STUDIES INTO THE INTERACTIONS BETWEEN OZONE POLLUTION AND HERBICIDES IN UK CROPS

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DECLARATION

The author has not been a registered candidate nor an enrolled student for another award of any other academic or professional institution during this research programme. Material contained in this thesis has not been used in any other submission for an academic award and is entirely the author's individual contribution. The author has attended appropriate lectures, seminars and conferences in partial fulfilment of the requirements of the degree.

Signed Janet Dixon
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Signed Gia Mills*
(Director of Studies) (*formerly Sanders)

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ABSTRACT

During the growing period, spring crops are treated with early post-emergence herbicides at times when episodes of ozone pollution are likely to occur. Therefore, there is the possibility of interactive effects between ozone and the herbicide. To investigate this likelihood, laboratory experiments were conducted in which two to three leaf seedlings of sugarbeet (*Beta vulgaris*), spring barley (*Hordeum vulgare*) and spring oilseed rape (*Brassica napus*) were exposed to a simulated two day ozone episode (100 nl l^{-1}, 7 h d^{-1}) and/or treatment with field rate herbicide (diclofop-methyl, clopyralid, phenmedipham, mecoprop-p and metazachlor).

A preliminary study was carried out to determine the response of the crops to various rates of herbicide application. Clopyralid exerted no significant effects on spring barley or sugarbeet. Diclofop-methyl had no significant effects on the spring barley cvs. Tyne and Nugget, but decreased shoot dry weight in Sherpa and Corgi. Treatment with fenpropimorph, for the control of powdery mildew, produced varied results dependent on the cultivar. Mecoprop-p reduced the shoot dry weight of the barley cultivar Nugget. Phenmedipham reduced the shoot dry weight of all 3 sugarbeet cvs. Application of diclofop-methyl did not affect shoot dry weight of the sugarbeet cultivars, Amethyst, Celt and Saxon. Shoot dry weight of oilseed rape was not affected by any of the herbicides and only diclofop-methyl induced visible symptoms of injury. Damage caused by diclofop-methyl was in the form of round chlorotic areas, indicative of contact injury.

Exposure to a simulated two day ozone episode (100 nl l^{-1}, 7 h d^{-1}) did not have consistent effects on shoot dry weight of oilseed rape and barley. Ozone reduced shoot dry weight in sugarbeet only when the plants were older (25 d after sowing) at the time of exposure.

Growth analyses indicated interactive effects in sugarbeet (cv. Saxon) treated with ozone and phenmedipham and spring oilseed rape (cvs. Starlight and Galaxy) treated with ozone and clopyralid. The remaining experiments all revealed no more or less than additive effects of the herbicides and ozone treatment.

Physiological and biochemical studies were then undertaken to determine the nature of the interaction between ozone pollution and phenmedipham in sugarbeet cv. Saxon. Exposure to phenmedipham alone or ozone followed by phenmedipham

reduced net photosynthesis by over 56 % and stomatal conductance by 49 % compared to the control. However, reductions in total chlorophyll and carotenoid content were both intermediate between ozone (small reduction) and phenmedipham (large reduction), although the interactive effect was not significant. Membrane leakage studies indicated that the response of leaves treated with ozone plus phenmedipham was between that of the herbicide and ozone. Determinations of the leachate cation content indicated treatment with phenmedipham increased the leakage of sodium, potassium and magnesium from leaf tissue, whilst ozone had no effect on leakage of cations.

Ozone increased the activities of the antioxidant scavengers, monodehydroascorbate reductase and guaiacol peroxidase, 3 d after exposure, in sugarbeet. Phenmedipham initially elevated the activities of monodehydroascorbate reductase, glutathione reductase, guaiacol peroxidase and glutathione *S*-transferase and decreased the content of glutathione.

When exposed to ozone prior to the application of phenmedipham, the activities of all measured antioxidant enzymes, except SOD, were elevated 2 d after herbicide treatment. Furthermore, some of the enzymes (monodehydroascorbate reductase, glutathione reductase and guaiacol peroxidase) exhibited increases in activity that were greater than the additive effects of the individual treatments after 1 d. This response was reversed 2 d after phenmedipham treatment. Protein contents exhibited a less than additive interaction between days 2 and 4, whilst glutathione reductase, catalase and guaiacol peroxidase also exhibited a less than additive interaction 4 d after herbicide application. Since physiological effects (photosynthetic rate, stomatal conductance, membrane leakage) were not greater in plants treated with ozone and phenmedipham, this might suggest that ozone was increasing the titre of the enzymes sufficiently, to lead to an increased tolerance to phenmedipham damage.

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Part I

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Dixon, J., Hull, M.R., Cobb, A.H., & Sanders, G.E., (1996) A study of antagonism between the herbicide phenmedipham and ozone in sugarbeet (*Beta vulgaris* L. cv Saxon). Pesticide Science **46** (3) : 286-287

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December 1993	Society of Chemicals and Industry (SCI) Conference Agrochemicals and Pharmaceuticals, London	
March 1994	UN ECE International Cooperative Programme on Crops Task Force Meeting, Rome, Italy	
March 1994	CAPER, University of Newcastle upon Tyne	presentation
April 1995	CAPER, Sheffield University	poster
March 1995	SCI Bioactive Molecules, Smithkline Beecham, London	poster
June 1995	5th International Conference on Acid Deposition, Gothenburg, Sweden	poster
July 1995	EMAGE, Nottingham Trent University	poster
November 1995	British Crop Protection Conference Weeds, Brighton	poster

ABBREVIATIONS

ACCase	acetyl coenzyme A carboxylase
AI	active ingredient
APX	ascorbate peroxidase
ATP	adenosine triphosphate
BSA	bovine serum albumin
CAT	catalase
CDNB	1-chloro-2,4-di-nitrobenzene
CF	charcoal filtered
CoA	acetyl coenzyme A
cv(s).	cultivar(s)
d	day
DHA	dehydroascorbate
DHAR	dehydroascorbate reductase
DMRT	diethylene triamine penta acetic acid
DNOC	4,6-dinitro-o-cresol
DTPA	diethylene triamine penta acetic acid
Fd	ferrodoxin
FNR	ferrodoxin NADP reductase
GDA	glutaraldehyde
GPOD	guaiacol peroxidase
GR	glutathione reductase
GSH	reduced glutathione
GSSG	oxidised glutathione
GST	glutathione-S-transferase
h	hour
ha	hectare
His 215	histidine 215 of the D_1 protein
IAA	Indole acetic acid
IRGA	infra red gas analysis
LHC	light harvesting complex
MDA	malondialdehyde
MDHA	monodehyroascorbate
MDHAR	monodehydroascorbate reductase
mRNA	messenger ribonucleic acid
NBT	nitro blue tetrazolium
NIAB	National Institute of Agricultural Biology
NO	nitric oxide
NO_2	nitrogen dioxide
O_3	ozone
OD	outside diameter
OEC	oxygen evolving complex
OSR	oilseed rape
PAR	photosynthetically active radiation
PC	plastocyanin
Phaeo	phaeophytin
PPFD	photosynthetic photon flux density
PQ	plastoquinone
PTFE	poly tetra fluoro ethane
PVPP	polyvinyl polypyrrolidone
Q_A & Q_B	plastoquinones
RNA	ribonucleic acid
RuBisCO	ribulose 1,5-bisphosphate carboxylase/oxygenase
Ser 264	serine 264 of the D_1 protein
SOD	superoxide dismutase
TBA	thiobarbituric acid
Tr	tyrosine molecule
u.v.	ultra violet

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CHAPTER 1 - INTRODUCTION

Spring-sown crops, such as sugarbeet, oilseed rape and spring barley are sprayed with pesticides at a time when ozone episodes are likely to occur (QUARG, 1993). There is therefore the potential that ozone may influence plant responses to pesticide application and *vice versa*. For example, the fungicide benomyl and ozone are known to interact antagonistically in *Phaseolus vulgaris* L. (Pell, 1976), i.e. benomyl protects against ozone. Interactions between herbicides and ozone were first observed by Carney *et al* (1973). Various interactions were noted with benefin (antagonistic) and pebulate (synergistic) in tobacco (*Nicotiana tabacum* L.).

At the present time, experiments conducted to assess ozone damage to crops throughout Europe do not take into account standard agricultural practices of pesticide use. There is, therefore, a clear need to research the manner in which pesticide application may influence plant responses to ozone and *vice versa*.

1.1 OZONE

The effect of ground level ozone pollution on agricultural crops has caused concern since the 1950's when it was implicated in the "weather flecking" of grapes (Richards *et al*, 1958) and tobacco (Heggested & Middleton, 1959). Since then, the effects of ozone have been characterised in several crop species (Hill *et al*, 1961; Heck *et al*, 1984; Temple, 1990). Ozone has been shown to decrease crop yields in susceptible species such as wheat (Fuhrer *et al*, 1992), beans (Schenone *et al*, 1992) and soybean (Miller *et al*, 1994). Moreover, the current problem may be exaggerated in the future. Preliminary results for London indicate that for the expected reduction in oxides of nitrogen (NO_x) of 50-60 %, annual mean ozone concentrations will increase by 3-4 ppb from current levels of around 10-15 ppb (Derwent, 1999).

1.1.1 Ozone Production

Ozone is a secondary pollutant formed by chemical reactions between the primary gaseous pollutant, nitric oxide (NO), the secondary pollutant nitrogen dioxide (NO_2) and other atmospheric components (volatile organic carbons (VOCs)). The two main sources of ozone are *in situ* production and stratospheric - tropospheric exchange. Conversely, ozone can be removed by photochemical destruction and deposition to the Earth's surface and oceans.

1.1.1.1 *In situ* Production

In situ production is thought to be the major source of ground level ozone (Colbeck, 1988). It results from the photolysis of nitrogen dioxide from car exhausts, heating and power generators to nitric oxide and atomic oxygen (O; equation 1):

$$NO_2 + hv\ (\lambda = 240\text{-}430\ nm) \rightarrow NO + O\ (j_1) \quad (1)$$

$$O + O_2 + M \rightarrow O_3 + M\ (k_2) \quad (2)$$

$$NO + O_3 \rightarrow NO_2 + O_2\ (k_3) \quad (3)$$

Where M is a molecule such as nitrogen or oxygen, which dissipates the reaction energy and prevents the redissociation of ozone; and j_1, k_2 and k_3 are the photolytic rate coefficients. The above scheme is highly simplified for ease of explanation. In unpolluted air, equations 1-3 are in balance and the following relationship holds true:

$$[O_3] = \frac{j_i\ [NO_2]}{k_3\ [NO]} \quad (4)$$

Consequently, ozone concentration depends on the ratio of nitrogen dioxide to nitric oxide (Colbeck, 1988). Nitric oxides can be oxidised to nitrogen dioxides by peroxy radicals (RO_2; equation 5) and other compounds, formed either naturally or from the photochemical degradation of volatile organic compounds (VOCs) of anthropogenic origin:

$$RO_2 + NO \rightarrow RO + NO_2 \quad (5)$$

The concentration of VOCs in the atmosphere is increasing due to the continued production and emission of large quantities of VOCs by cars, solvents (especially paint) and power stations, i.e. combustion of fossil fuels (PORG, 1993). Consequently in polluted air, reaction (3) is slowed and the equilibrium shifts towards net ozone production, resulting in the occurrence of potentially damaging ozone concentrations.

During the summer in temperate regions, when weather conditions are warm (>20°C), dry, sunny and still (1-3 m s^{-1}; typical of anticyclonic conditions in Western Europe), ozone concentrations can reach very high levels compared to normal background levels. For example, during June and August 1976 there were 40 d with anticyclonic conditions over North Western Europe, resulting in around 30 d with hourly ozone concentrations over 80 nl l^{-1} in London (Ball & Bernard, 1978). During this episode, the maximum hourly mean concentration at several sites in southern England exceeded 200 nl l^{-1}, with the highest (258 nl l^{-1}) occurring at Harwell, a rural site in Oxfordshire (PORG, 1987). In contrast, ozone concentrations may stay close to zero for several days during winter (Nov 1994 to Jan 1995; see Figure 1.1). This is due to generally higher winds,

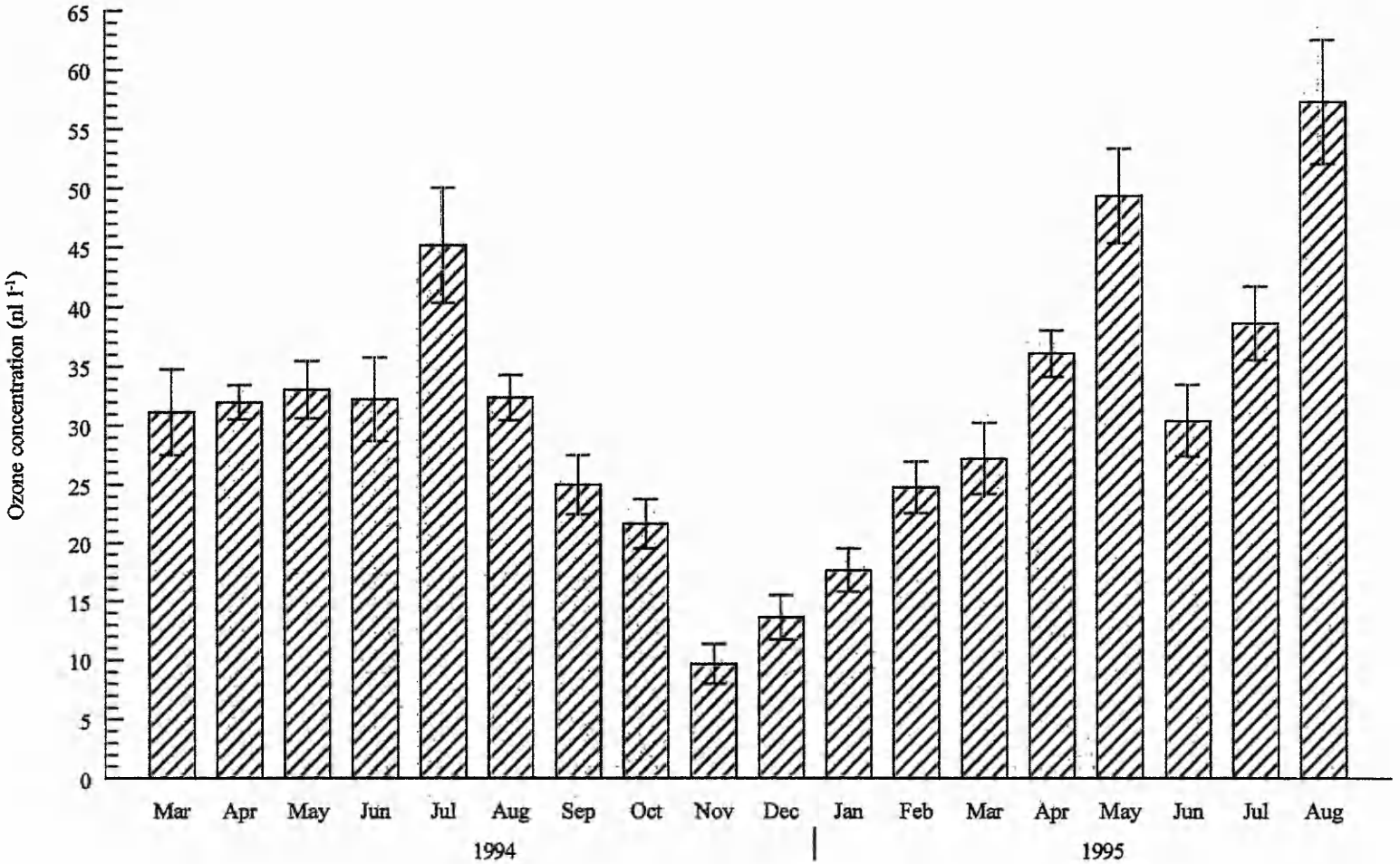


Figure 1.1 Seven hour monthly mean ozone concentrations at Nottingham (Nottingham Trent University, Clifton site, 200m from A453, 11.8km from M1; Dixon unpublished). Values are means ± se.

overcast conditions, shorter days, lower temperatures and lower levels of U.V. radiation (Colbeck, 1988).

1.1.1.2 Stratospheric-Tropospheric Exchange

Ozone formed in the upper atmosphere (reactions 6 and 7) is brought down to the troposphere by mixing between the two layers.

$$O_2 + hv\ (\lambda < 242\ nm) \rightarrow O_3 \quad (6)$$

$$O + O_2 + M \rightarrow O_3 + M \quad (7)$$

Episodes due to this exchange are of short duration and usually occur with strong surface winds (Heck *et al*, 1984). It is thought that only 0.7 and 0.3 % of episodes of 60 and 80 nl l^{-1}, respectively, are due to stratospheric incursion (Colbeck & Harrison, 1985).

