, 81-87.

Ashmore, M.R., Brown, V., Kristiansen, L., Shah, D. (1988). Effects of ambient air pollution, water stress and aphid pests on *Vicia faba*. In *The European Communities research project on open-top chambers. Results on agricultural crops, 1987-1988*. eds. J. Bonte & P. Mathy. COST/CEC Report No. 19, Pau, France. pp. 8-25.

Ashmore, M.R., Garretty, C., McHugh, F.M., Mepsted, R. (1988). Combined effects of ozone and acid mist on tree seedlings. In *Air Pollution and Ecosystems*. eds. P. Mathy, Reidel Publ. Co., Dordrecht. pp. 659-64.

Atkinson, C.J., Davies, W.J., Mansfield, T.A. (1989). Changes in stomatal conductance in intact aging wheat leaves in response to ABA. *J. Exp. Bot.*, 40, 1021-8.

Barnes, J.D. & Davison, A.W. (1988). The influence of ozone on the winter hardness of Norway spruce (*Picia abies* (L.) Karst.). *New Phytol.*, 108, 159-166.

Barnes, J.D., Davison, A.W., Booth, T.A. (1988). Ozone accelerates structural degradation of epicuticular wax on Norway spruce needles. *New Phytol.*, 110, 309-318.

Barnes, J.D., Eamus, D., Davison, A.W., Ro-Poulsen, H., Mortensen, L. (1990). Persistent effects of ozone on needle water loss and wettability in Norway spruce. *Environ. Pollut.*, 63, 345-363.

Bell, J.N.B. & Cox, R.A. (1975). Atmospheric ozone and plant damage in the United Kingdom. *Environ. Pollut.*, 8, 163-170.

Bennett, J.P. & Oshima, R.J. (1976). Carrot injury and yield response to ozone. *J. Amer. Soc. Hort. Sci.*, 101, 638-639.

Bennett, J.P. & Runeckles, V.C. (1977). Effects of low levels of ozone on growth of crimson clover and annual ryegrass. *Crop Sci.*, 17, 443-445.

Bennett, J.P., Oshima, R.J., Lippert, L.F. (1979). Effects of ozone on injury and dry matter partitioning in pepper plants. *Env. Exp. Bot.*, 19, 33-39.

Bennett, J.P., Resh, H.M., Runeckles, V.C. (1974). Apparent stimulations of plant growth by air pollutants. *Can. J. Bot.*, 52, 35-41.

Black, V.J. (pers. comm.). Department of Human Sciences, Loughborough University of Technology, Loughborough, Leics. LE11 3TU.

Black, C.R. & Black, V.J. (1979). The effects of low concentrations of SO_2 on stomatal conductance and epidermal cell survival in field bean (*Vicia faba* L.) *J. Exp. Bot.*, 30, 291-298.

Black, V.J. & Unsworth, M.H. (1980). Stomatal responses to sulphur dioxide and vapour pressure deficit. *J. Exp. Bot.*, 31, 667-677.

Black, V.J., Ormrod, D.P., Unsworth, M.H. (1982). Effects of low concentrations of ozone, singly, and in combination with sulphur dioxide on net photosynthesis rates of *Vicia faba* L. *J. Exp. Bot.*, 33, 1302-1311.

Blank, L.W. (1985). A new type of forest decline in Germany. *Nature*, 314, 311-314.

Bradford, K.J. & Hsiao, T.C. (1982). Physiological responses to moderate water stress. In *Encyclopedia of Plant Physiology Vol. 12b.* eds. O.L. Lange, P.S. Nobel, C.B. Osmond, H. Ziegler. Spinger-Verlag, Berlin. pp. 325-373.

Brown, J.M.B. (1953). Studies on British beechwoods. Forestry Commission Bulletin No. 20. HMSO, London.

Bucher, J.B., Schiller, G., Siegwolf, R.T.W. (1988). Effects of ozone and/or water stress on xylem pressure, transpiration and leaf conductance in fir (*Albies alba* Mill.). In *Relationships Between Above and Below Ground Influences of Air Pollutants on Forest Trees.* eds. J. Bervaes, P. Mathy & P. Evers. COST/CEC Report No. 16, CEC Brussels. pp. 83-88.

Cape, J.N. (1988). Air pollutants effects on conifer needle surfaces. In *Scientific Basis of Forest Decline Symptomatology.* eds. J.N. Cape & P. Mathy. COST/CEC Report No. 15, CEC, Brussels. pp. 149-159.

Coleman, J.S. & Jones C.G. (1988). Acute ozone stress on eastern cottonwood *Populus deltoides* Bartr. and the pest potential of the aphid *Chaitophorus populicola* Thomas (Homoptera: Aphidae). *Environmental Entomolgy*, 17, 207-212.

Colls, J.J., Sanders, G.E., Clark, A.G. (1988). Open-top chamber experiments on field-grown *Vicia faba* L. at Sutton Bonnington (1987-1988). In *The European Communities research project on open-top chambers. Results on agricultural crops, 1987-1988.* eds. J. Bonte & P. Mathy. COST/CEC Report No. 19, Pau, France. pp. 43-72.

Cooley, D.R. & Manning, W.J. (1987). The impact of ozone on assimilate partitioning in plants: a reveiw. *Environ. Pollut.*, 47, 95-113.

Costonis, A.C. & Sinclair, W.A. (1969). Ozone injury to *Pinus strobus. J. Air Pollut. Contr. Assoc.*, 19, 867-872.

Darrall, N.M. (1989). The effect of air pollutants on physiological processes in plants. *Plant Cell Environ.*, 12, 1-30.

Davies, W.J., Rodriguez, J.L., Fiscus, E.L. (1982). Stomatal behaviour and water movement through roots of wheat plants treated with abscissic acid. *Plant Cell Environ.*, 5, 485-493.

Davison, A.W., Barnes, J.D. (1986). Effects of winter stress on pollutant responses. In *How Are The Effects of Air Pollutants on Agricultural Crops Influenced by The Interaction With Other Limiting Factors?* Proc. COST-CEC Workshop, Roskilde, Denmark. pp. 16-32,

Day, W. & Legg, B.J. (1983). Water relations and irrigation response. In *The Faba Bean (Vicia faba L.).* ed. P.D. Hebblethwaite. Butterworths, London. pp. 217-231.

Dugger, M.Jr., Taylor, O.C., Cardiff, E., Thompson, C.R. (1962). Stomatal action in plants as related to damage from photochemical oxidants. *Plant Physiol.*, 37, 487-491.

Eamus, D., Barnes, J.D., Mortensen, L., Ro-Poulson, H., Davison, A.W. (1990). Persistent stimulation of CO_2 assimilation and stomatal conductance by summer ozone fumigation in Norway spruce. *Environ. Pollut.* 63, 365-379.

Elkiey, T. & Ormrod, D.P. (1979). Leaf diffusion resistance responses of three petunia cultivars to ozone &/or sulfur dioxide. *J. Air Pollut. Contr. Assoc.*, 29(6), 622-625.

Fink, S. (1988). Histology and histochemistry of leaf fall. In *Scientific Basis of Forest Decline Symptomatology*. eds. J.N. Cape & P. Mathy. COST/CEC Report No. 15, CEC, Brussels. pp. 39-48.

Fitter, A.H. & Hay, R.K.M. (1983). Environmental Physiology of Plants. Academic Press, London.

Flagler, R.B. & Younger, V.B. (1982). Ozone and sulphur dioxide effects on tall fescue I: Growth and yield responses. *J. Environ. Qual.*,11, 386-389.

Fluckiger, W. & Braun, S. (1986). Effects of air pollutants on insects and host plant/insect relationships. In *How Are The Effects of Air Pollutants on Agricultural Crops Influenced by The Interaction With Other Limiting Factors?* Proc. COST-CEC Workshop, Roskilde, Denmark. pp. 43-59.

Fowler, D. & Cape, J.N. (1982). Air pollutants in agriculture and horticulture. In *Effects of Gaseous Air Pollution in Agriculture and Horticulture*. eds. M.H. Unsworth & D.P. Ormrod. Butterworth Scientific, London. pp. 3-26.

Freer-Smith, P.H. & Dobson, M.C. (1989). Ozone flux to *Picea sitchensis* (Bong) Carr and *Picea abies* (L) Karst during short episodes and the effects of these on transpiration and photosynthesis. *Environ. Pollut.*, 59, 161-176.

Freer-Smith, P.H. & Taylor, G. (1988). Abiotic factors, other than air polltion, responsible for premature yellowing and necrosis of tree foliage. In *Scientific Basis of Forest Decline Symptomatology*. eds. J.N. Cape & P. Mathy. COST/CEC Report No. 15, CEC, Brussels. pp. 107-125.