1.1.2 Measurements of Ozone Concentrations

Ozone concentrations can be expressed as ppb (parts per billion (1×10^9)), as nl l^{-1} or as $\mu g\ m^{-3}$, where 1 ppb = 1 nl l^{-1} = 0.52 $\mu g\ m^{-3}$. There are several different ways to depict ozone concentrations:

i) single event - 1 h or 7 h maxima (peak concentration over one hour or 7 hours, Figure 1.2 line a); second highest daily maximum 1 h concentration. These descriptors give an indication of the peak level only and do not provide data on the duration of the episode or the general concentration.

ii) mean - 7/8/12 h daily means (average of the concentrations over several hours). Measured from 10 am to 5 pm (7 h, Figure 1.2 line b), 9 am to 5 pm (8 h, Figure 1.2 line c) and 8 am to 8 pm (12 h, Figure 1.2 line d); seasonal 7/8 h daily mean (average of the 7 or 8 h daily means over a growing season (April to September or May to July)). Exposures expressed as means take into account longer term concentrations and seasonal means do this to an even greater extent. However, such procedures tend to smooth-out the highest concentrations that can occur during episodes.

iii) cumulative - seasonal sum of hourly concentrations (SUM00; total amount of ozone over the entire season weighted equally). This does not take into account the fact that high concentrations for a short time have more effect on vegetation than prolonged periods at low concentrations.

iv) concentration weighting - AOT40 [mainly used in Europe] (sum of all mean hourly concentrations over a threshold of 40 nl l^{-1}; Figure 1.2 e (shaded area)); SUM06, SUM08, SUM10 [mainly used in America] (seasonal sum of hourly concentrations at or above 60, 80 and 100 nl l^{-1} respectively); HRS08 (total hours with a concentration at or

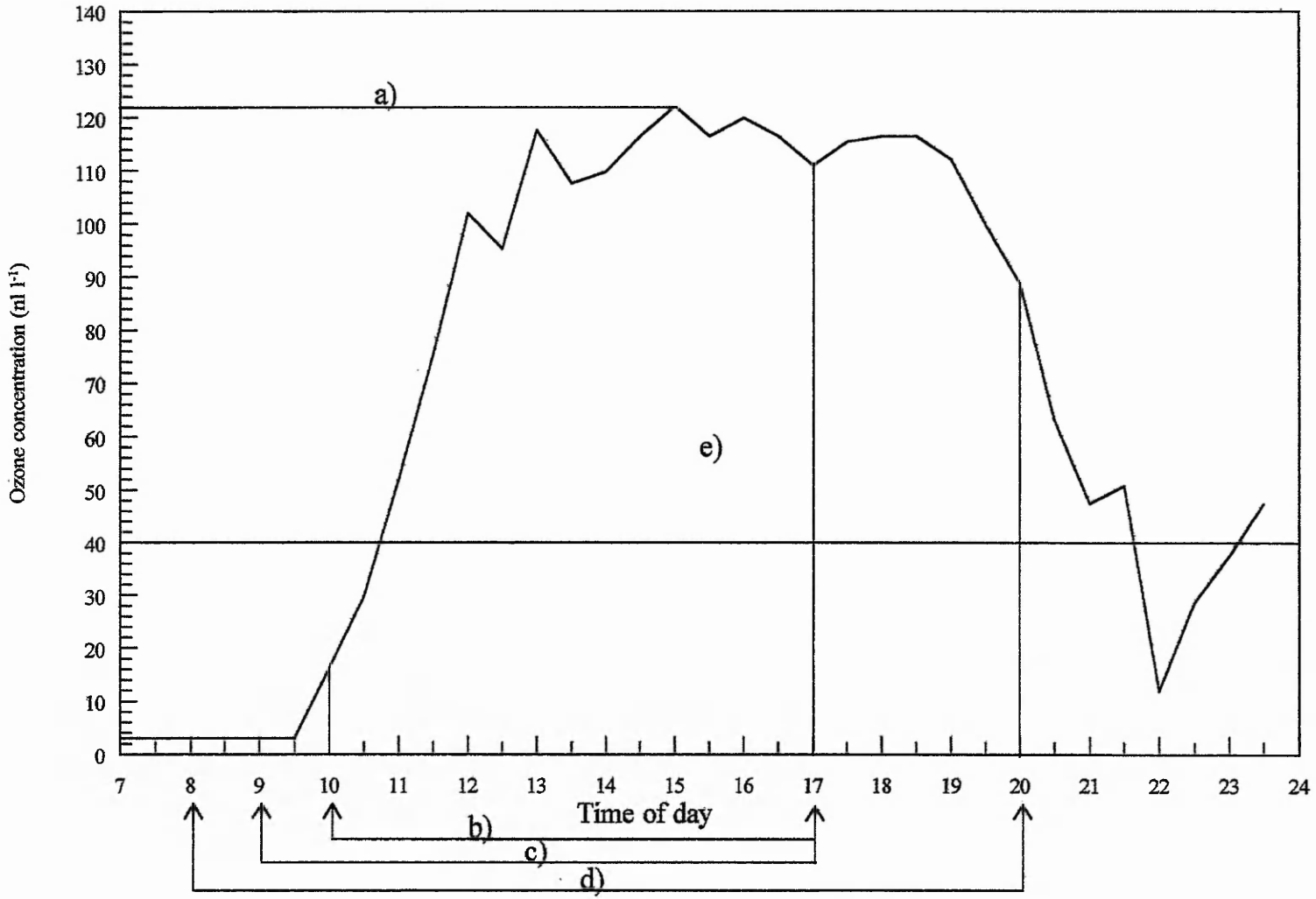


Figure 1.2 Measurements of ozone concentrations. Data from 5th May 1995 at Nottingham (Nottingham Trent University, Clifton site, 200m from A453, 11.8km from M1; Dixon unpublished). Explanation of figure in text.

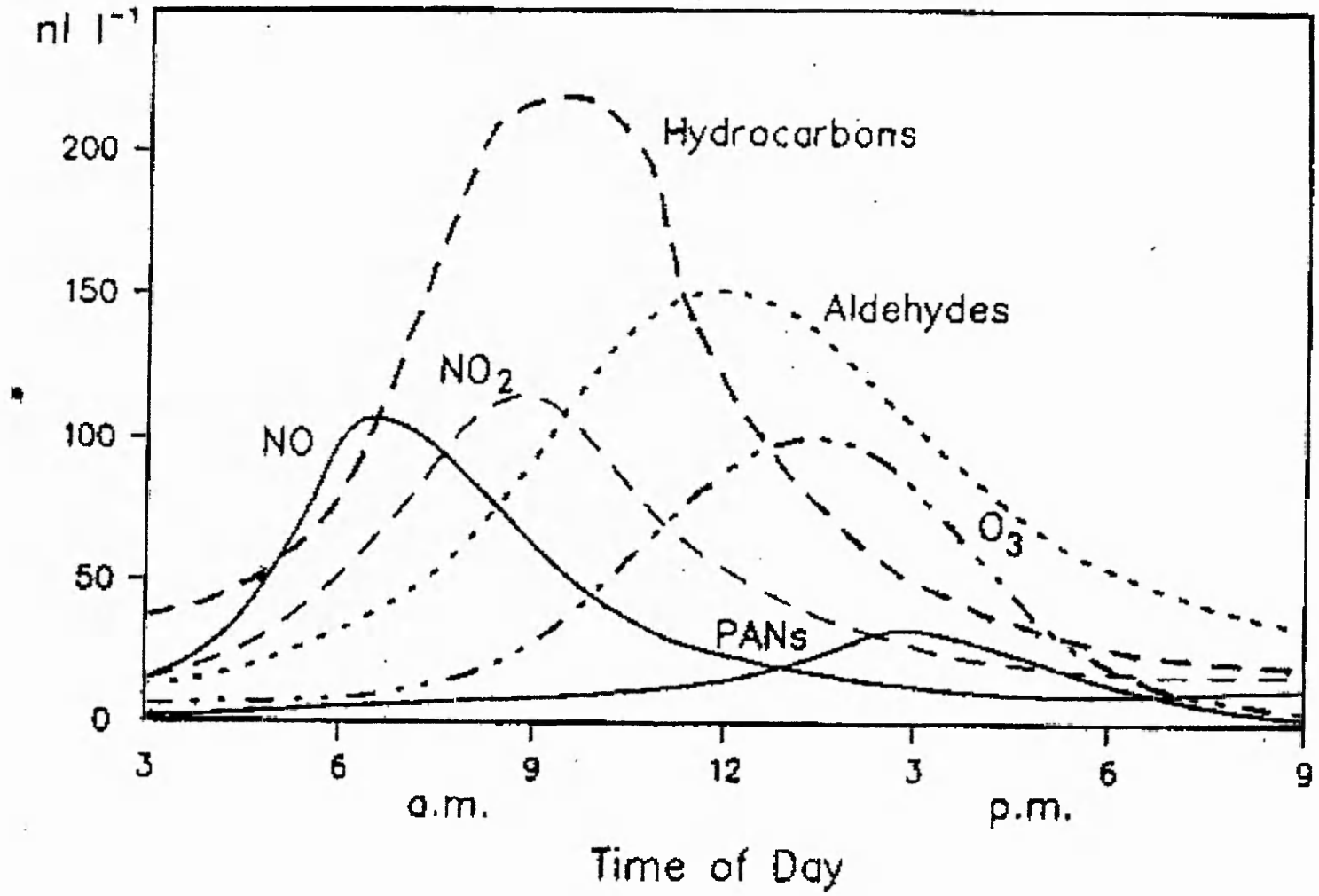


Figure 1.3 The diurnal patterns of pollutant distribution in urban areas (Wellburn, 1994).

concentrations commonly increase and cause a transient decrease in ozone concentrations (Colbeck, 1988). As the volume of traffic decreases there is a slow rise in the concentration of nitrogen dioxide and associated with this the ozone concentration increases, typically attaining peak levels between 1200h and 1500h. From 1800h onwards, ozone concentrations gradually fall, presumably reflecting an increase in nitric oxide concentrations during the evening rush hour (QUARG, 1993). The reduction in light and the relatively increasing importance of deposition to surfaces also contribute to the decreased ozone concentration after this time. Figure 1.4 shows the diurnal variations occurring at Nottingham (Nottingham Trent University, Clifton site, approx. 200 m from A453) from 1st June 1994 to 30th May 1995. Generally, the Nottingham data tend to follow the standard patterns of diurnal exposure observed in urban areas. Concentrations were around 30-35 nl l^{-1} between 1200 and 1800h except in July 1994 and May 1995 where the monthly mean during this time was around 50-55 nl l^{-1}. National air quality warnings were issued in these months on days when 7 h mean daily concentrations were above 100 nl l^{-1} (Edwards, 1995).

Mean ozone concentrations in urban sites are generally lower than at nearby suburban and rural areas, due to the amount of nitric oxide produced by vehicles (QUARG, 1993). However, the introduction of catalytic converters in cars will decrease the amount of nitric oxide produced and therefore may increase the amount of ozone in urban areas. Ozone and its precursors may be carried hundreds of miles and this can lead to increased ozone concentrations during the night (0200-0400h) in rural areas (QUARG, 1993). At rural sites, ozone concentrations during the summer show a normal diurnal variation, whereas during the winter concentrations remain almost constant (Colbeck, 1988).

1.1.4 Exposure of Plants to Ozone

To expose plants to gaseous pollutants several techniques may be adopted each with advantages and disadvantages.

i) Closed chamber systems; usually consisting of a number of chambers (closed boxes) made of an inert material such as perspex, through which air is passed. A larger more expensive version is the solardome, utilising solar radiation as the light source. The relevant gas (e.g. ozone, sulphur dioxide, nitrogen dioxide or carbon dioxide) is injected into the air stream prior to entry into the chamber. The pollutants and microclimate are monitored within the chamber, with the option of controlling several of the variables, such as pollutant concentration, temperature, photon flux density and relative humidity. Closed

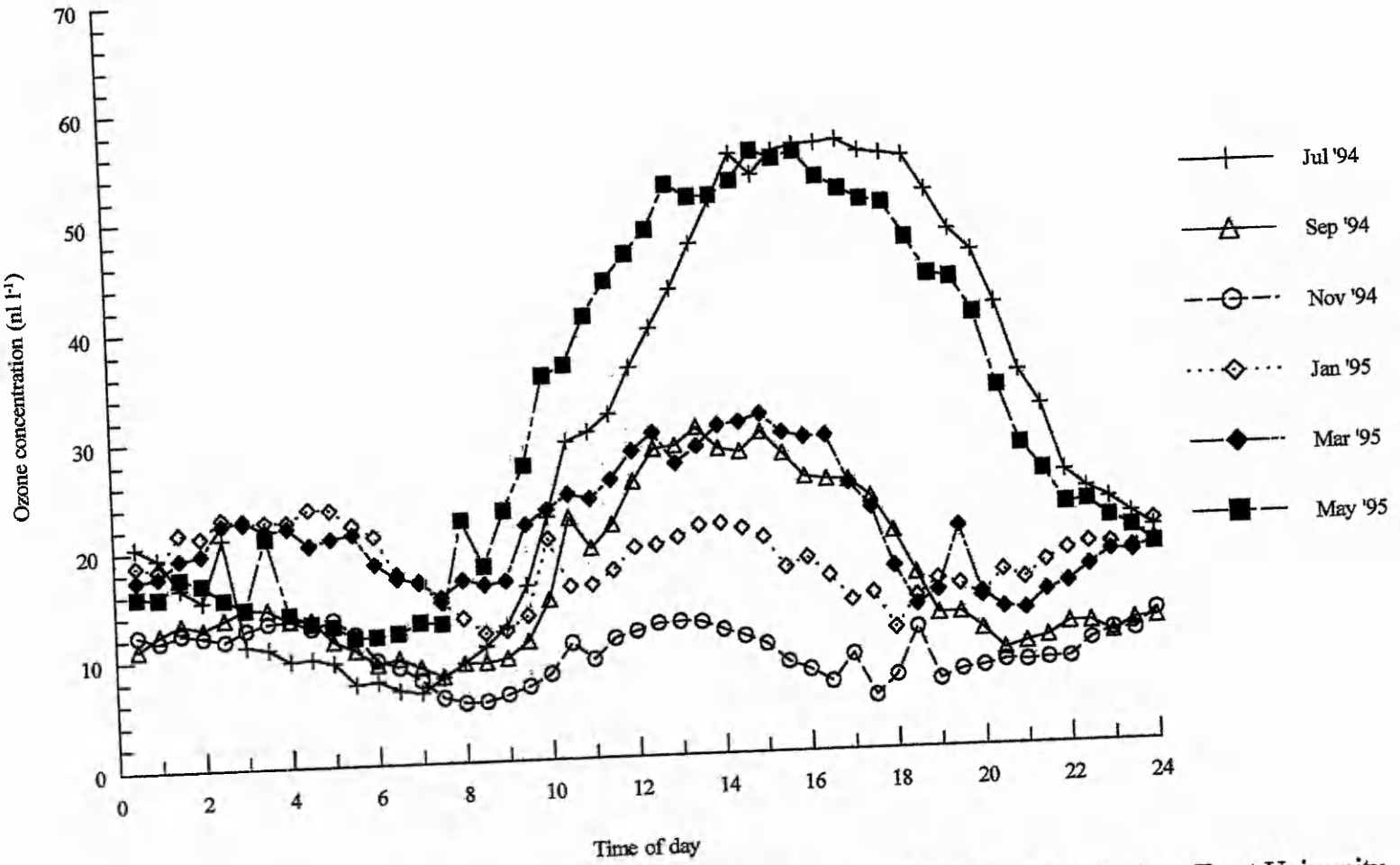


Figure 1.4 Diurnal variations in ozone concentrations from July '94 to May '95 at Nottingham (Nottingham Trent University, Clifton site, 200m from A453, 11.8km from M1; Dixon unpublished). Values are 5 min means measured every 30 min and averaged for the respective month.

chamber systems are often used for short-term studies such as those investigating the effects of acute doses of a pollutant. Advantages of these systems include their relative low cost to construct and maintain. However, closed chambers are also the least representative of field conditions.

ii) Open exposure systems; consist of 2 types

a) Open top chambers; These consist of cylindrical chambers with no roof (hence open-top) which fit over the top of either pots of plants or field sown plants. Air is blown from soil level up through the top of the chamber. Open top chambers are used for longer-term studies (eg. growing season) to determine chronic effects of pollutants. Conditions within the chambers are closer to those in the field, although chamber effects can be large, including rain shadows and raised temperatures compared to ambient air plots.

b) Field exposure systems; Systems are either low cost, as in the case where plants are exposed to ambient air with no addition of pollutants, whilst monitoring the pollution and climatic data (UN-ECE, 1995) or very expensive where pollutants are added to ambient concentrations through the use of complex computer controlled rings placed around the plants. These systems represent conditions within the field. However, unless equipment monitors all pollutants and other stresses, e.g. water and temperature, then data from these systems may not be reliable.

After consideration of the use of the system and other practical constraints (cost/space/maintenance) a closed chamber exposure system was constructed at Nottingham Trent University.

1.1.5 Effects of Ozone on Plants

Plant species show variation in sensitivity to ozone and visible damage can occur in some species at concentrations as low as 60 nl l^{-1}. Several reviews of the effects of ozone have been published (Heath, 1980; Roberts *et al*, 1983; Malhotra & Khan, 1984; Cooley & Manning, 1987; Krupa & Manning, 1988; Darrall, 1989; Heagle, 1989). An underlying trend seems to suggest large differences in sensitivity depending on species (Hill *et al*, 1961), cultivar (Temple, 1990) and developmental stage (Reiling and Davison, 1992). Experiments must therefore be carefully defined in order to obtain reproducible results.

1.1.5.1 Ozone Injury

Ozone injury has been classified into visible and non-visible (Heath, 1980). Visible injury is the amount of leaf area noticeably altered from its normal morphological form

(Heath, 1980). Non-visible injury is described as biochemical or physiological alterations resulting in lowered plant productivity without any apparent visible symptoms (Heath, 1980). It has been suggested that once ozone is inside the leaf, it passes through the intercellular spaces and reacts with the tissue, which may cause flecking-type injury over small areas (Heath, 1994b). Visible ozone injury typically comprises a scattered distribution of roughly symmetrical, chlorotic flecking, which develops between veins (Wellburn, 1994). These injuries may become reddish or bronzed due to enhanced anthrocyanin production or tannin formation. The appearance of visible injury has been used to assess differences in ozone sensitivity of various species and cultivars. Visible injury on 10 cultivars of spring wheat was variable between varieties and had very little correlation with the effects on growth (Barnes *et al*, 1990). This apparent lack of correlation with sensitivity or yield reductions was also observed in 4 cultivars of tomato (Temple, 1990).

The appearance of visible symptoms of ozone damage (i.e. localised cell death) are often associated with induction of defence-related genes in response to ozone, leading to analogies between the reaction of plants to ozone and the hypersensitive responses induced by many biotic pathogens (Sandermann *et al*, 1998). For example, ozone-induced increases in phenolic metabolism, resembling elicited defence responses, occurred with effects characteristic of browning reaction and wound responses in soybean after exposure to 100nl l^{-1} ozone for 13 days (Booker & Miller, 1998).

1.1.5.2 Growth and Yield

The effects of ozone on the growth and yield of many species of crops have been studied (see reviews by Heath, 1980; Roberts *et al*, 1983; Malhotra & Khan, 1984; Cooley & Manning, 1987; Krupa & Manning, 1988; Darrall, 1989; Heagle, 1989). In general, susceptible species show decreases in growth and/or yield in response to exposure to realistic ozone concentrations. Wheat (*Triticum aestivum* L.) is particularly susceptible to ozone and has been the subject of many studies. For example, yield reductions of 7 and 22 % were observed in cultivar Drabant, in response to 42 and 56 nl l^{-1} ozone (7 h seasonal mean; Pleijel *et al*, 1991); 100 nl l^{-1} ozone (8 h seasonal mean) reduced grain yield by 57 % in cultivar Albis (Lehnherr *et al*, 1987); Ten Greek cultivars of spring wheat, introduced between 1932 and 1980, exposed to 90 nl l^{-1} showed a decrease in the mean relative growth rate which was negatively correlated with the year of introduction (Barnes *et al*, 1990). Effects of ozone on wheat yield largely result from a decrease in the number of

grains produced, although the size and quality of individual grains may also be affected (Fuhrer *et al*, 1992; Selldén & Pleijel, 1993).

The sensitivity of other cereals, such as barley (*Hordeum vulgare* L.) and oats (*Avena sativa* L.), to ozone is not as great as that of wheat. No consistent growth or yield reductions were observed in response to 39-111 nl l^{-1}, whilst visible injury was only seen at the highest concentration (Adaros *et al*, 1991b). No visible effects were observed at 45 nl l^{-1} (7 h seasonal mean; Pleijel *et al*, 1992) or 94 nl l^{-1} (7 h seasonal mean; Temple *et al*, 1985). A reduction in the number of grains per ear has been shown in oats, but this was not converted to effects on yield (Pleijel, 1993).

Beans (*Phaseolus vulgaris* L.) are generally considered sensitive to ozone, although there may be considerable variation between cultivars. In sensitive genotypes, large reductions in yield have been observed in response to relatively low ozone concentrations. Decreases in yield of 31 % have been reported in response to 43 nl l^{-1} 7 h seasonal mean ozone at a rural site and 50 nl l^{-1} at an urban site (Schenone *et al*, 1992), while decreases of 35 and 48 % in biomass and pod weight, respectively, were observed in cultivar Rintintin in response to 110 nl l^{-1} ozone (Bender *et al*, 1990).

Increased premature senescence at 61 nl l^{-1} ozone may have accounted for an observed yield loss of 27 % in spring rape (*Brassica napus* L.) cultivar Callipso (Adaros *et al*, 1991a). This has also been observed at 75 nl l^{-1} in 5 cultivars (8 h mean; Johnsen *et al*, 1987). Several studies have been conducted on soybean (*Glycine max* L. Merr.) which have shown reductions in growth and yield (Mulchi *et al*, 1988; Miller *et al*, 1994). While other studies have revealed no significant effects of ozone (Smith *et al*, 1987). Other studies have shown the importance of compensatory flexibility in response to pollutant-induced losses of reproductive sites, for example, through increased numbers and dry weight of seed in a tolerant cv of *Brassica napus* (Bosac *et al*, 1998).

Ozone generally decreases the growth and yield of sensitive genotypes. To affect crop yields, ozone episodes must occur at susceptible times during the life cycle of the crop. In the case of cereals, the most damaging episodes may coincide with grain filling (Selldén & Pleijel, 1993).

1.1.5.3 Photosynthesis

Ozone generally decreases whole plant photosynthesis in sensitive species. The reductions have been shown to be correlated with increases in ozone concentration and decreases in growth and yield (Reich & Amundson, 1985). Correlations with growth and

yield have also been observed in *Phaseolus vulgaris* at an urban (50 nl l^{-1}) and a rural (43 nl l^{-1} 7 h seasonal mean) site in Italy (Schenone *et al*, 1994). Maximum reductions in photosynthesis (40 % urban, 23 % rural) occurred at the time of pod ripening, which may have had a direct influence on yield. Stomatal conductance was also reduced in this study, although this only occurred late in the season.

Stomatal conductance has been shown to be increased, decreased or to be unaffected by exposure to ozone (Darrall, 1989), and the full suite of responses has been observed in 12 cultivars of *P. vulgaris* in response to 400-500 nl l^{-1} for 75-135 min (Guzy & Heath, 1993). Sensitive varieties exhibited relatively higher inherent stomatal conductances, whilst those of tolerant cultivars were lower. Reductions in stomatal conductance have generally been observed in wheat (Balaguer *et al*, 1995), bean (Schenone *et al*, 1994), barley (Rowland-Bamford, *et al*, 1989) and soybean (Reich *et al*, 1985). Stomatal closure may result from effects on photosynthesis, increasing the internal carbon dioxide concentration, rather than direct effects on the stomatal apparatus itself (Farage *et al*, 1991; Lehnherr *et al*, 1988; Reiling & Davison, 1994).

The reduction in photosynthesis in response to exposure to ozone could have two causes. Firstly, a decrease in photosynthetic rate may be due to the closure of stomata after an ozone-induced loss of permeability of the guard cells (Heath 1994b). Secondly, several studies on wheat, have noted reductions in ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO), activity and content. Plants in these studies were exposed to 100 nl l^{-1} (8 h seasonal mean; 50 d; Lehnherr *et al*, 1987) and 150 nl l^{-1} (7h mean; 16 d; Nie *et al*, 1993). Observations indicated that the reductions in RuBisCO content, pigments and photosynthetic capacity were due to ozone-induced premature senescence. A further study indicated that the reduction in photosynthetic rate was due to a reduction in the quantity of active RuBisCO (Farage & Long, 1999). Ozone accelerated the normal decline in the activity and quantity of RuBisCO in the fully expanded leaves of poplar (*Populus maximowizii x trichocarpa* NE 388) and radish (*Raphanus sativus* L.) and this was coupled with a rapid yellowing and abscission of leaves (Pell *et al*, 1992). An investigation of the chronology of events attributed the reduction in carbon dioxide assimilation to a decline in RuBisCO content, rather than a decrease in stomatal conductance which either did not occur or occurred after the reduction of photosynthesis. Similar reductions in content and activity of RuBisCO have been observed in soybean (Reid *et al*, 1998).

Effects on photosynthetic rate may or may not be dependent on the age of the leaf or plant. Studies conducted on cereals seem to indicate age dependence. Nie *et al* (1993) split the leaf into 3 sections: the youngest at the base of the leaf, the middle and the oldest at the leaf tip. The oldest section of the leaf was the most sensitive to ozone, showing reductions in photosynthetic rate and chlorophyll and protein contents. Similarly, in oat the oldest active leaf was the most sensitive to 150 nl l^{-1} ozone for 2 h, although recovery of photosynthesis occurred after 19 h (Myhre *et al*, 1988).

Photosynthetic parameters, such as photosynthetic rate and chlorophyll content, of sensitive species are affected by ozone. The RuBisCO content of the leaf seems to be influenced through the acceleration of leaf senescence by ozone, since no effect has been shown on RuBisCO synthesis. Stomatal conductance has been reported to both increase and decrease.

1.1.5.4 Membrane Damage

Ozone is thought to dissolve rapidly in the water within the intercellular spaces, where the relative humidity approaches 100%. It reacts with water to form active oxygen species, such as superoxide, hydroxyl radicals, hydrogen peroxide and singlet oxygen (Kanofsky & Sima, 1991; Heath, 1994b). The formation of such species prior to symptom appearance has been demonstrated in *Pisum sativum* and *Phaseolus vulgaris* using electron spin resonance (Mehlhorn *et al*, 1990). When these radicals reach the plasma membrane, they can initiate lipid peroxidation through reactions with unsaturated fatty acids (Heath & Castillo, 1988) or increase leakiness by inhibiting the pumps and transporters in the membrane (Dominy & Heath, 1985).

After ozone has entered the leaf, the first response is an increase in the passive permeability of potassium ions and a depolarization of the membrane potential (Heath & Castillo, 1988). Secondly, active sugar transport becomes inhibited, passive permeability increases further and a decrease occurs in the energy sources by depletion of ATP. Increases have been observed in the permeability of glucose and deoxyglucose in *P. vulgaris* in response to 400 nl l^{-1} for 1 h (Perchorowicz & Ting, 1974). These increases were not due to enhanced metabolic activity within the plant. Increases in electrolyte loss from rice and bean were observed in response to exposure to 250 nl l^{-1} for 10 d (Tripathi *et al*, 1990). In some species, the alterations in leakage returned to control levels within 72 h indicating a mechanism of recovery from the stress (McKersie *et al*, 1982; Swanson *et al*, 1982).

Lipid and sulfhydryl oxidation products, such as malondialdehyde (MDA), also accumulate. For example, wheat treated with 500 nl l^{-1} ozone for 6 h developed typical visible symptoms of injury. These effects were coupled with increases in solute leakage, the concentration of free fatty acids and MDA measured by the thiobarbituric acid (TBA) assay (Mackay *et al*, 1987). There was no decrease in the degree of fatty acid unsaturation providing no evidence of fatty acid peroxidation. Direct modification of protein sulfhydryls within the membrane can lead to a decline in transport and a change in the fluidity of the membrane. This allows ozone or its reaction products to penetrate further and react with proteins and sulfhydryl groups normally buried deep within the membrane (Heath, 1987). As a result of this, membrane vesiculation and ultimately cell lysis occur (Swanson *et al*, 1982). In contrast, a study on *Vicia faba* exposed to 150 nl l^{-1} ozone for 4 h, showed increased leakage and concentrations of TBA reactive substances prior to the onset of visible injury (Guidi *et al*, 1999).

1.2 HERBICIDES

It is necessary to control weeds for several reasons. Firstly, weeds compete with the crop for water, nutrients and light. Secondly, weeds may harbour pests and diseases. Thirdly, the use of agricultural machinery can become very difficult or impossible if some plants (e.g. *Polygonum aviculare* L. (knotgrass) and *Chenopodium album* L. (fat hen)) are growing within the crop. Fourthly, some weeds can reduce the quality of the crop by having seeds which are difficult to separate from the crop. These include *Avena fatua* L. in barley and wheat, *Lolium temulentum* L. and *Agrostemma githago* L. in flour-quality cereals and *Solanum nigrum* L. in peas.

Bordeaux mixture and copper sulphate were the first chemicals used to control weeds at the turn of the century (Hassall, 1990). The introduction of the synthetic herbicides 4,6-dinitro-*o*-cresol (DNOC), 2,4-D and MCPA prior to World War II prepared the way for the complete mechanisation of farm practices and the use of cereal mono-cultures (Cobb, 1992). Chemical weed control has now almost completely replaced hand weeding, since it is cheaper, more effective, less damaging to the crop and less weed seeds are brought to the surface of the soil. Herbicides may be classified into several groups according to their chemical structure and mode of action. This study concentrates on 5 herbicides with 3 contrasting modes of action. These are phenmedipham (photosystem II inhibitor), clopyralid and mecoprop-p (auxin-type) and diclofop-methyl and metazachlor (graminicides).

1.2.1 Photosystem II Inhibitors

Over half of the herbicides currently in use either block or divert photosynthetic electron transport as shown in Figure 1.5. Several groups of herbicides are known to inhibit photosystem II by preventing plastoquinone from binding to the Q_B site on the D_1 protein, where Q_B binds *via* two hydrogen bridges at His 215 and Ser 264 (Halliwell, 1991). There are two families of photosystem II herbicides. The serine family include those with a carbonyl or equivalent group (e.g. ureas, triazines and carbamates) which are orientated towards Ser 264. Whilst those inhibitors with a phenol group (hydroxybenzonitriles and nitrophenols) bind to His 215 and are termed the histidine family (Trebst, 1987). By binding to the D_1 protein both groups of herbicides prevent electrons from passing to plastoquinone, therefore excitation energy can not travel any further than Q_A (Cobb, 1992). The excess energy results in the photochemical destruction of carotenoids, which normally quench triplet chlorophyll and singlet oxygen during photoinhibition. Chlorophyll molecules are destroyed in turn, leading to the excitation energy being passed to oxygen molecules generating active oxygen species (section 1.3). The enzymes which scavenge these oxygen species become overloaded leading to peroxidation. Unsaturated fatty acids (particularly linoleic (18:2) and linolenic (18:3)) are susceptible to free radical attack. Several forms of active oxygen can be produced, resulting in the initiation of lipid peroxidation, and the generation of lipid hydroperoxides, alkoxy radicals, lipid alcohols and lipid radicals. Eventually, through the chain of lipid peroxidation the breakdown products ethane and malondialdehyde are formed.

Phenmedipham (methyl 3-(3-methylcarbaniloyloxy)carbanilate) is a carbamate herbicide which is a member of the serine family (Figure 1.6). It is the major post-emergent herbicide used to control broad leaved and grass weeds in sugarbeet, *Beta* spp. (red beet, fodder beet and mangels) and strawberries (*Fragaria* spp Duch.; Edwards, 1968; Proctor, 1993). In susceptible species, photosynthesis is blocked almost immediately and plants die *via* lipid photoperoxidation. Electron transport is also blocked in tolerant species, such as sugarbeet, but plants recover after a few days (Prodoehl *et al*, 1992). In isolated chloroplasts, phenmedipham strongly inhibits electron transport by 50 % at 2×10^{-8} M (Ravanel *et al*, 1990) and by 100 % at 2.1×10^{-7} M in spinach (*Spinacea oleracea* L.; Macherel *et al*, 1982). Studies have shown that sugarbeet metabolises phenmedipham into two metabolites much more quickly than the susceptible *Brassica napus* L. (Davies *et al*, 1990). The less polar of these two metabolites had properties

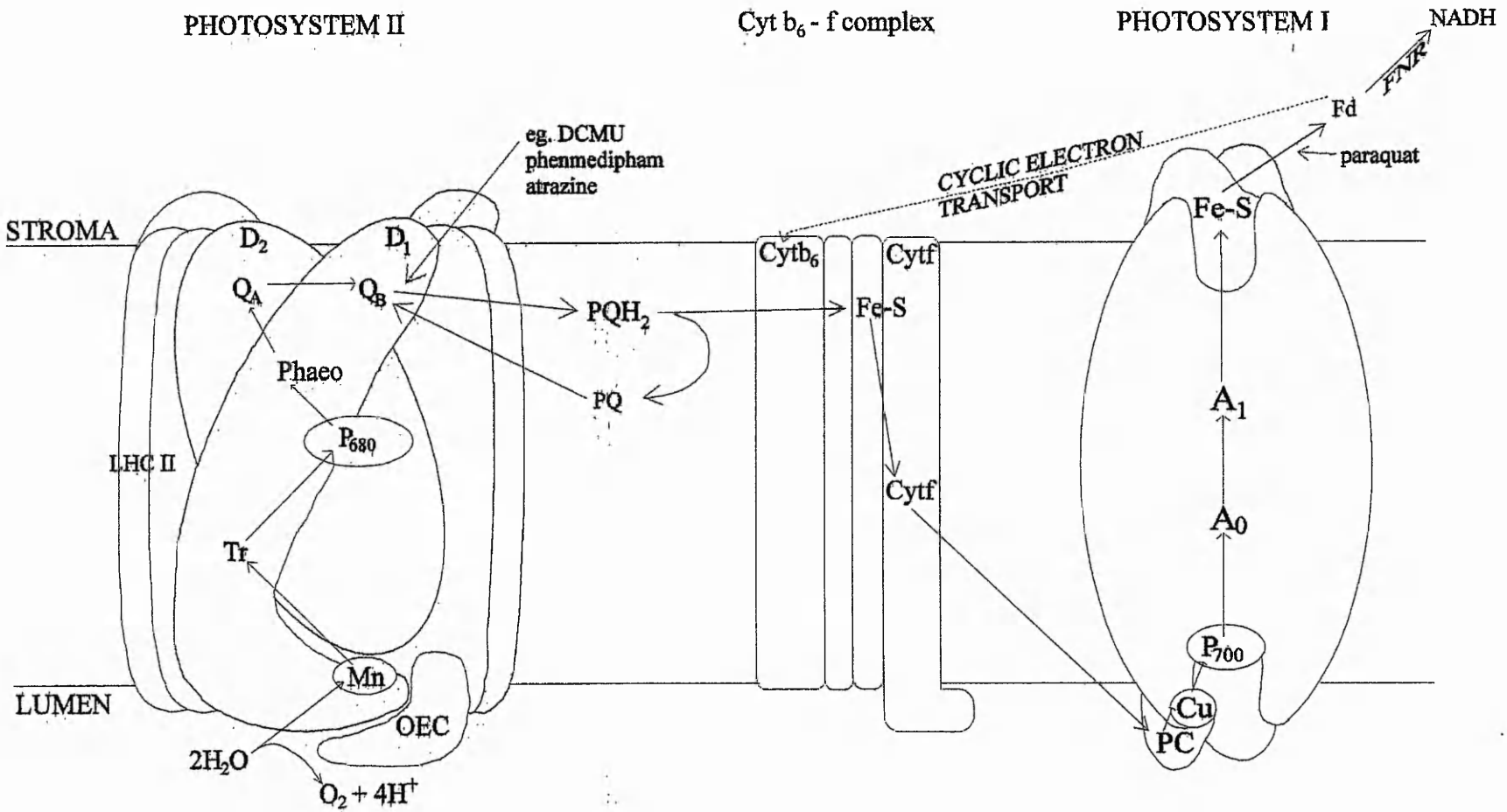


Figure 1.5 Photosystems I and II in the thylakoid membrane of chloroplasts. OEC, oxygen evolving complex; Tr, tyrosine molecule; Q_A & Q_B, plastoquinones; D_1 & D_2, proteins; P_{680} reaction centre of PS II; Phaeo, phaeophytin; PQ, plastoquinone; Fe-S,protein; PC, plastocyanin; P_{700} , reaction centre of PS I; A_0 & A_1 electron acceptors; Fd, ferrodoxin; FNR, ferrodoxin NADP reductase; LHC II, light harvesting complex II (After Hall & Rao, 1988, Cobb, 1992).