Fuhrer, J. & Grandjean, A. (1988). Chlorophyll loss and ethylene biosynthesis in needles (*Abies alba*) and leaves (*Triticum aestivum*). In *Scientific Basis of Forest Decline Symptomatology*. eds. J.N. Cape & P. Mathy. COST/CEC Report No. 15, CEC, Brussels. pp. 282-287.

Gane, A.J., King, J.M., Gent, G.P. (1975). Pea and bean growing handbook Vol. II, Beans. Processors & Growers Research Organisation, Peterborough, U.K.

Garrec, J.P. (1988). Etude des surfaces foliares des arbres deperissants. Effect de l'ozone et des pluies acides sur les cuticles et implication dans le phenomene de leaching. In *Air Pollution and Forest Decline*. IUFRO, Geneva. pp. 14-30

Guderian, R. (1985). Air pollution by photochemical oxidants. Springer-Verlag, Berlin.

Harkov, R. & Brennan, E. (1980). The influence of soil fertility and water stress on the ozone response of hybrid poplar trees. *Phytopathol.*, 70, 991-994.

Havranek, W.M. & Benecke, U. (1978). The influence of soil moisture on water potential, transpiration and photosynthesis of conifer seedlings. *Plant Soil*, 49, 91-103.

Heagle, A.S. (1977). Effect of ozone on parasitism of corn by *Helminthosporium maydis*. *Phytopathol.*, 67, 616-618.

Heagle, A.S., Flagler, R.B., Patterson, R.P., Lesser, W.M., Shafer, S.R., Heck, W.W. (1987). Injury and yield response of soybean to chronic doses of ozone and soil moisture deficit. *Crop Sci.*, 27, 1016-1024.

Heagle, A.S., Heck, W.W., Lesser, V.M., Rawlings, J.O. (1987). Effects of daily ozone exposure duration and concentration fluctuation on yield of tobacco. *Phytopathol.*, 77, 856-862.

Heagle, A.S., Kress, L.W., Temple, P.J., Kohut, R.J., Miller, J.E., Heggestad, H.E. (1987). Factors influencing ozone dose-yield response relationship in open-top field chamber studies. In *Assessment of Crop Loss from Air Pollutants*. eds. W.W. Heck, O.C. Taylor & D.T. Tingey. Elsevier Applied Science, London & New York. pp. 141-180.

Heagle, A.S., Miller, J.E., Heck, W.W., Patterson, R.P. (1988). Injury and yield response of cotton to chronic doses of ozone and soil moisture deficit. *J. Environ. Qual.*, 17, 627-635.

Heagle, A.S., Rebbeck, J., Shafer, S.R., Blum, U., Heck, W.W. (1989). Effects of long-term ozone exposure and soil moisture deficit on growth of a ladino clover- tall fescue pasture. *Phytopathol.*, 79, 128-136.

Heck, W.W., Taylor, D.C., Adams, R., Bingham, G., Miller, J., Preston, E., Weinstein, L. (1982). Assessment of crop loss to ozone. *J. Air Pollut. Contr. Assoc.*, 32, 353-361.

Heggestad, H.E., Gish, T.J., Lee, E.H., Bennett, J.H., Douglass, L.W. (1985). Interaction of soil moisture stress and ambient ozone on growth and yields of soybeans. *Phytopathol.*, 75, 472-477.

Hibben, C.R. & Taylor, M.P. (1975). Ozone and sulphur dioxide effects on the lilac powdery mildew fungus. *Environ. Pollut.* ,9, 107-114.

Hogsett, W.E., Plocher, M., Wildman, V., Tingey, D.T., Bennett, J.P. (1985). Growth response of 2 varieties of slash pine seedlings to chronic ozone exposures. *Can. J. Bot.*, 63, 2369-2376.

Hughes, P.R., Laurence, J.A. (1984). Relationship of biochemical effects of air pollution on plants to environmental problems: insect and microbial interactions. In *Gaseous Air Pollution and Plant Metabolism.* eds. M.J. Koziol & F.R. Whately. Butterworths Scientific, London. pp. 361-377.

James, R.L., Cobb, F.W., Miller, P.R., Parmeter, J.R. (1980). Effects of oxidant air pollution on susceptibility of pine roots to *Formes annosus*. *Phytopathol.*, 80, 560-563.