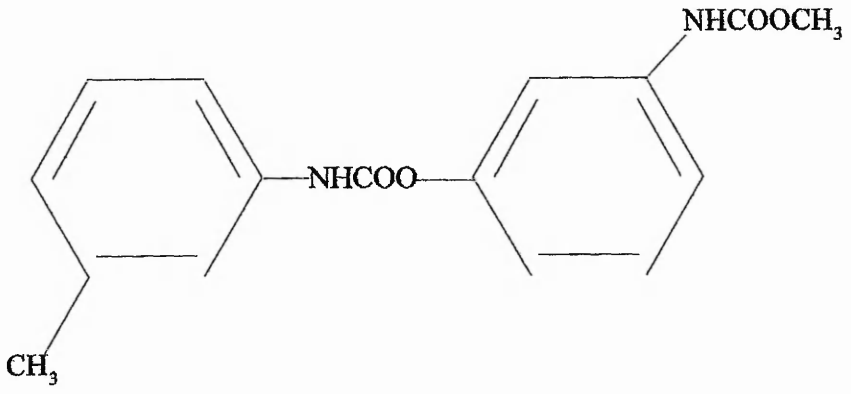
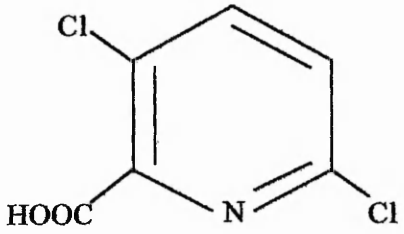


Figure 1.6 Chemical structure of phenmedipham (methyl3-(3-methylcarbaniloyloxy)carbanilate).

a)



b)

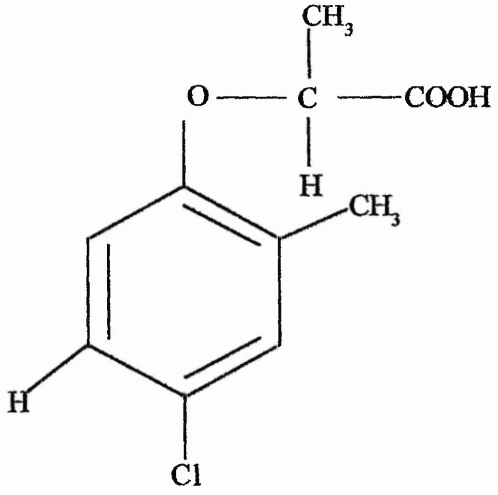


Figure 1.7 Chemical structures of a) clopyralid (3,6-dichloro-2-pyridinecarboxyic acid) and b) mecoprop (2'-(methyl-4-chlorophenoxy) propionic acid.

which indicated it was derived from phenmedipham by a single hydroxylation and monoglycosylation step. This was also a precursor of the second more polar metabolite.

1.2.2 Auxin-type Herbicides

Auxin-type herbicides are synthetic auxins and although the molecular basis of their activity is unknown, the morphological symptoms produced are indicative of excessive auxin response (Cobb, 1992). These herbicides can be split into five groups, which all possess a free carboxyl group: phenoxyalkanoic acids (e.g. 2,4-D, mecoprop); benzoic acids (e.g. dicamba, chloramben); aromatic carboxymethyl derivatives (e.g. benazolin, indole acetic acid (IAA)); pyridine derivatives (e.g. clopyralid, picloram) and quinoline carboxylic acids (e.g. quinclorac).

Normal IAA concentrations are around 1-100 mg kg^{-1} fresh weight, hence for a plant weighing 10 g the amount of auxin will be 10-100 ng. 2,4-D is applied at 0.2-2.0 kg ha^{-1} and a single plant may intercept 100 mg, which is clearly an overdose (Cobb, 1992). Plants die through an excess of these auxin-type herbicides leading to uncontrolled growth. There are 3 stages of symptom development in susceptible species (Coupland, 1994). Firstly, within 1 day of spraying, changes occur in cation permeability, for example an enhanced accumulation of potassium ions in guard cells, which results in increased stomatal conductance. This increased permeability results from the enhanced activity of plasma membrane ATPases (Cleland, 1987). It is thought that the herbicide competes with auxin for an auxin-binding protein on the plasma membrane. An auxin-binding protein from maize has been characterised and located in the lumen of the endoplasmic reticulum and on the outside of the plasma membrane (Venis & Napier, 1995). Soluble reducing sugars and amino acids increase in concentration coupled with elevated mRNA synthesis and large increases in the rate of protein synthesis. At this stage, the evolution of ethylene may be detected (Coupland, 1994). Within 7 d of treatment, stem, petiole and leaf epinasty can be observed, induced by ethylene evolution, and apical dominance may be lost. Ten days after herbicide treatment, there is commonly complete disruption of the intracellular membranes, resulting in organelle breakdown and cell death. The extent to which the above processes are affected depends on the species and the age of the plant as well as the type of auxin analogue (Sanders & Pallett, 1987).

Uptake and movement of these herbicides in susceptible and tolerant species has shown very little correlation with selectivity. Similar variations have been observed in studies of metabolism. The primary modes of metabolism seem to be through conjugation

with glutathione and sugars, ring hydroxylation at the C4 position and side-chain cleavage.

Clopyralid (3,6-dichloro-2-pyridinecarboxyic acid) (Figure 1.7a) is used in sugarbeet, red beet, fodder beet, mangels, cereals, oilseed rape, vegetable *Brassicae*, turnips (*Brassica napa* L.), swedes (*Brassica napobrassica* L.), onions (*Allium cepa* L.), maize (*Zea mays* L.) and strawberries to kill annual, and some perennial, broad leaved weeds. Selectivity between a tolerant species (sugarbeet) and a sensitive species (scentless mayweed; *Matricaria perforata* Merat.) is not due to differences in uptake, movement or metabolism (Thompson & Cobb, 1986). Studies on auxin herbicide-resistant and -susceptible wild mustard (*Sinapis arvensis* L.) biotypes have indicated differences in the binding of [^{3}H]IAA to auxin-binding proteins, which are correlated with the effects of various auxin herbicides on whole plants (Webb & Hall, 1995).

Mecoprop (2'-(2-methyl-4-chlorophenoxy)propionic acid) (Figure 1.7b) is recommended for use in wheat, barley, oats and grasslands to control broad leaved weeds. Studies conducted in wheat have indicated that 4x the rate applied in the field causes a reduction in root and shoot growth; roots developing a large number of short swollen laterals which gradually recover (Whipps & Greaves, 1986). Resistant (R) and susceptible (S) biotypes of *Stellaria media* L. have been shown to exhibit no differences in mecoprop uptake or movement (Lutman & Heath, 1990). In fact, the two biotypes initially show similar symptoms, although the R biotype gradually recovers. Mecoprop may be detoxified more quickly in R biotypes than S biotypes *via* the production of hydroxylated mecoprop derivatives (Coupland, 1994).

1.2.3 Graminicides

These herbicides help to solve a major problem in cereals, that of grass weeds. Graminicides can be split into five groups: thiocarbamates (eg. triallate, EPTC); chloroacetamides (eg. alachlor, metazachlor); alaninopropionates (eg. flamprop-methyl); aryloxyphenoxypropionates (eg. diclofop-methyl, fluazifop-butyl) and cyclohexanediones (eg. sethoxydim). Two of the groups, the aryloxyphenoxypropionates and the cyclohexanediones, compete for the same site on the strategic enzyme in fatty acid biosynthesis, Acetyl coenzyme A carboxylase (ACCase); reversibly inhibiting the enzyme. The other groups act at other sites in the process of fatty acid biosynthesis. Thiocarbamates inhibit elongases, preventing the production of long-chain saturated fatty acids, such as suberin and cuticular waxes from stearate (Fuerst, 1987). The

chloroacetamides may prevent the elongation of palmitate and the desaturation of oleate in green algae (Weisshaar *et al*, 1988). Graminicides commonly result in contact damage on treated leaves because most of the applied dose remains at the site of application (Carr *et al*, 1986). Growth stops within 2 d, after de-esterification to the acid form. It is this form which moves in the phloem and xylem and accumulates in meristematic tissue, where it results in chloroplast break-down and, ultimately, plant death within 2-3 weeks.

Selectivity depends on the metabolism of the herbicide within the plant (Fuerst & Lamoureux, 1992). Thiocarbamates are activated by sulphoxidation. Susceptible species can not detoxify the resulting sulphoxides and eventually die. Tolerant species conjugate the sulphoxides with glutathione. Chloroacetamides are also conjugated to glutathione in tolerant species. Rapid hydrolysis of alaninopropionates to the active acid form accounts for the susceptibility of some species. In tolerant plants, the process occurs much more slowly and is followed by glycosylation. After rapid de-esterification, the aryloxyphenoxypropionates are glucosylated in susceptible plants and aryl hydroxylated in tolerant species (Shimabukuro, 1990). Cyclohexanediones are detoxified by sulphoxidation, aryl hydroxylation and molecular rearrangement in tolerant species.

Metazachlor (2-chloro-N-(2,6-dimethyl-phenyl)-N-(1H-pyrazol-1ylmethyl) acetamide) (Figure 1.8a) is recommended for use in oilseed rape, swedes, turnips, hardy ornamentals and fruit trees to control broad leaved weeds and some grass weeds. It is thought that metazachlor interferes with fatty acid metabolism by alkylating key enzymes in fatty acid biosynthesis or by alkylating coenzyme A and therefore interfering with coenzyme A metabolism (Fuerst, 1987).

Diclofop-methyl (methyl 2-[4-(2,4-dichlorophenoxy)phenoxy]propanoate) (Figure 1.8b) is widely used to control grass weeds in cereals and broad leaved crops. The main sites of action are rapidly differentiating cells in the meristem. The primary mechanism underlying its action is the inhibition of fatty acid biosynthesis through effects ACCase in the chloroplasts and plastids of non-green tissue. Diclofop acid has been shown to be more effective in inhibiting the incorporation of ^{14}C-acetate into fatty acids than diclofop-methyl (Hoppe & Zacher, 1985). Reduced uptake of ^{14}C-acetate might also be explained by reduced cellular metabolic activity with increased herbicide injury. The secondary mechanism underlying its action is the dissipation of transmembrane proton gradient in plant cells caused by increases in the proton permeability of the plasmalemma (Wright & Shimabukuro, 1987). Depolarisation occurs within 10-12 minutes at 100 mM diclofop-methyl and there is no recovery in oat, slow recovery in wheat and no effect on mung bean

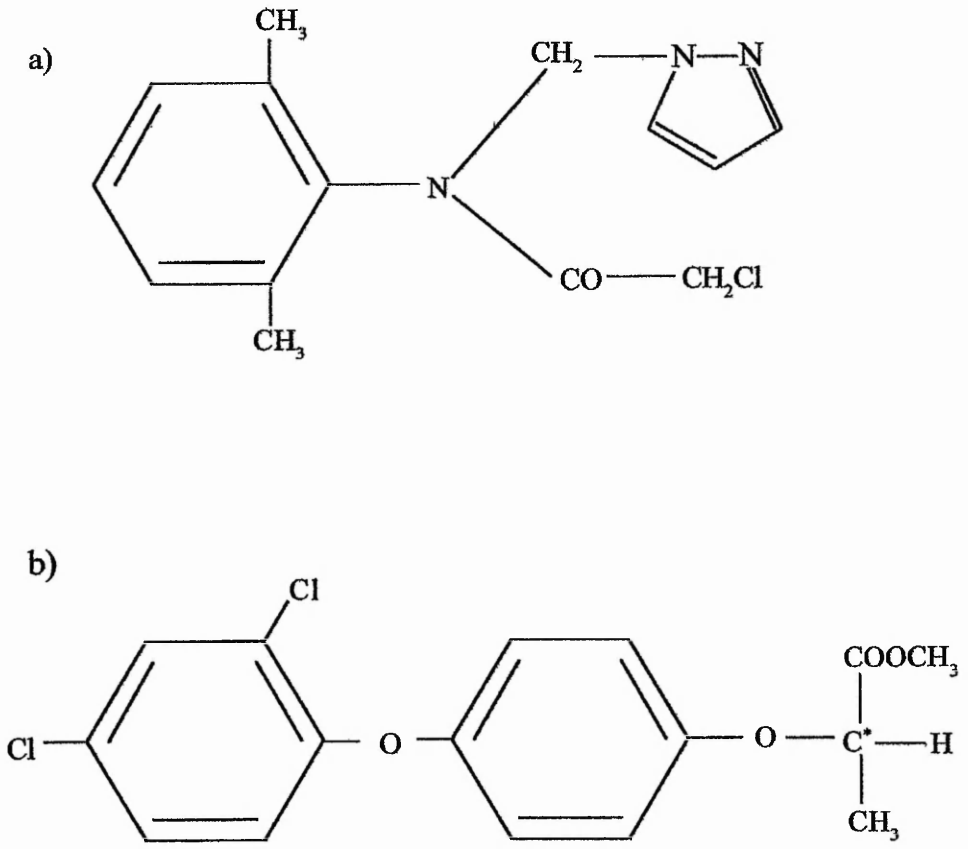


Figure 1.8 Chemical structures of a) metazachlor (2-chloro-N-(2,6-dimethyl-phenyl)N -(3H-pyrazol-1ylmethyl)acetamide) and b) diclofop-methyl (methyl2-[4-(2,4-dichlorophenoxy) -phenoxy]propanoate).

(Wright & Shimabukuro, 1987). Selectivity of diclofop-methyl seems to be due to differential metabolism (Fuerst & Lamoureux, 1992). Incorporation of ^{14}C-acetate into leaf lipids in wheat treated with diclofop-methyl has been shown to be inhibited as much as in susceptible species, although recovery occurred within 4 d (Shimabukuro, 1990). Injury resulting from diclofop-methyl application may also depend on crop growth stage. For example, grain yield of two-row barley was only reduced if the plants were treated at the two tiller stage (growth stage 22; M^{c}Mullan, 1993).

1.3 CELLULAR PROTECTION MECHANISMS

Both ozone and photosynthetic inhibitor herbicides act by the generation of active oxygen species, such as superoxide, hydroxyl or organic peroxides, hydrogen peroxide and singlet oxygen. To combat these potentially damaging oxygen species, plants contain several enzymatic and non-enzymatic protective mechanisms (Figure 1.9). These include scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT) and general peroxidases (GPOD), in addition to ascorbate (Vitamin C), reduced glutathione (GSH), α-tocopherol (Vitamin E) and carotenoids. The ascorbate-glutathione system is mainly found in the chloroplast but has also been identified in the mitochondria and peroxisomes of pea leaves (Jiménez *et al*, 1997).

1.3.1 Superoxide Dismutase (E.C. 1.15.1.1)

These are a group of metalloenzymes that catalyse the disproportionation of superoxide free radicals. They are found in all aerobic organisms and occur in three molecular forms - CuZn-SOD, Mn-SOD and Fe-SOD. These are distinguished according to the metal co-factor essential for activity. CuZn-SOD has been isolated from the stroma and membranes of chloroplasts (Hayakawa *et al*, 1984). The matrix of mitochondria and chloroplast membranes and thylakoids contain Mn-SOD (Bridges & Salin, 1981; Bennett *et al*, 1984; White & Scandalios, 1988). However, Fe-SOD is found mainly in animal systems, although some anaerobic cells and some higher plants may contain it, for example *Ginko biloba* (Duke & Salin, 1985). SOD catalyses the following reaction:

$$2O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + O_2 \qquad (11)$$

thereby removing superoxide which may form more-reactive oxygen species if left unchecked. SOD can occur in water-soluble and membrane-bound states. When membrane bound, it is thought to alter the structure and the susceptibility to chemical attack (Bennett *et al*, 1984).

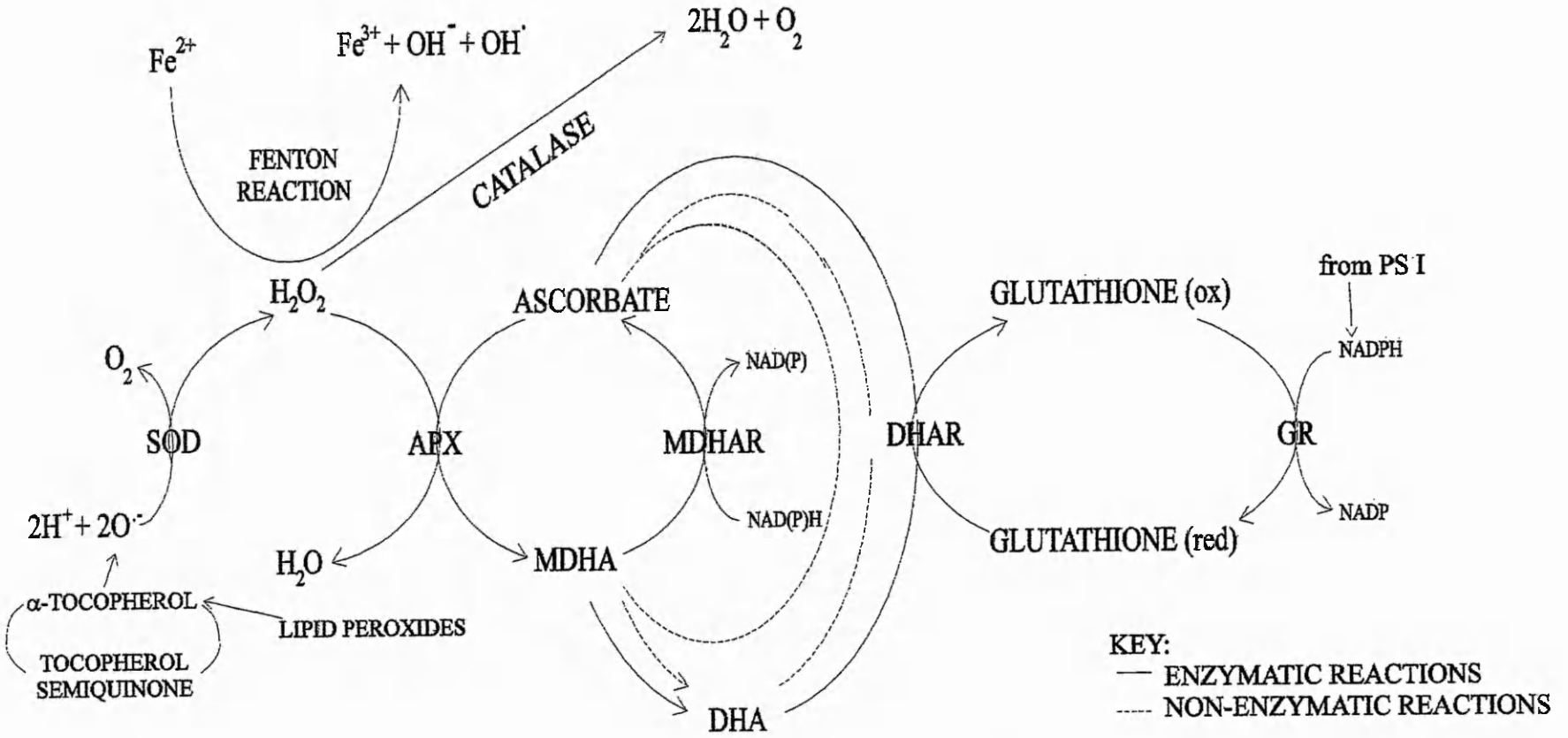


FIGURE 1.9 Enzymatic and non-enzymatic protective mechanisms in plants including the ascorbate-glutathione pathway (Hossain *et al*, 1984) (SOD: superoxide dismutase; APX: ascorbate peroxidase; MDHAR: monodehydroascorbate reductase; DHAR: dehydroascorbate reductase; MDHA: monodehydroascorbate; GR: glutathione reductase; DHA: dehydroascorbate)

1.3.2 Catalase (EC.1.11.1.6)

Catalase (H_2O_2:H_2O_2-oxidoreductase) catalyses the dismutation of hydrogen peroxide:

$$2H_2O_2 \rightarrow 2H_2O + O_2 \quad (12)$$

Catalase is important, when in combination with SOD, to prevent the accumulation of H_2O_2 and production of highly reactive hydroxyl radicals. Activity is located in the peroxisomes, glyoxysomes, cytosol and mitochondria (Scandalios, 1993). In leaves exposed to excessive light there is a rapid turnover of catalase (Feierabend *et al*, 1992). Several isozymes of catalase exist, which are differentially affected by stress conditions and accumulate in different tissues or cells (Willekens *et al*, 1995).

1.3.3 General Peroxidases (EC.1.11.1.7)

In vitro, haem-containing peroxidase activity can be measured using a wide range of substrates, such as guaiacol and coniferyl alcohol. However, *in vivo* these substrates are not known and hence the contribution of peroxidases to damage prevention is not clear. Peroxidases catalyse the following reaction:

$$RH_2 + H_2O_2 \rightarrow R + 2H_2O \quad (13)$$

where RH_2 is the reducing substrate.

1.3.4 Ascorbate (Vitamin C)

Ascorbate is found in chloroplasts, cytosol, vacuole and apoplast at high concentrations (Foyer *et al*, 1983; Foyer *et al*, 1991; Polle *et al*, 1990). Chloroplastic ascorbate accounts for 20 - 40% of the total content in leaf mesophyll cells (Gillham & Dodge, 1986). The concentration of apoplastic ascorbate varies between 10-2000μM dependent on species and growing conditions (Lyons *et al*, 1999). Ascorbate has several functions including the regeneration of α-tocopherol and zeaxanthin, and the removal of hydrogen peroxide generated in the light:

$$H_2O_2 + 2 \text{ ascorbate} \rightarrow 2H_2O + 2 \text{ monodehydroascorbate} \quad (14)$$

This reaction is catalysed by ascorbate-specific peroxidases (APX; EC. 1.11.1.7; Figure 1.9) in chloroplasts, cytosol and apoplast (Nakano & Asada, 1981). However, APX is also inhibited by H_2O_2 (Hossain & Asada, 1984). Cytosolic and chloroplastic isozymes of ascorbate peroxidase have been distinguished, in addition to a thylakoid bound APX which is distinct from the other forms (Chen & Asada, 1989; Miyake & Asada, 1992). A further form of APX has been localised on mitochondrial membranes

in peas (Jiménez *et al*, 1997), although the apoplastic form(s) has not yet been characterised. Ascorbate regenerates through the spontaneous disproportionation of monodehydroascorbate (MDHA) to dehydroascorbate (DHA) followed by non-enzymic reduction by reduced glutathione (GSH) to ascorbate:

$$2\ GSH + DHA \rightarrow GSSG + ascorbate \qquad (15)$$

or through a reaction catalysed by dehydroascorbate reductase (DHAR; E.C. 1.8.5.1; Nakano & Asada, 1981; Jablonski & Anderson, 1981). Regeneration of ascorbate can also occur through the reduction of MDHA, catalysed by monodehydroascorbate reductase (MDHAR; E.C. 1.6.5.4; Hossain *et al*, 1984) or by direct reduction of MDHA by electrons from the photosynthetic electron transport chain (Foyer *et al*, 1994). Apoplastic washing fluid has been generally found to contain peroxidase, SOD and ascorbate, but not glutathione or any of the enzymes required to facilitate the regeneration of ascorbate to its oxidised form (Lyons *et al*, 1999).

Ascorbate also reacts with superoxide at comparable rates to SOD (Nishikimi, 1975):

$$2O_2^{\cdot-} + 2H^+ + ascorbate \rightarrow DHA + 2H_2O_2 \qquad (16)$$

1.3.5 Glutathione

Reduced glutathione (GSH) participates in the reduction of DHA to ascorbate either enzymatically or non-enzymatically. A sulphydryl group of cysteine forms a disulphide bond with a second molecule of GSH on oxidation, generating oxidised glutathione (GSSG) and mediating antioxidant activity. GSH accounts for 65-70% of total glutathione in leaves. Glutathione plays a major role in the transport of sulphur and is also involved in the detoxification of herbicides through conjugation.

Chloroplasts contain only 10 % of the total glutathione pool, the remaining 90 % occurring in the cytosol (Bielawski & Joy, 1986; Gillham & Dodge, 1986). Glutathione reductase (GR; E.C. 1.6.4.2) catalyses the reduction of oxidised glutathione. This reaction is highly dependent on NADPH derived from photosystem I. Several isozymes of GR have been distinguished in spinach (Guy & Carter, 1984). In peas, the majority of GR occurs in the chloroplast stroma of (52 & 75% in young and mature leaves respectively), with smaller amounts in the cytosol and mitochondria (Bielawski & Joy, 1986).

1.3.6 Effects of Ozone on Antioxidative Systems

Once inside the plant, ozone reacts with water in the intercellular spaces to form active oxygen species, such as superoxide, hydroxyl or organic peroxides, hydrogen peroxide and singlet oxygen (Kanofsky & Sima, 1991). Formation of these reactive species has been demonstrated in *Pisum sativum* and *Phaseolus vulgaris* (Mehlhorn *et al*, 1987). The impacts of ozone on cellular antioxidative systems are dependent upon ozone concentration (Decleire *et al*, 1984; Nouchi, 1993); age of the plant tissue (Price *et al*, 1990; Bender *et al*, 1994); genotype (Tanaka *et al*, 1985) and the localisation of the antioxidative systems (Castillo *et al*, 1984; 1987; Castillo & Greppin, 1988).

1.3.7 Effects of Herbicides on Antioxidative Systems

Several groups of herbicides can induce the formation of active oxygen species. Inhibitors of photosystem II, such as atrazine, diuron and phenmedipham, produce active oxygen species (Halliwell, 1991). Inhibitors of carotenoid biosynthesis, such as norfluazon, fluridone and aminotriazole, accelerate the photodegradation of chlorophyll and so are termed 'bleaching herbicides'. Carotenoids minimise the production of singlet oxygen and quench it if it is formed. Aminotriazole has been shown to inhibit catalase activity (Halliwell, 1991) and increase the amount of reduced glutathione in leaf tissue (Smith, 1985). Redox-active herbicides, such as paraquat and diquat, which are reduced by electron acceptors of photosystem I, produce radical cations and react with oxygen to form superoxide and hydrogen peroxide (Shaatiel *et al*, 1988). The diversion of electrons to paraquat reduces the production of NADPH and renders the ascorbate-glutathione cycle inoperative. Paraquat-tolerant cultivars of *Lolium perenne* L. have higher activities of catalase, superoxide dismutase and guaiacol peroxidase (Harper & Harvey, 1978). Cultivars of *Conyza bonariensis* (L.) Cronq. which were tolerant to paraquat were also resistant to sulphur dioxide, atrazine and acifluorfen (Shaatiel *et al*, 1988).

1.4 OZONE * HERBICIDE INTERACTIONS

Several environmental factors influence the efficacy of herbicides. These include temperature (phenmedipham; Preston & Biscoe, 1982); air pollution (ozone; Carney *et al*, 1973) and other pesticides (diclofop-methyl and chlorsulfuron; Liebl & Worsham, 1987). Studies are undertaken during the pre-registration period of the compound to determine any interactions with meteorological factors such as light, temperature and rainfall. Air pollution effects are not taken into account during these studies, although it is clear from previous experiments that this may be necessary.

The effects of ozone in combination with various herbicides on the growth and yield of several species have been studied and are summarised in Table 1.1. The potential for interactions depends on several factors. Firstly, the timing of application of the herbicide relative to the occurrence of high concentrations of the pollutant influences the nature of the interaction. For example, velvetleaf (*Abutilon theophrasti* Medic.) produced an additive response when exposed to 200 nl l^{-1} ozone followed by chlorsulfuron, whilst the interaction was antagonistic when the treatments were reversed (Hatzios & Yang, 1983). Secondly, species sensitivity to both the herbicide and the pollutant affects the interaction. For example, two cultivars of tobacco showed different responses to chloramben and pebulate when treated prior to 300 nl l^{-1} ozone (2 x 1.5 h; Carney *et al*, 1973). Pre-treatment with chloramben, which effects RNA synthesis and protein metabolism, resulted in synergistic and additive effects on Delhi 34 (ozone tolerant) and White Gold (ozone sensitive), respectively; indicating an apparent loss of ozone tolerance in Delhi 34. Treatment with pebulate prior to ozone produced additive and synergistic interactions, respectively, in Delhi 34 and White Gold. In a later study, the response of two other cultivars of tobacco to treatment with pebulate prior to ozone exposure was not consistent over three seasons (Reilly & Moore, 1982). Thirdly, the concentration of ozone influences the nature of the interaction. Atrazine treatment (3.5 kg AI ha^{-1}) of maize (*Zea mays*) followed by 200 nl l^{-1} (36 h over 3 weeks) ozone resulted in additive effects on dry weight, whilst an antagonistic interaction was observed with exposure to 300 nl^{-1} ozone (Mersie *et al*, 1990). Finally, other environmental factors may affect the interaction, such as photosynthetic photon flux density (PPFD) and temperature. For example, exposure of tomato (*Lycopersicon esculentum* Mill.) to 300 nl^{-1} ozone for 1 h prior to metribuzin treatment at low PPFDs produced an additive interaction, whereas at high PPFDs the interaction was antagonistic (Phatak & Proctor, 1976).

It should be noted that these studies were conducted on crops that were economically important in the USA and Canada, employing herbicides most commonly used in those crops. The experiments concentrated on the effects on crop growth and yield, although Hodgson and co-workers (Hodgson *et al*, 1973, 1974; Hodgson & Hoffer, 1977) also studied effects on the metabolism of diphenamid in tomato and pepper (*Capsicum frutescens* L.). The concentrations of ozone used in all of these studies were high compared with those normally experienced under UK conditions. Very little is known about the potential for interactions in the UK. Furthermore, only a small amount

Table 1.1 Previous work on ozone interactions with herbicides.

Species	Herbicide [a]	Ozone Conc ($nl\ l^{-1}$) [b]	Interaction [c]	Sequence [d]	Reference
Maize	Atrazine (3.5)	200 (36 h over 3 d) 300	Add Ant	H/O_3	Mersie et al, 1990
Maize Bean Soybean	Metolachlor (0.1, 0.5, 1.0 ppm)	200/400 (6h) 400	Syn Add/Ant	H/O_3	Mersie et al, 1989
Tomato	Metribuzin (0.28, 0.56)	75 (1-3 h) 150 300	Ant/Add Syn/Add Add	O_3/H	Phatak & Proctor, 1976
Tomato Tobacco	Trifuralin (2.24) Pebulate (8.96) Benefin (2.8)	300 (2 x 1.5 h)	Add Syn/Add Ant	H/O_3	Carney et al, 1973
Tobacco	Isopropalin (1.7) Diphenamid (4.5) Pebulate (4.5)	44 (monthly mean; 4 h max = 150)	Ant Ant Not consistent	H/O_3	Reilly & Moore, 1982
Sorghum Velvetleaf	Chlorosulfuron (0.06, 0.12)	100, 200 (6 h) 200	Add Add Ant Add	H/O_3 O_3/H H/O_3 O_3/H	Hatzios & Yang, 1983

Notes:
[a] application rates in parenthesis (kg AI ha^{-1} unless otherwise stated);
[b] exposure regime in parenthesis;
[c] interactions add = additive;
ant = antagonistic;
syn = synergistic;
[d] H = herbicide;
O_3 = ozone.

of work has been conducted on the physiological mechanisms underlying interactions between herbicides and ozone.

1.5 AIMS OF THE STUDY

The aims of this study were to:

i) determine the effects of 5 post-emergent herbicides, with various modes of action, on cultivars of 3 UK spring-sown crops treated at the 2-3 leaf stage in the glasshouse;

ii) discover the nature of interaction between ozone pollution and herbicide application in these crops;

iii) ascertain the physiological basis for a selected interaction through observations of photosynthesis, membrane leakage and ion leakage from the tissue, and

iv) establish the biochemical nature of this interaction through the use of assays for active oxygen scavenging enzymes.

CHAPTER 2: MATERIALS AND METHODS

2.1 PLANT MATERIAL

Spring barley (*Hordeum vulgare* L. cvs. Sherpa, Corgi, Tyne, and Nugget), spring oilseed rape (*Brassica napus* L. cvs. Galaxy and Starlight), obtained from Westcrop Ltd (Warminster, UK), and sugarbeet (*Beta vulgaris* L. cvs. Saxon, Celt and Amethyst), obtained from British Sugar (Peterborough, UK) were used throughout the project. Seed was sown in J. Arthur Bowers multipurpose compost in pots of 7 cm diameter, 385 cm^3 soil volume and the plants were raised in either a growth cabinet (Fitotron, Sanyo, Loughborough, UK) at 21°C/10°C, 50% relative humidity and 180 µmol m^{-2} s^{-1} PAR, 14h daylength or under glasshouse conditions (22°C, 14h daylength, natural light supplemented with sodium halide lamps during the winter). Plants were thinned to 2 per pot 7-10 d prior to treatment at the 2-3 leaf stage (approximately 21 d after sowing). Pots were returned to the growth cabinet after treatment.

2.2 EXPOSURE OF PLANTS TO OZONE

2.2.1 Exposure System

The ozone exposure system (Figure 2.1) consisted of four 0.8 m x 0.8 m x 0.8 m perspex chambers. Air was initially drawn into the glasshouse from outside by a 0.37 kW centrifugal fan (Air Control Installations Ltd) and forced through a Purafil and charcoal filter (Jones & Attwood Ltd., Stourbridge, U.K.) to remove ambient ozone, oxides of nitrogen and sulphur dioxide. The filtered air supply then passed through polythene drainpipes and flexible canvas ducting into the exposure chambers. Airflow was measured at 2.4-2.6 m^3 min^{-1} with no significant difference between the chambers (Balls, 1996). Ozone was generated by passing zero grade oxygen (source Air Products, Walton-on-Thames, UK) around a UV lamp (Light O_3 Clean A/S, Denmark) and was introduced into the air-intake pipes though Poly Tetra Fluoro Ethane (PTFE) tubing prior to the air entering the chambers. Stainless steel fine-metering needle valves (maximum flow rate 1.3 l min^{-1}) were used to control the amount of ozone entering individual chambers. A third tube from the generator acted as an overflow to vent excess ozone to the outside of the glasshouse. To ensure thorough mixing of the air, an 8 cm minifan (Radio Spares, Corby, UK) was fixed perpendicular to the air inlet at the top of each chamber. Using this mixing system, uniform concentrations of ozone were achieved throughout individual

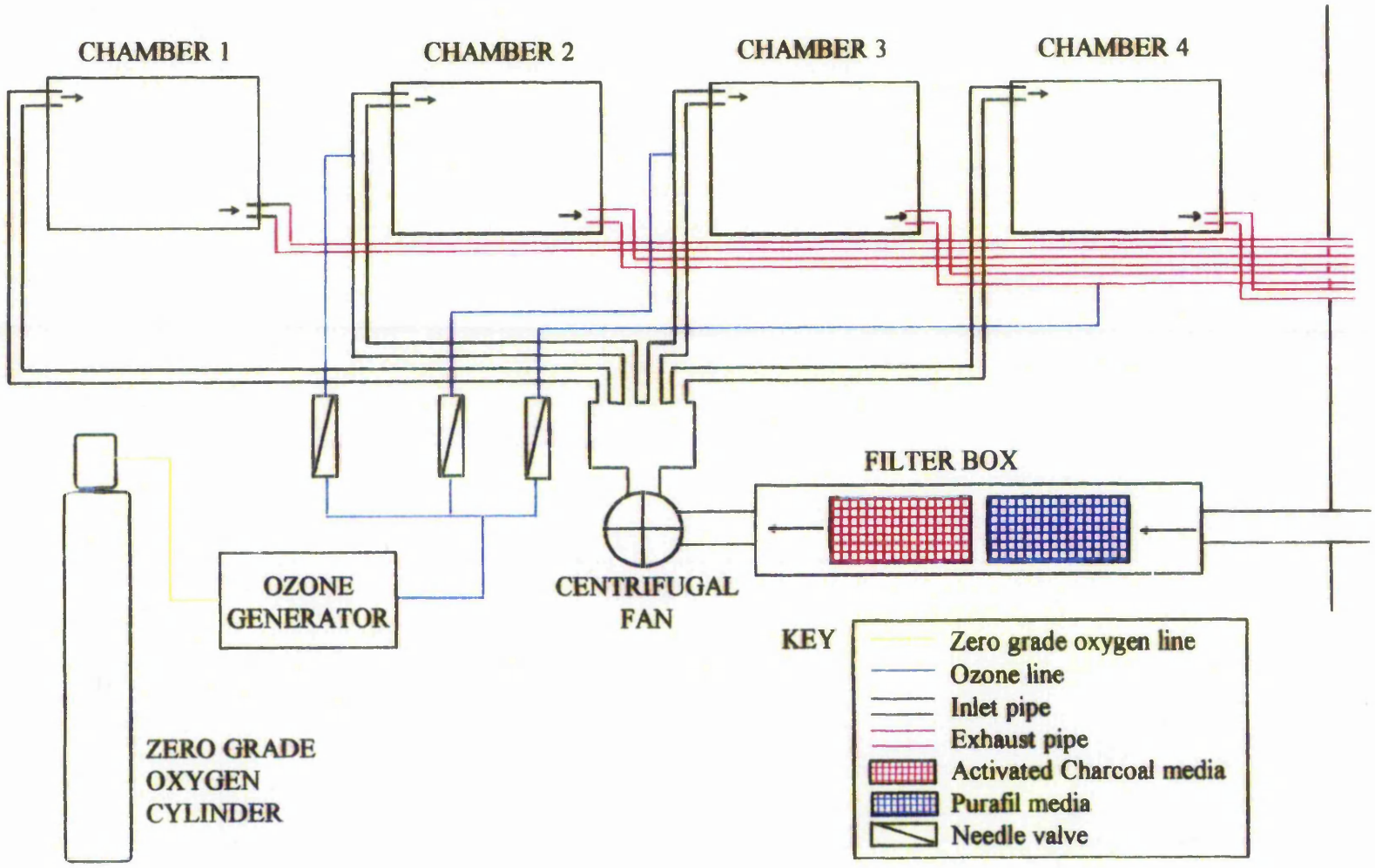


Figure 2.1 Layout of the closed chamber exposure system, airflow and ozone introduction into the chambers.

chambers (Balls, 1996). Temperature, photon flux density (ambient illumination during the summer months, supplemented with sodium halide lamps during the winter) and relative humidity (ambient) within the chambers were routinely monitored.