Jensen, K.F. (1982). An analysis of the growth of silver maple and eastern cottonwood seedlings exposed to ozone. *Can. J. For. Res.*, 12, 420-424.

Johnson, A.H. (1983). Red spruce decline in the northeastern U.S.: hypothesis regarding the role of acid rain. *J. Air Pollut. Contr. Assoc.*, 33, 1049-1054.

Karhu, M. & Huttunen, S. (1986). Errosion effects of air pollution on needle surfaces. *Water Air & Soil Pollution*, 31, 417-423.

Keller, T. & Hasler, R. (1984). The influence of a fall fumigation with ozone on the stomatal behaviour of spruce and fir. *Oecologica*, 64, 284-286.

Kerstiens, G. & Lendzian, K.J. (1989). Interactions between ozone and plant cuticles. II Water permeability. *New Phytol.*, 112, 21-27.

Kozlowski, T.T. (1982). Water supply and tree growth, part 1: Water deficits. *Forestry Abstracts*, 43, 57-85.

Kramer, P.J. (1983). Water relations of plants. Academic Press. New York.

Krause, G.H.M., Arndt, U., Brandt, C.J., Bucher, J., Kenk, G., Matzner, E. (1986). Forest decline in Europe: development and possible causes. *Water, Air & Soil Pollution*, 31, 647-668.

Kress, L.W. & Skelly, J.M. (1982). Response of several eastern forest tree species to chronic doses of ozone and NO_2. *Plant Disease*, 66, 1149-1152.

Krizek, D.T. (1985). Methods of inducing water stress in plants *Hort. Science*, 20, pp. 1028-1038.

Lechowicz, M.J. (1987). Resource allocation by plants under air pollution stress: Implications for plant-pest-pathogen interactions. *Bot. Reveiw*, 53, 281-300.

Leonardi, S. (pers comm). GSF-Munchen, Institut fur Biochemische Pflanzenpathologie, D-8042, Neuherberg.

Lonsdale, D., Hickman, I.T., Mobbs, I.D., Mathews, R.W. (1989). A quantitive analysis of beech health and pollution across southern Britain. *Naturwissenschaften*, 76, 571-573.

Mansfield, T.A. (1988). Factors determining root:shoot partitioning. In *Scientific Basis of Forest Decline Symptomatology*. eds. J.N. Cape & P. Mathy. COST/CEC Report No. 15, CEC, Brussels. pp. 171-181.

Mansfield, T.A. & Freer-Smith, P.M. (1984). The role of stomata in resistance mechanisms. In *Gaseous Air Pollutants and Plant Metabolism*. eds. M.J. Koziol & F.R. Whately. Butterworths Scientific, London. pp. 131-146.

Matkin, O.H. & Chandler, P.A. (1957). The U-C type soil mixes. In *The UC system for producing healthy container-grown plants*. ed. K.F. Baker. University of California Agricultural Experimental Station, Extension Service Manual No. 23. pp. 68-85.

McLaughlin, S.B., (1985). Effects of air pollutants on forests: a critical reveiw. *J. Air Pollut. Contr. Assoc.*, 35, 512-534.

McLaughlin, S.B. & McConathy, R.K. (1983). Effects of SO_2 and O_3 on allocation of ^{14}C-labelled photosynthate in *Phaseolus vulgarus*. Plant Physiol., 73, 630-635.

McLaughlin, S.B., McConathy, R.K., Durick, D., Mann, L.K. (1982). Effects of chronic air pollution stress on photosynthesis, carbon allocation and growth of white pine trees. *Forest Sci.*, 28, 60-70.

McLaughlin, S.B. & Taylor, G.E. (1981). Relative humidity: important modifier of pollutant uptake by plants. *Science*, 211, 167-169.

McNeill, S. & Whittaker, J.B. (1990). Air pollution and tree-dwelling aphids. In *Population Dynamics of Forest Insects.* ed. S. Leather. (in press).

Melhorn, H. & Wellburn, A.R. (1987). Stress ethylene determines plant sensitivity to ozone. *Nature*, 327, 417-418.

Miller, C.A. & Davis, D.D. (1981). Effect of temperature on stomatal conductance and ozone injury of pinto bean leaves. *Plant Disease*, 65, 750-751.