In the monitoring system (Figure 2.2), air was sampled from the 2 ozone chambers, one charcoal filtered (CF) chamber, and external air, through PTFE pipes. Ozone concentrations were measured at plant height (approximately 0.2 m above the base of the chamber), through a sample line that was situated 0.4 m from the front and rear of the exposure chamber. Sample lines consisted of 6 mm OD tubing, sealed at the open end, with 16 1.0 mm^2 holes 2.5 cm apart, along the 0.8 m of the tube within the chamber. Each sample was drawn through a PTFE filter, to remove any particulate material, to a 2-way, PTFE, normally closed solenoid valve (Biochem International). These were controlled by a switching box (MFI 100 switching unit, CIL Group Ltd, Lancing, UK), which was linked in turn to an MFI 1010 data logger (CIL Group Ltd). Readings were taken from each sample for 5 min in every 30 min cycle. From the solenoids, the air-sample passed to an ozone analyser (Dasibi 1003 PC, Glendale, USA). Ozone concentration readings in analogue form were logged by the MFI 1010 data logger unit, which converted the signal to digital values. These were then averaged for the last 3 min of each 5 min period. This data was then communicated to a 286 personal computer (Tiny Computers, Redhill, UK) and stored on floppy disc for later analysis. The temperature (Shielded T type thermocouples, Thermocouple Instruments Ltd) in all of the glasshouse compartments, chambers and ambient air; the percentage relative humidity (humitter sensors; Vaisala) and the photon flux density (PAR meter; Skye Instruments, Llandrindod Wells, UK) in one chamber, were logged continuously and averaged over 30 min by the MFI 1010 data logger unit. The data were then analysed using Microsoft Excel v.5.

2.2.2 Pollutant and Microclimate Conditions during Exposure of Plants

Data recorded during the exposure of plants for various experiments within this study are presented in Table 2.1. These figures indicate that the exposure regime was reproducible over the 2.5 years the system was in operation, although there was some variation in factors that could not be controlled such as PPFD, temperature and relative humidity.

2.2.2.1 Ozone Concentrations in CF and CF + O_3 Chambers

Variability of the CF + O_3 concentration inside the chambers was due to several factors (Table 2.1). The system was such that when set at 100 nl l^{-1} per chamber under one

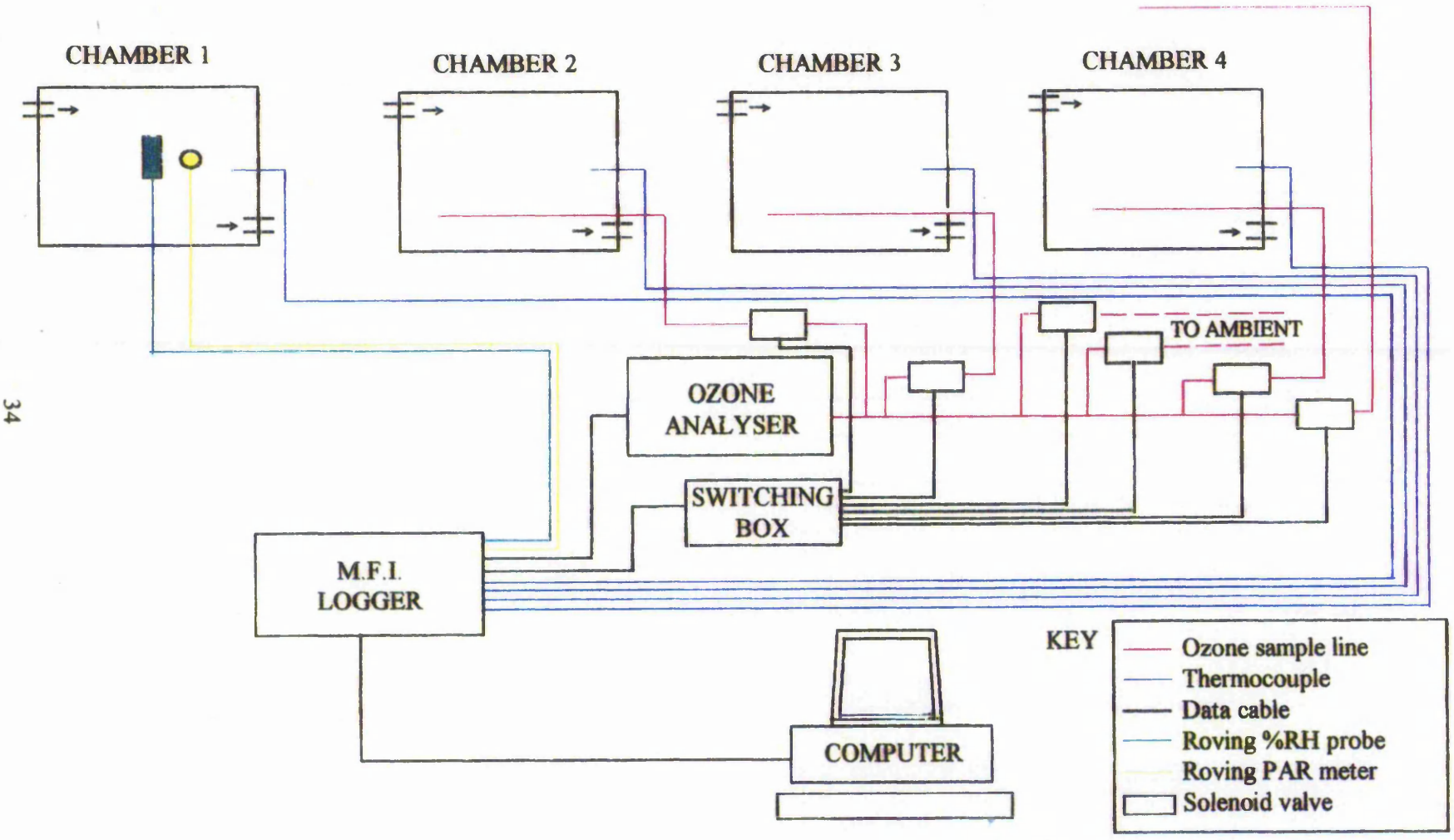


Figure 2.2 Layout of the closed chamber exposure system, ozone concentration and microclimate monitoring.

Table 2.1 Operational variation of some parameters during experiments to determine the physiological and biochemical basis of interactions between ozone and phenmedipham. Values are means ± s.e., where means are the average of all 7 h values for both chambers and n = 8-24.

Experimental Parameter	Interactions Growth Analysis (Ch. 4)	Photo-synthesis (Ch. 5)	Membrane and ion leakage (Ch. 5)	Electron microscopy (Ch. 5)	Antioxidant enzymes (Ch. 6)
CF O_3 conc (nl l^{-1})	9.3 ± 2.4	10.7 ± 3.4	12.7 ± 2.3	15.0 ± 2.4	8.7 ± 2.6
CF + O_3 conc (nl l^{-1})	113.5 ± 12.0	96.5 ± 4.3	91.6 ± 4.6	93.5 ± 2.2	107.1 ± 6.6
Chamber Temp. (°C)	22.2 ± 1.4	25.4 ± 1.0	20.8 ± 0.7	22.2 ± 1.4	22.9 ± 1.6
% Relative Humidity	49.0 ± 4.1	59.8 ± 4.5	64.2 ± 6.0	57.9 ± 1.4	53.9 ± 3.8
PAR (μmol m^{-2} s^{-1})	160.0 ± 39.7	220.8 ± 36.0	175.9 ± 9.3	157.6 ± 22.6	222.6 ±33.4

set of environmental conditions, if the meteorological conditions changed the following day, adjustments had to be made. Adjustments were usually made after an initial warm-up period of approximately 1 h and again around 1400-1500 h. Therefore ozone concentrations depended on fine manual control by needle valves. The amount of material in the chamber also affected the ozone concentration. For example, if large numbers of plants were exposed at any time, ozone concentrations were difficult to maintain at 100 nl l^{-1}, due to absorption to surfaces (soil, pots and leaf tissue). The exposure system was checked at regular intervals, both for safety and to observe the 5 min mean concentrations in the chambers which were displayed once every 30 min.

2.2.2.2 Exposure to ambient ozone prior to treatment

Plants were grown in the glasshouse or growth chambers prior to the start of experiments and were therefore subject to natural fluctuations in ambient ozone. Figure 2.3 illustrates the variability in ambient ozone concentrations over 7 months at the beginning of 1995. Concentrations of ozone were very low from January to April, whilst higher episodes occurred from May onwards. Plants were therefore subject to more ozone prior to treatment during experiments conducted in late Spring and Summer. For example, during the growth period of one set of plants in July/August 1994, 7 h daily means for ambient ozone concentrations were greater than 40 nl l^{-1} for 11 d out of 25. Six days prior to the planned ozone exposure, a natural episode occurred reaching a maximum concentration on 128 nl l^{-1} with a 7 h mean of 116 nl l^{-1}. A further episode with 7 h mean concentration of around 70 nl l^{-1} for 2 d occurred between the intended exposure and the application of the herbicide. These episodes had direct effects on the experiments conducted (antioxidant enzyme assays) since it was observed that enzymes were elevated in all plants including those in the CF chamber. For this reason, plants that were between 10 and 28 d old when an ambient episode (7 h mean > 60 nl l^{-1}) occurred were not used in experiments, as they were considered to have been exposed to too much ozone during the growing period.

2.2.2.3 Microclimate conditions

Temperatures during exposure to ozone in the present study were around 22°C, relative humidities 57 % and PAR 187 μmol m^{-2} s^{-1} (Table 2.1). Conditions were relatively stable, although the most unstable was the PAR, which depended on the time of year of the exposure. Plants were generally grown up in the growth chambers, which gave stable conditions before and after exposure to ozone.

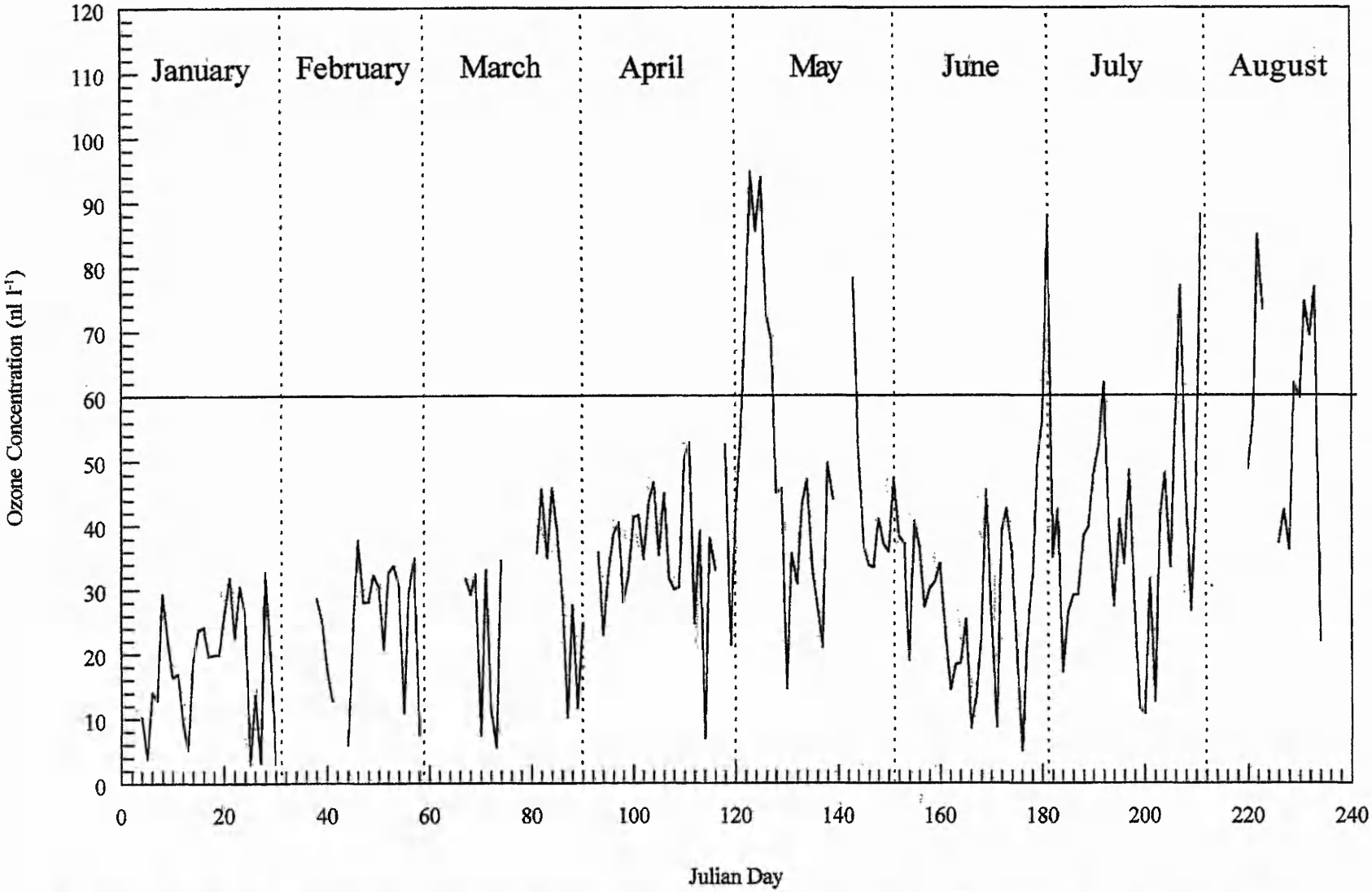


Figure 2.3 7 h daily mean ozone concentrations at Nottingham (NTU, Clifton site, 200 m A453, 11.2 km M1) from January to August 1995. Gaps indicate missing data.

2.3 HERBICIDE TREATMENTS

The herbicides were applied as commonly used formulated products (Table 2.2). A laboratory pot sprayer (Mardrive Bioevaluation Unit, Stockport, U.K.; Teejet 80° flat fan nozzle, 240 l water ha^{-1}, 3 bar pressure) was used to apply the products at 1 of 4 rates: control, half field-rate, field-rate and twice field-rate. Control pots were sprayed with distilled water, since spraying control plants with a formulation blank may have affected later experiments. Sprayed plants were allowed to dry prior to returning them to the growth cabinet or glasshouse bench, to prevent cross contamination between the treatments.

2.4 OZONE * HERBICIDE INTERACTIONS

Experiments to quantify interactions were conducted according to one of the following procedures:

(i) At the 2-3 leaf stage (approximately 21 d after sowing) plants were treated with herbicide at field-rate. Plants were allowed to dry prior to returning them to the growth cabinet or glasshouse bench. Three days later the plants were exposed to approximately 100 nl l^{-1} ozone for 7 h d^{-1} for 2 d.

(ii) The above procedure was carried out in reverse, i.e. ozone exposure followed 3 d later by the application of field-rate herbicide.

Initial studies were carried out using procedure (i). Sugarbeet plants were also treated following procedure (ii) to test for a timing effect on the interaction. All subsequent experiments were performed using procedure (ii). The number of plants used for each treatment remained the same for all of the experiments, namely 8 plants (4 pots) per treatment per chamber. This gave a total of 64 plants in each experiment (8 plants x 4 treatments x 2 replicate chambers).

A significant interaction would be indicated by a two- or three-way ANOVA result with a p value of less than 0.05. An antagonistic interaction occurs where one or both treatments are not exerting their full effect, resulting in an effect which is closer to the control than the additive effect. A synergistic interaction occurs where the effects of the two treatments together is the opposite of that for antagonistic interactions, i.e. the effect is greater than the two individual treatments added together (and hence further away from the control than the additive effect).

Table 2.2 Herbicides used during the study

Active ingredient (AI)	Product name	Company	Field-rate (kg AI ha^{-1})	Crop used
Clopyralid	Dow Shield	DowElanco	0.07 0.10 0.10	Spring Barley, Oilseed Rape, Sugarbeet
Diclofop-methyl	Hoegrass	Hoechst	0.95 1.14 1.14	Spring Barley, Oilseed Rape, Sugarbeet
Mecoprop-p	Duplosan New System CMPP	BASF	1.38	Spring Barley
Metazachlor	Butisan S	BASF	0.75	Oilseed Rape
Phenmedipham	Betanal E	AgrEvo	1.14	Sugarbeet

2.5 STATISTICAL ANALYSIS

All experiments (except herbicide dose response experiments) consisted of 2 replicates of 4 treatments, namely control (CF), ozone alone, herbicide alone and ozone and herbicide. For each treatment, 4 pots each containing 2 plants were used. The number of repeats of each experiment are presented in each chapter. Chamber means were used as replicates in all experiments except the herbicide dose response study, where pot means were used as replicates.

One-way ANOVA was conducted, using Unistat version 4 for Windows, to determine differences between, for example, herbicide dose and control (Chapter 3) and two-way ANOVA was conducted where two treatments (e.g. ozone and herbicide) were used. Where ANOVA indicated that the null hypothesis of no difference between treatments could be rejected, a Duncan's Multiple Range Test was conducted to determine where the treatment means were significantly different. Further details on statistical analyses are included in the individual chapters wherever necessary.

2.5.1 Duncan's Multiple Range Test

(Gomez & Gomez, 1984). After an ANOVA has indicated significant differences between means, the mean square of the errors (MS_E = variance) is used to determine significant differences *via* the following equation:

$$SSR_P = r_p \sqrt{\frac{MS_E}{n}}$$

where SSR_P = shortest significant range

r_p = least significant studentised range (obtained from table)

MS_E = error mean square from ANOVA

n = common no of replicates per treatment

γ = degrees of freedom for MS_E

Two means are considered significantly different if the difference between the means is greater than the SSR_P.

CHAPTER 3: HERBICIDE DOSE/RESPONSES

3.1 INTRODUCTION

Spring-sown crops were selected after consideration of their general use in the UK and their responses to ozone pollution. Barley and oilseed rape are more sensitive to ozone than sugarbeet, although visible injury occurs on all 3 crops (Ogata & Maas, 1973; Ashmore and Onal, 1984; Adaros et al, 1991a). Wheat is more sensitive to ozone than barley. However, spring wheat is grown on a considerably smaller hectarage than spring barley (Anon, 1989). Winter-sown crops would be treated with early post-emergence herbicides at a time when ozone episodes are not likely to occur, therefore reducing the possibility of interactions. Cultivars of these spring-sown crops were chosen according to recommendations by the National Institute of Agricultural Biology (NIAB) and their use by farmers (NIAB, 1991).

Sugarbeet was grown on around 175,000 ha in 1995 in the UK (Knott *et al*, 1995). On average, sugarbeet fields are treated with a three-spray herbicide programme, including a pre-emergence spray of chloridazon (applied alone or in mixture with ethofumesate) or metamitron (Anon, 1994). The critical time for weed control is between emergence and the 6-8 true leaf stage of the crop. Post-emergence herbicides used in sugarbeet include phenmedipham, metamitron, lenacil and clopyralid. Major weeds include *Elymus repens* (L.) Gould (couch grass), *Chenopodium album* L. (fathen), *Fallopia convolvulus* (L.) A. Löve (bindweed), *Cirsium arvense* (L.) Scop. (creeping thistle) and *Solanum tuberosum* L. (volunteer potato).

The practise of growing spring barley has been declining since 1980 due to an increase in the popularity of winter cereals. Around 12,300 ha of spring barley using 57 varieties, were sown in 1995 (Blake, 1996). Major weeds of cereals include *Stellaria media* (L.) Vill., *Matricaria perforata* Mérat. (scentless mayweed), *Poa annua* L. (annual meadowgrass), *Polygonum aviculare* L. (knotgrass), *Elymus repens*, *Cirsium arvense* and *Convolvulus arvensis* L. (field bindweed). The herbicides which can be used to control these are pre-emergence - pendimethalin; pre- or post-emergence - chlorsulfuron, tri-allate; post-emergence - bromoxynil/ioxynil, dichlorprop, clopyralid, 2,4-D, diclofop-methyl.

After an initial literature search, five widely used early post-emergence herbicides were chosen for use in this study. The compounds differed in their mode of action and were formulated as single active ingredient products to reduce the number of interactions studied. A detailed introduction to these herbicides is included in Chapter 1.

It is known that plants grown in the glasshouse are generally more susceptible to stresses than field-grown plants. It was therefore necessary to determine the effect of the herbicides on glasshouse-grown crop plants, prior to looking at interactions between the herbicides and ozone pollution. This study also served to determine the relative sensitivity and variability of the cultivars and give an indication of the time-course of injury development on the plants and the types of injury occurring. The aim of these experiments was also to reveal problems with the husbandry of the plants and so prevent the possibility of problems in later experiments. For example, the susceptibility of plants to pests and diseases present in the glasshouse. These preliminary experiments gave the opportunity to make an informed choice of the crop/cultivar and herbicides for further study.

3.2 MATERIALS AND METHODS

Four spring barley cultivars (Tyne, Nugget, Sherpa and Corgi) and 3 sugarbeet cultivars (Amethyst, Celt and Saxon) were grown as described earlier (Chapter 2, section 2.1). Pots were labelled prior to sowing seed and the position of the pots during the experiments was fully randomised. At the 2-3 leaf stage, plants were sprayed at multiples of field-rate (0, 0.5, 1, 2) with 1 of 4 herbicides (clopyralid, diclofop-methyl, mecoprop-p or phenmedipham; Chapter 2, section 2.3). The mean of 2 plants per pot was treated as a replicate. There were 4 replicates of each treatment, and all results were tested using analysis of variance (ANOVA). If this proved significant at the 5 % level, then Duncan's Multiple Range tests were carried out as described in Chapter 2, section 2.5.

Plants were checked daily for visible symptoms and an estimate of the amount of visible injury made as a percentage of leaf area (where 0 = no injury, 50 = 50 % of leaf was chlorotic/necrotic and 100 = leaf dead or completely chlorotic/necrotic). An Arc-Sin transformation was carried out on the data prior to analysis of variance. Fourteen days after spraying, the plants were harvested by excision at soil level for leaf area determination. Shoot dry weights were obtained by drying the harvested plant material in an oven (80°C) for 2-3 d until the weights remained constant. During the early part of this experiment spring barley was treated with fenpropimorph (Corbel, Ciba-Geigy) at field-rate (0.75 kg AI ha^{-1}) prior to application of diclofop-methyl or mecoprop-p, in an attempt to control powdery mildew (*Erysiphe graminis* D.C. ex Merat.).

3.3 RESULTS

3.3.1 Clopyralid on Barley

Clopyralid had no significant effect on shoot dry weight of the 4 cultivars studied (Table 3.1 and Appendix 1.1). The herbicide did not induce any visible injury on the plants.

3.3.2 Diclofop-methyl on Barley

The shoot dry weights of Sherpa and Corgi significantly decreased ($p = 0.005$ and $p < 0.001$, respectively) with increasing herbicide concentration (Figure 3.1 and Appendix 1.2). In contrast, Tyne and Nugget did not show a significant response to diclofop-methyl.

Diclofop-methyl produced injury symptoms on those leaves present at the time of spraying, indicative of contact injury, with chlorotic areas appearing within 2-5 d (Plate 3.1). At field-rate, chlorotic lesions merged to cover approximately 20-25 % of the second leaf (Plate 3.2). The amount of injury on the plants also reiterated the differences in response of the 4 cultivars (Table 3.2; Appendix 1.2.1). Tyne and Nugget had very little injury on the second leaf even at high concentrations of herbicide, whilst Sherpa and Corgi had large amounts of injury on the second leaf. A significant interaction occurred between diclofop-methyl and the fungicide, fenpropimorph, for herbicide injury in Corgi (Appendix 1.2.1). New growth was unaffected, except the tip of the third leaf that was emerging at the time of herbicide treatment.

When sprayed with fenpropimorph 3 d before diclofop-methyl treatment, shoot dry weights of the fungicide alone treatment were reduced significantly in Sherpa ($p = 0.032$; Appendix 1.2.2), whilst this did not occur in Corgi (Table 3.3). There were no significant interactions between fenpropimorph and diclofop-methyl on shoot dry weight of either cultivar (Appendix 1.2.2). The plants did not have any powdery mildew visible on the leaves at the time of harvest. Typical symptoms of powdery mildew are shown in Plate 3.3 to give a comparison between this and the effects of the herbicides on spring barley.

3.3.3 Mecoprop-p on Barley

Mecoprop-p decreased the shoot dry weight of Nugget ($p = 0.042$; Appendix 1.3; Figure 3.2) with increasing herbicide concentration. There were no significant effects on the remaining cultivars in response to mecoprop-p. Injury symptoms consisted of chlorotic lesions on the sprayed leaves, similar to those of diclofop-methyl (Plate 3.4). These symptoms may be attributed to contact action. Treatment with fenpropimorph prior to application of mecoprop-p increased the effects of the herbicide on Sherpa, whilst

Table 3.1 Effects of clopyralid on the shoot dry weights of 4 spring barley cultivars applied at various rates (0, 0.035, 0.07 and 0.14 kg AI ha^{-1}). Harvested 14 d after treatment with clopyralid. Values are means ± standard error, where n = 4. No significant treatment effects were detected by one-way ANOVA for any of the cultivars (Appendix 1.1).

Cultivar	Shoot Dry Weight (g)			
	Clopyralid Concentration (kg AI ha^{-1})			
	0	0.035	0.07	0.14
Sherpa	0.282 ± 0.033	0.235 ± 0.021	0.305 ± 0.005	0.298 ± 0.022
Corgi	0.282 ± 0.042	0.285 ± 0.024	0.258 ± 0.026	0.318 ± 0.017
Tyne	0.275 ± 0.025	0.313 ± 0.038	0.275 ± 0.072	0.238 ± 0.032
Nugget	0.313 ± 0.024	0.300 ± 0.029	0.275 ± 0.032	0.275 ± 0.014

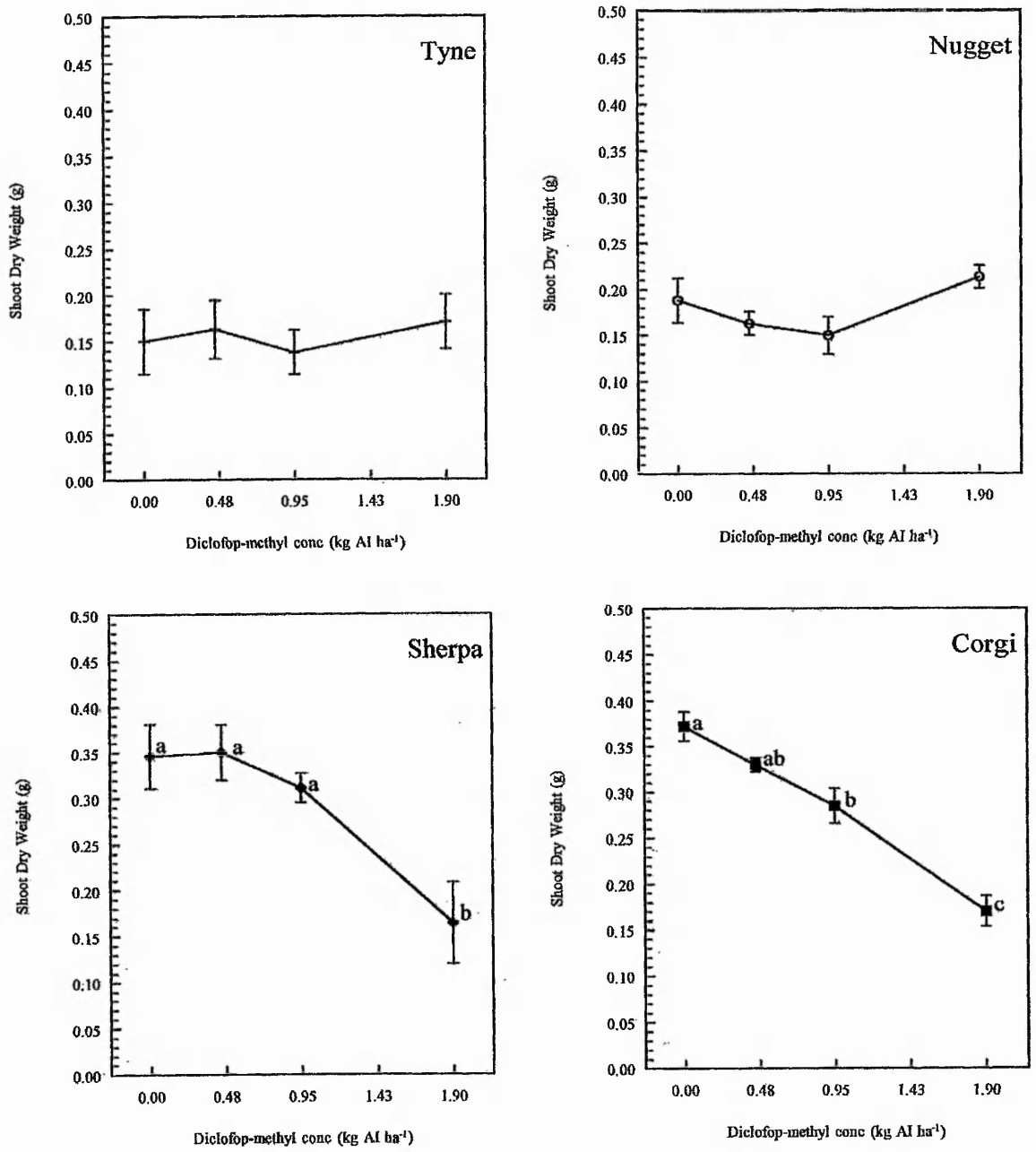


Figure 3.1 Effects of different concentrations of diclofop-methyl on the shoot dry weights of 4 spring barley cultivars. Values are means, where n = 4 and bars represent 2 standard errors. Different letters represent significant differences ($p<0.05$) detected using DMRT. Statistical analyses are presented in Appendix 1.2.

Plate 3.1 Diclofop-methyl (1.9 kg AI ha^{-1}) symptoms on spring barley cv. Sherpa. Note: chlorotic /necrotic areas (arrow); new growth pulled the third leaf away from the stem (double arrow).

Plate 3.2 Diclofop-methyl symptoms on spring barley cv. Sherpa. Left: control; Right: diclofop-methyl (0.95 kg AI ha^{-1}). Note: herbicide treated plants slightly smaller than the controls; chlorotic /necrotic areas on oldest leaves (arrow).

Table 3.2 Effects of the fungicide fenpropimorph (0.75 kg AI ha^{-1} 3 d prior to herbicide treatment) on herbicide injury on the second leaf of spring barley treated with various rates of diclofop-methyl (0, 0.48, 0.95, 1.9 kg AI ha^{-1}). Injury scored 14 d after treatment with herbicide, where 0 = no injury, 50 = 50% of the leaf chlorotic or necrotic and 100 = leaf dead or completely chlorotic /necrotic. Values are means, where n = 4 or 8. Different letters indicate significant differences ($p \leq 0.05$) between means within the same cultivar, calculated by Duncan's Multiple Range Test. (See Appendix 1.2.1)

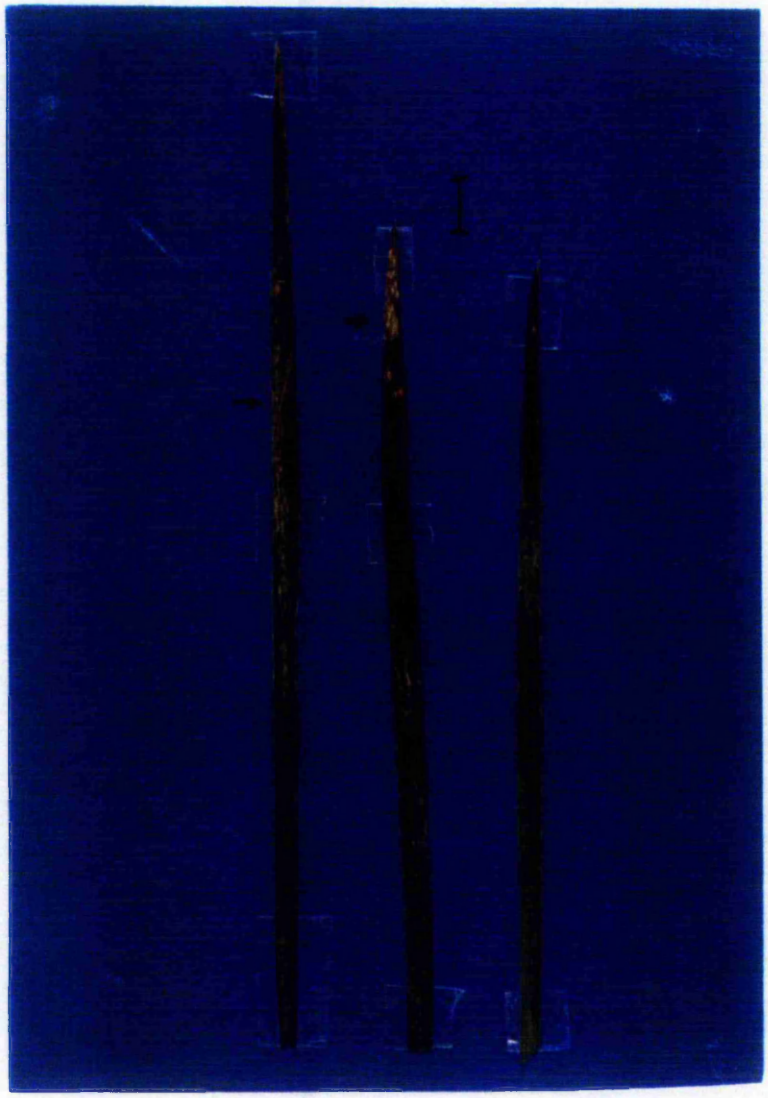
Cultivar	Diclofop-methyl Concentration (kg AI ha^{-1})	Visible Injury Score (0-100)	
		- fenpropimorph	+ fenpropimorph
Sherpa	0	0	0
	0.48	8 a	8 ab
	0.95	46 c	29 bc
	1.90	64 d	71 d
Corgi	0	0	0
	0.48	3 a	1 a
	0.95	29 b	25 b
	1.90	49 c	70 d
Tyne	0	0	not determined
	0.48	15	
	0.95	14	
	1.90	13	
Nugget	0	0	not determined
	0.48	4 a	
	0.95	18 b	
	1.90	16 b	

Table 3.3 Effects of fenpropimorph (0.75 kg AI ha^{-1}) on the shoot dry weight of spring barley cultivars Sherpa and Corgi, applied 3 d prior to treatment with various rates of diclofop-methyl (0, 0.48, 0.95, 1.9 kg AI ha^{-1}). Harvested 14 d after treatment with herbicide. Values are means, where n = 4. Different letters indicate significant differences at the 5% level within the column and cultivar (Duncan's Multiple Range Test, see Appendix 1.2.2).

Cultivar	Diclofop-methyl Concentration (kg AI ha^{-1})	Shoot Dry Weight (g)	
		- fenpropimorph	+ fenpropimorph
Sherpa	0	0.346 a	0.245 a
	0.48	0.239 b	0.220 ab
	0.95	0.219 bc	0.190 ab
	1.90	0.153 c	0.160 b
Corgi	0	0.299 a	0.276 a
	0.48	0.256 a	0.236 ab
	0.95	0.229 a	0.280 a
	1.90	0.158 b	0.196 b

Plate 3.3 Powdery mildew (*Erisiphye graminis*) symptoms on spring barley.

Note: pustules surrounded by areas of chlorosis (arrow). Bar = 1cm.