Miller, E.J., Heagle, A.S., Vozzo, S.F., Philbeck, R.B., Heck, W.W. (1989). Effects of ozone and water stress seperately and in combination on soybean yield. *J. Environ. Qual.*, 18, 330-336.

Miller, J.E., Patterson, R.P., Heagle, A.S., Pursley, W.A., Heck, W.W. (1988). Growth of cotton under chronic ozone stress at two levels of soil moisture. *J. Environ. Qual.*, 17, 635-643.

Mooney, H.A. & Winner, W.E. (1988). Carbon gain, allocation and growth as affected by atmospheric pollutants. In *Air Pollution and Plant Metabolism.* eds. S. Schulte-Hostede, N.M. Darrall, L.W. Blank & A.R. Wellburn. Elsevier Applied Science, London & New York. pp. 272-287.

Morey, P.R. (1978). How trees grow. Edward Arnold. London.

Moser, T.J., Tingey, D.T., Rodecap, K.D., Rossi, D.J., Scott Clark, C. (1987). Drought stress applied during the reproductive phase reduced ozone-induced effects in bush bean. In *Assessment of Crop Loss from Air Pollutants.* eds. W.W. Heck, O.C. Taylor & D.T. Tingey. Elsevier Applied Science, London & New York. pp. 345-364.

Myhre, A., Forberg, E., Aares, H., Nilsen, S. (1988). Reduction of net photosynthesis in oats after treatment with low concentrations of ozone. *Environ. Pollut.*, 53, 265-271.

Ormrod, D.P. (1973). Ozone stimulation of growth. *Hort. Science*, 8, 36.

Olszyk, D.M. & Tibbitts, T.W. (1981a). Stomatal responses and leaf injury of *Pisum sativum* L. with SO_2 and O_3 exposures. I Influence of pollutant level and leaf maturity. *Plant Physiol.*, 67, 539-544.

Olszyk, D.M. & Tibbitts, T.W. (1981b). Stomatal response and leaf injury of *Pisum sativum* L. with SO_2 and O_3 exposures. II Influence of moisture stress and time of exposure. *Plant Physiol.*, 67, 545-549.

Oshima, R.J., Bennett, J.P., Braegelmann, P.K. (1978). Effect of ozone on growth and assimilate partitioning in parsley. *J. Amer. Soc. Hort. Sci.*, 103, 348-350.

Oshima, R.J., Braegelmann, P.K., Flagler, R.B., Teso, R.R. (1979). The effects of ozone on the growth, yield and partitioning of dry matter in cotton. *J. Environ. Qual.*, 8, 474-479.

Peterson, C.E., Mattson, K.G., Hickler, R.A. (1989). Seedling response to sulfur, nitrogen and associated pollutants. Report No. EPA/600/3-89/081. E.P.A., Corvallis.

Power, S., Ling, K., Ashmore, M.R. (1989). Beech health and air pollution in southern Britain. In *Poster Abstracts for the International Congress on Forest Decline Research: State of Knowledge and Perspectives*. 2-6 October, 1989. Lake Constance, FRG. pp. 43-44.

Prinz, B. (1983). Gedanken zum stand der diskussion uber die ursache der Waldschaden in der Bundesrepublik Deutschland. *Forst-und Holzwirt*, 38, 460-467.

Prinz, B., Krause, G.H.M., Jung, K.D. (1987). Development and causes of novel forest decline in Germany. In *Effects of Atmospheric Pollutants on Forests, Wetlands and Agricultural Ecosystems*. eds. T.C. Hutchinson & K.M. Meena. Springer-Verlag Berlin. pp. 1-24.

Prinz, B., Krause, G.H.M., Stratman, H. (1982). Forest damage in the Federal Republic of Germany. LIS Report No. 28, LIS, Essen, CEGB translation T14240.

Pye, J.M. (1988). Impact of ozone on the growth and yield of trees: a review. *J. Environ. Qual.*, 17, 347-360.

Reich, P.B. (1983). Effects of low concentrations of O_3 on net photosynthesis, dark respiration and chlorophyll contents in aging hybrid poplar leaves. *Plant Physiol.* 73, 291-296.

Reich, P.B. (1987). Quantifying the response of plants to ozone: a unified explanation. *Tree Physiol.* 3, 63-91.

Reich, P.B. & Lassoie, J.P. (1984). Effects of low level O_3 exposure on leaf diffusive conductance and water-use efficiency in hybrid poplar leaves. *Plant Cell Environ.*, 7, 661-668.

Reich, P.B. & Lassoie, J.P. (1985) Influence of low concentrations of ozone on growth, biomass partitioning and leaf senescence in young hybrid poplar plants. *Environ. Pollut.*, 39, 39-51.

Reich, P.B., Lassoie, J.P., Amundson, R.G. (1984). Reduction in growth of hybrid poplar following field exposure to low levels of ozone and (or) sulphur dioxide. *Can. J. Bot.*, 62, 2835-2841.

Reich, P.B., Schoettle, A.W., Amundson, R.G. (1985). Effects of low concentrations of ozone, leaf age and water stress on leaf diffusive conductance and water use efficiency in soybean. *Physiol. Plant.*, 63, 58-64.

Reich, P.B., Schoettle, A.W., Amundson, R.G. (1986). Effects of O_3 and acidic rain on photosynthesis and growth in sugar maple and northern red oak seedlings *Environ. Pollut.*, 40, 1-15.

Reich, P.B., Schoettle, A.W., Raba, R.M., Amundson, R.G. (1986). Response of soybean to low concentrations of ozone: I Reductions in leaf and whole plant net photosynthesis and leaf chlorophyll content. *J. Environ. Qual.*, 15, 31-36.

Reinert, R.A. & Gooding, G.V. (1978). Effect of ozone and tobacco streak virus alone and in combination on *Nicotiana tabacum*. *Phytopathol.*, 68, 15-17.

Rich, S. & Turner, N.C. (1972). Importance of moisture on stomatal behaviour of plants subjected to ozone. *J. Air Pollut. Contr. Assoc.*, 22, 718-721.

Roloff, A. (1985). Untersuchungen zum vorzeitigen laubfall und zur diagnose von trockenschaden in buchenbestanden. *Augemeine Forst Zeitschrift*, 40, 157-160.

Rowland-Bamford, A.J., Coghlan, S., Lea, P.J. (1989). Ozone-induced changes in CO_2 assimilation, O_2 evolution and chlorophyll a fluorescence transients in barley. *Environ. Pollut.*, 59, 129-140.

Schonherr, J. (1976). Water permeability of isolated cuticular membranes: the effect of cuticular waxes on diffusion of water. *Planta*, 131, 159-164.

Sharp, R.E. & Davies, W.J. (1975). Solute regulation and growth by roots and shoots of water stressed maize plants. *Planta*, 147, 43-49.

Skarby, L. & Sellden, G. (1984). The effects of ozone on crops and forests. *Ambio*, 13, 68-72.

Skeffington, R.A., Roberts, T.M. (1985). The effects of ozone and acid mist on Scots pine saplings. *Oecologia*, 65, 201-206.

Sutinen, S., Wallin, G., Skarby, L., Sellden, G. (1988). Effects of long term exposure of ozone on ultrastructure and net photosynthesis of Norway spruce, *Picea abies*. In *Relationship Between Above and Below Ground Influences of Air Pollutants on Forest Trees*. eds. J. Bervaes, P. Mathy & P. Evers, COST/CEC Report No. 16, CEC Brussels. pp. 251-259.

Takemoto, B.K., Bytnerowicz, A., Olszyk, D.M. (1988). Depression of photosynthesis, growth and yield in field-grown green pepper (*Capsicum annuum* L.) exposed to acidic fog and ambient ozone. *Plant Physiol.*, 88(2), 477-482.

Taylor, G. (pers. comm.). Forestry Commission, Forest Research Station, Alice Holt Lodge, Wrecclesham, Farnham, Surrey. GU10 4LD.

Taylor, G. & Dobson, M.C. (1989). Photosynthetic characteristics, stomatal responses and water relations of *Fagus sylvatica*: impact of air quality at a site in southern Britain. *New Phytol.*, 113, 265-273.

Taylor, G., Dobson, M.C., Freer-Smith, P.H., Davies, W.J. (1989). Tree physiology and air pollution in southern Britain. Research Information Note, 145. Forestry Commission, Forest Research Station, Alice Holt Lodge, Wrecclesham, Farnham, Surrey. GU10 4LD.

Temple, P.J., Benoit, L.F. Lennox, R.W., Reagan, C.A. & Taylor, O.C. (1988). Combined effects of ozone and water stress on alfafa growth and yield. *J. Environ. Qual.* 17, 108-113.

Temple, P.J., Kupper, R.S., Lennox, R.L., Rohr, K. (1988). Physiological and growth responses of differentially irrigated cotton to ozone. *Environ. Pollut.*, 53, 255-263.

Temple, P.J., Taylor, O.C., Benoit, L.F. (1985a). Effects of ozone on yield of two field-grown barley cultivars. *Environ. Pollut.* 39, 217-225.

Temple, P.J., Taylor, O.C., Benoit, L.F. (1985b). Cotton yield responses to ozone as mediated by soil moisture and evaporation. *J. Environ. Qual.*, 14, 55-60.

Tingey, D.T. & Hogsett, W.E. (1985). Water stress reduces ozone injury via a stomatal mechanism. *Plant Physiol.*, 77, 944-947.

Tingey, D.T. & Taylor, G.E.Jr. (1982). Variation in plant response to ozone: A conceptual model of physiological events. In *Effects of Gaseous Air Pollution in Agriculture and Horticulture*. eds. M.H. Unsworth & D.D. Ormrod. Butterworth, London. pp. 113-138.

Tingey, D.T., Heck, W.W., Reinert, R.A. (1971). Effect of low concentrations of ozone and sulphur dioxide on foliage, growth and yield in radish. *J. Amer. Soc. Hort. Sci.*, 96, 369-371.

Tingey, D.T., Standley, C., Field, R.W. (1976). Stress etheylene evolution: a measure of ozone effects on plants. *Atmos. Environ.*, 10, 969-974.

Tingey, D.T., Thutt, G.L., Gumpertz, M.L., Hogsett, W.E. (1982). Plant water status influences ozone sensitivity of bean leaves. *Agriculture & Environment*, 7, 243-254.

Tingey, D.T., Wilhour, R.G., Standley, C. (1976). The effect of chronic ozone exposures on the metabolic content of ponderosa pine seedlings. *For. Sci.*, 22, 234-241.

Trimble, J.L., Skelly, J.M., Tolin, S.A., Orcutt, D.M. (1982). Chemical and structural characterization of the needle epicuticular wax of two clones of *Pinius strobus* differing in sensitivity to ozone. *Phytopathol.*, 72, 652-656.

Tseng, E.C., Seiler, J.R., Chevone, B.I. (1988). Effects of ozone and water stress on greenhouse-grown fraser fir seedling growth and physiology. *Environ. & Exp. Bot.*, 28, 37-41.

UK PORG, (1987). Ozone in the United Kingdom. United Kingdom Photochemical Oxidants Review Group. HMSO, London.

UK TERG, (1988). The effects of acid deposition on the terrestrial environment in the United Kingdom. United Kingdom Terrestrial Effects Review Group. HMSO, London.

Vargo, R.H., Pell, E.J., Smith, S.H. (1978). Induced resistance to ozone injury of soybean by tobacco ringspot virus. *Phytopathol.*, 68, 715-719.

Wallin, G., Skarby, L., Sellden, G. (1986). Photosynthesis, respiration and transpiration of scots pine, *Pinus sylvestris*, exposed to ozone in open top chambers. In *Direct Effects of Wet and Dry Deposition on Forest Ecosystems, in Particular Canopy Interactions.* COST/CEC Report No. 4, CEC Brussels. pp. 117-121.

Warren Spring Laboratory (1989). National Ozone Episode 10 to 25 June, 1989. WSL, Gunnels Wood Road, Stevenage, Herts., U.K.

Whittaker, J.B., Warrington, S. (1990). Effects of atmospheric pollutants on interactions between insects and their food plants. *Environ. Pollut.* (in press).

Willmer, C.M. (1983). Stomata. Longman, London and New York.

Winner, W.E., Gillespie, C., Wen-Shame Shen, Mooney, H.A. (1988). Stomatal responses to SO_2 and O_3. In *Air Pollution and Plant Metabolism.* eds. S. Schulte-Hostede, N.M. Darrall, L.W. Blank & A.R. Wellburn. Elsevier Applied Science, London & New York. pp. 255-271.

Wookey, P.A. (pers. comm.). Department of Biological Sciences, University of Lancaster, Lancaster. LA1 4YQ.

Zhang, J., Davies, W.J. (1989). Abscisic acid produced in the dehydrating roots may enable the plant to measure the water status of the soil. *Plant Cell Environ.*, 12, 73-81.