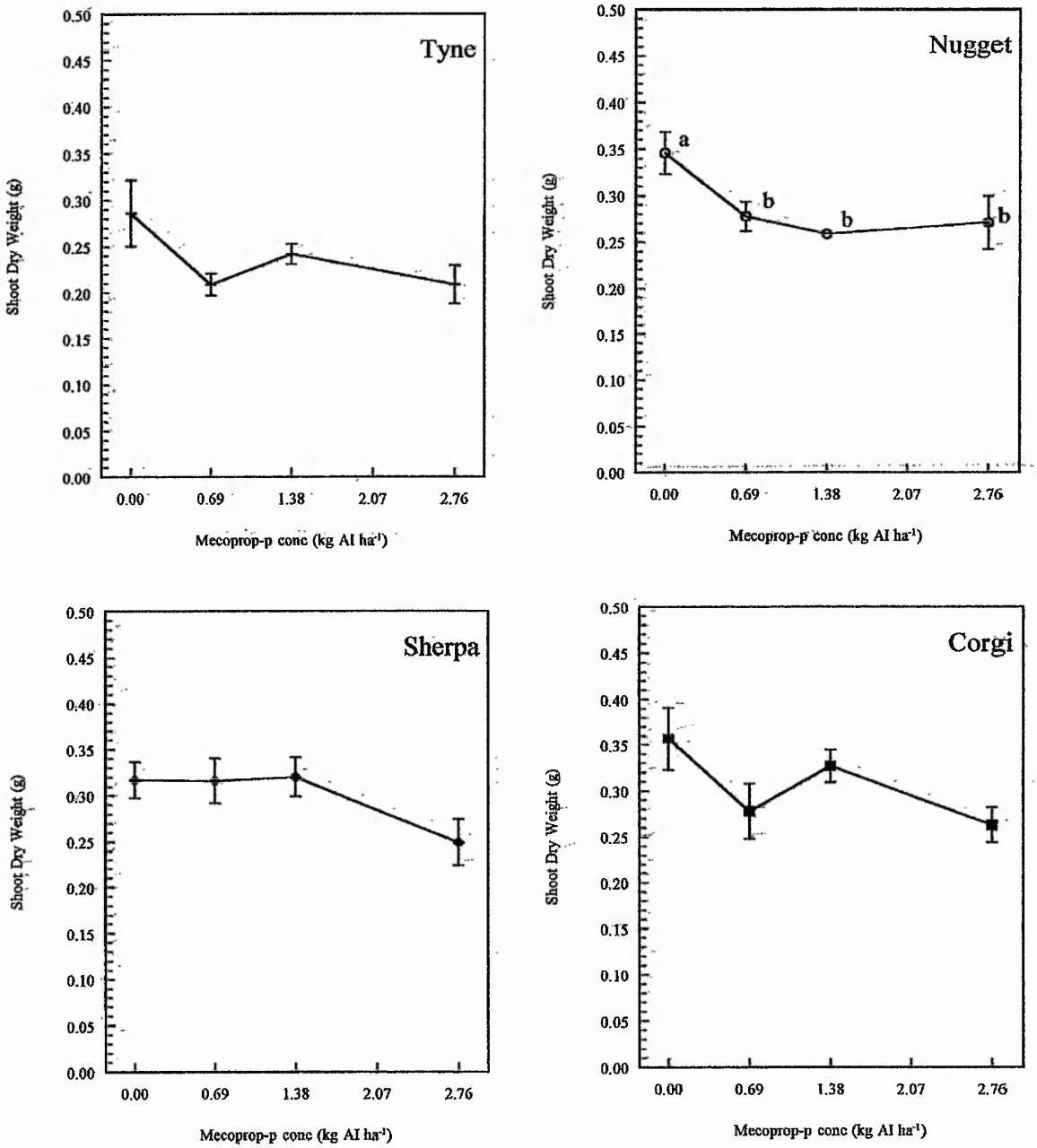
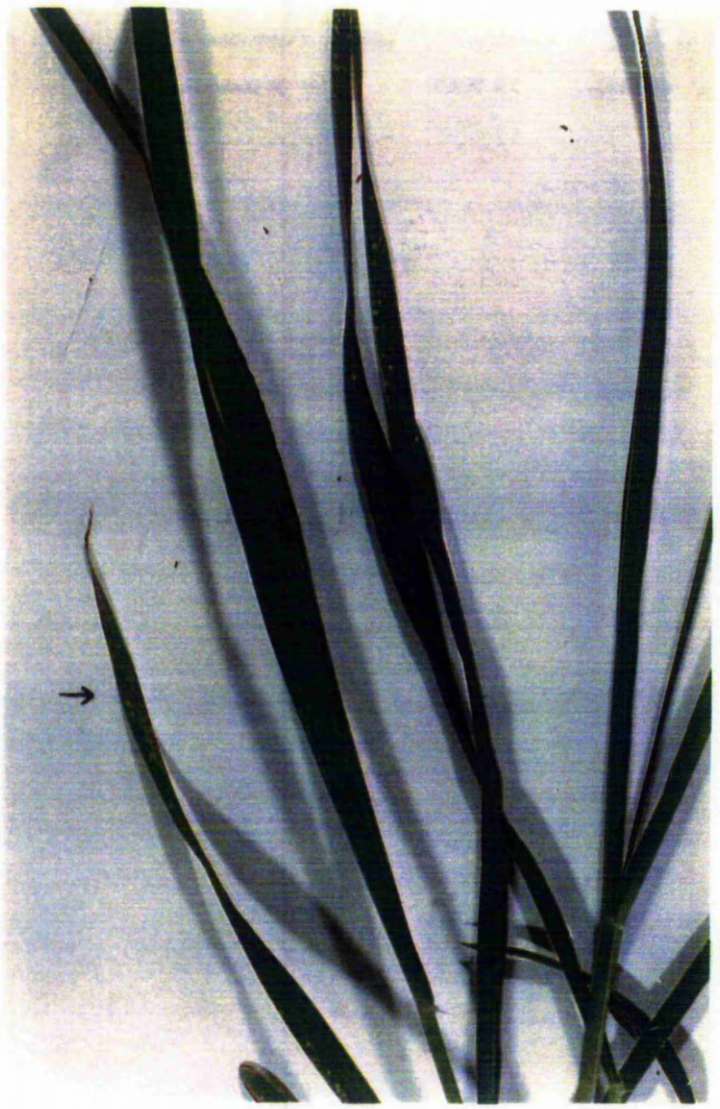


Figure 3.2 Effects of different concentrations of mecoprop-p on the shoot dry weights of 4 spring barley cultivars. Values are means, where n = 4 and bars represent 2 standard errors. Different letters represent significant differences ($p<0.05$) detected using DMRT. Statistical analyses are presented in Appendix 1.3.

Plate 3.4 Mecoprop-p (2.76 kg AI ha^{-1}) symptoms on spring barley cv. Sherpa, 13 d after treatment. Note: necrotic areas on leaves (arrow).



Corgi, Tyne and Nugget were affected similarly with or without the fungicide (Table 3.4; Appendix 1.3.2).

3.3.4 Clopyralid on Sugarbeet

Clopyralid did not produce any effects on shoot dry weights (Table 3.5; Appendix 1.4). The plants showed no indications of injury resulting from treatment with the herbicide.

3.3.5 Diclofop-methyl on Sugarbeet

Increasing the diclofop-methyl concentration did not significantly affect shoot dry weight of any cultivar (Table 3.6; Appendix 1.5). The herbicide produced small necrotic lesions on those leaves which had been sprayed (Plate 3.5).

3.3.6 Phenmedipham on Sugarbeet

Phenmedipham treatment produced a significant reduction (Amethyst $p < 0.001$; Celt $p = 0.010$; Saxon $p = 0.003$; Appendices 1.6 & 1.6.1) in shoot dry weight (Figure 3.3) in all 3 cultivars. Plants were visibly smaller at field-rate and twice field-rate. Symptoms were chlorotic spots that merged to form large areas covering 20-40 % of the sprayed leaves (Plate 3.6). Injury appeared 1-3 d after treatment.

3.4 DISCUSSION

The main objectives of this preliminary study were six-fold:

i) to determine the effects of each of the herbicides alone on the crops and cultivars selected,

ii) to establish the relative sensitivities of the cultivars under investigation,

iii) to characterise the response of glasshouse-grown plants to differing rates of herbicides,

iv) to give an indication of the time-course of symptom development on the plants and the types of injury occurring,

v) to give an indication of any other problems that may have been detrimental in later studies, (e.g. husbandry)

vi) to facilitate the choice of crops, cultivars and herbicides for further study.

The herbicides chosen for study are all widely used in British agriculture and so it was expected that there would be very little response of the crops to the compounds. However, several of the herbicides produced injury symptoms and growth reductions in the plants under investigation. The significance of the reductions depended upon the cultivar and the rate of herbicide application.

Treatment with diclofop-methyl and mecoprop-p resulted in injury and reductions in

Table 3.4 Effects of fenpropimorph (0.75 kg AI ha^{-1}) on the shoot dry weight of spring barley cultivars Sherpa and Corgi, applied 2 d prior to treatment with various rates of mecoprop-p (0, 0.69, 1.38, 2.76 kg AI ha^{-1}). Harvested 14 d after treatment with herbicide. Values are means ± standard error, where n = 4. Different letters indicate significant differences at the 5% level within the column and cultivar (Duncan's Multiple Range Test, see Appendix 1.3.2).

Cultivar	Mecoprop-p Concentration (kg AI ha^{-1})	Shoot Dry Weight (g)	
		- fenpropimorph	+ fenpropimorph
Sherpa	0	0.3127 a	0.4428 a
	0.69	0.3113 a	0.2904 b
	1.38	0.3198 a	0.2478 b
	2.76	0.2486 a	0.2102 b
Corgi	0	0.3571 a	0.3257 a
	0.69	0.2782 a	0.2578 a
	1.38	0.3293 a	0.2607 a
	2.76	0.2866 a	0.2509 a
Tyne	0	0.2862 a	0.2705 a
	0.69	0.2090 b	0.2044 b
	1.38	0.2420 ab	0.2372 ab
	2.76	0.2091 c	0.1801 b
Nugget	0	0.3469 a	0.3850 a
	0.69	0.2781 b	0.3191 ab
	1.38	0.2593 b	0.2735 b
	2.76	0.2709 b	0.2863 b

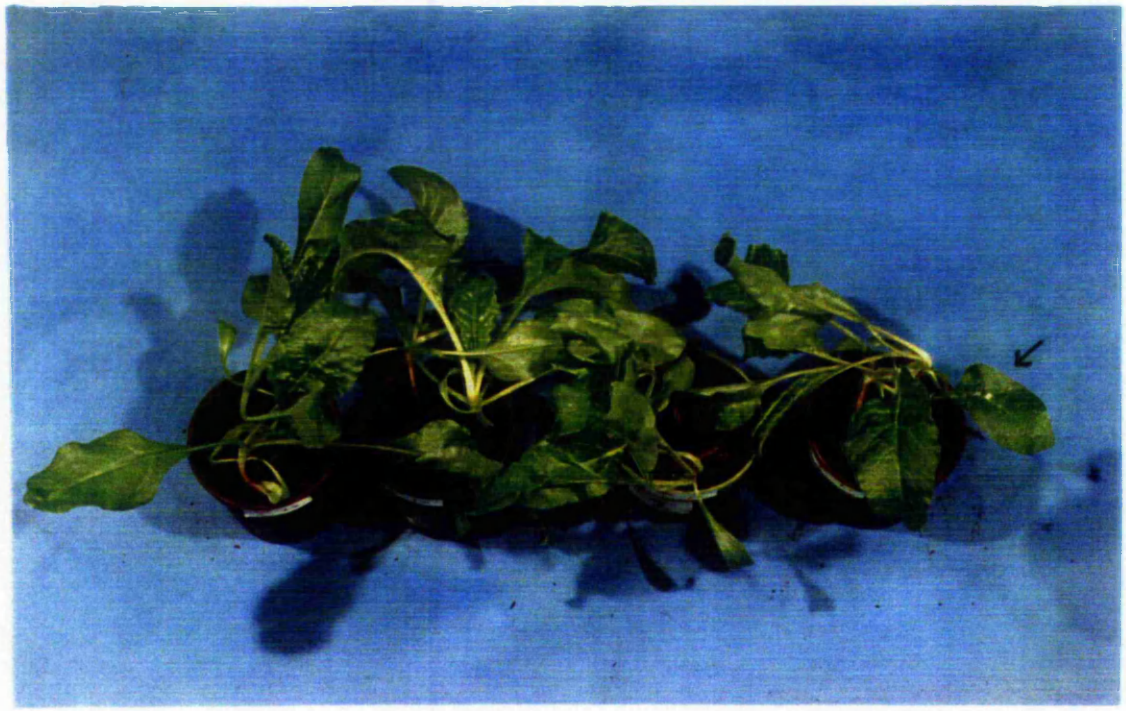
Table 3.5 Effects of clopyralid on the shoot dry weights of 3 sugarbeet cultivars applied at various rates (0, 0.05, 0.1 and 0.2 kg AI ha^{-1}). Harvested 14 d after treatment with clopyralid. Values are means ± standard error, where n = 4. No significant treatment effects were detected by one-way ANOVA for any of the cultivars (Appendix 1.4).

Cultivar	Shoot Dry Weight (g)			
	Clopyralid Concentration (kg AI ha^{-1})			
	0	0.05	0.1	0.2
Amethyst	0.298 ± 0.023	0.280 ± 0.011	0.286 ± 0.058	0.288 ± 0.027
Celt	0.230 ± 0.023	0.210 ± 0.011	0.186 ± 0.025	0.226 ± 0.016
Saxon	0.263 ± 0.018	0.275 ± 0.020	0.196 ± 0.011	0.258 ± 0.025

Table 3.6 Effects of diclofop-methyl on the shoot dry weights of 3 sugarbeet cultivars applied at various rates (0, 0.57, 1.14 and 2.28 kg AI ha^{-1}). Harvested 14 d after treatment with diclofop-methyl. Values are means ± standard error, where n = 4. No significant treatment effects were detected by one-way ANOVA for any of the cultivars (Appendix 1.5).

Cultivar	Shoot Dry Weight (g)			
	Diclofop-methyl Concentration (kg AI ha^{-1})			
	0	0.57	1.14	2.28
Amethyst	0.238 ± 0.030	0.230 ± 0.005	0.175 ± 0.028	0.189 ± 0.016
Celt	0.185 ± 0.028	0.215 ± 0.023	0.149 ± 0.014	0.153 ± 0.014
Saxon	0.323 ± 0.019	0.284 ± 0.048	0.190 ± 0.035	0.248 ± 0.023

Plate 3.5 Effects of diclofop-methyl (0, 0.57, 1.14 and 2.28 kg AI ha^{-1}, left to right respectively) on sugarbeet cv. Saxon, 13 d after treatment. Note: chlorotic/necrotic areas on leaves of 2.28 kg AI ha^{-1} plants (far right; arrow).



0 0.57 1.14 2.28 kg AI ha^{-1}

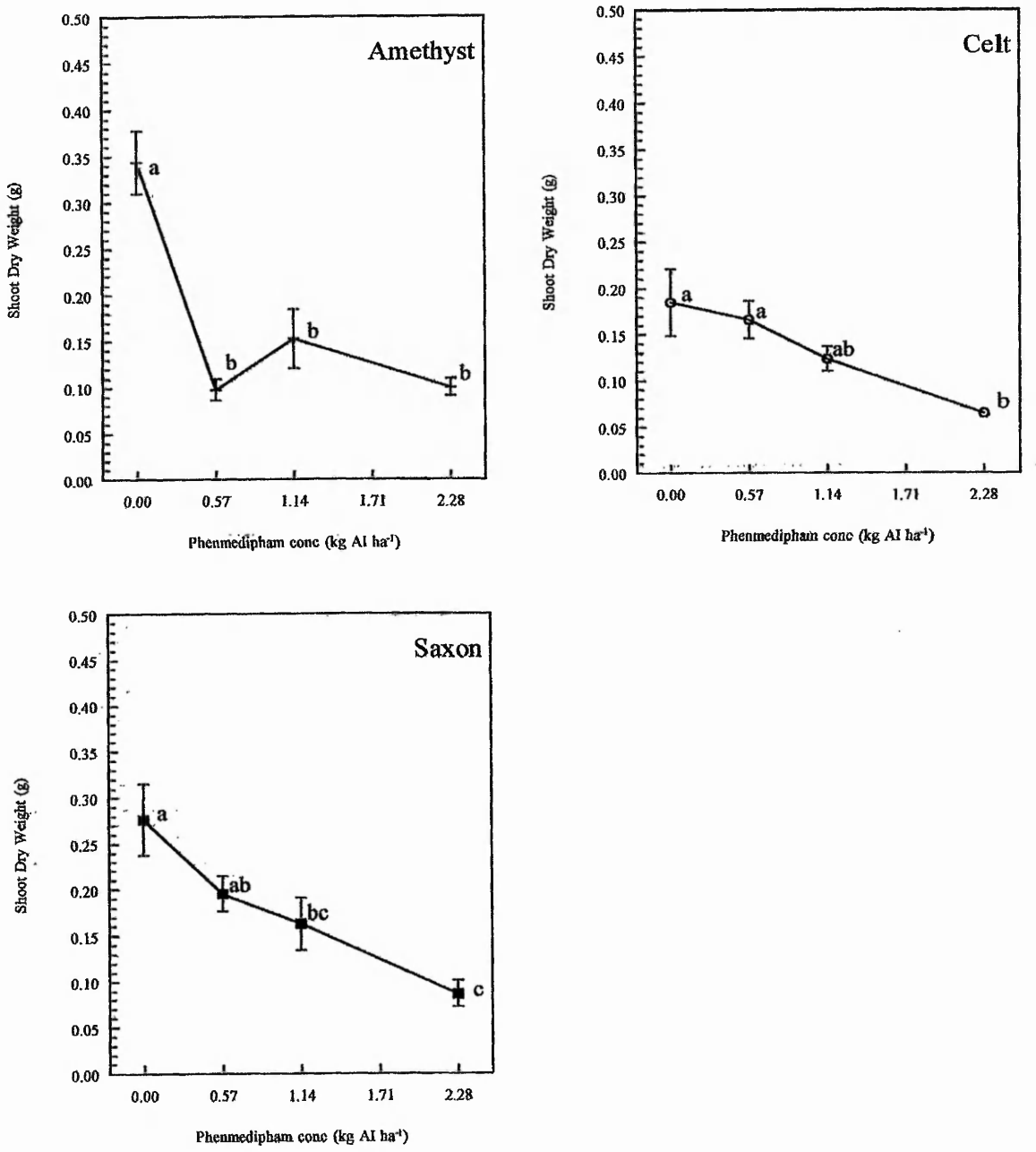
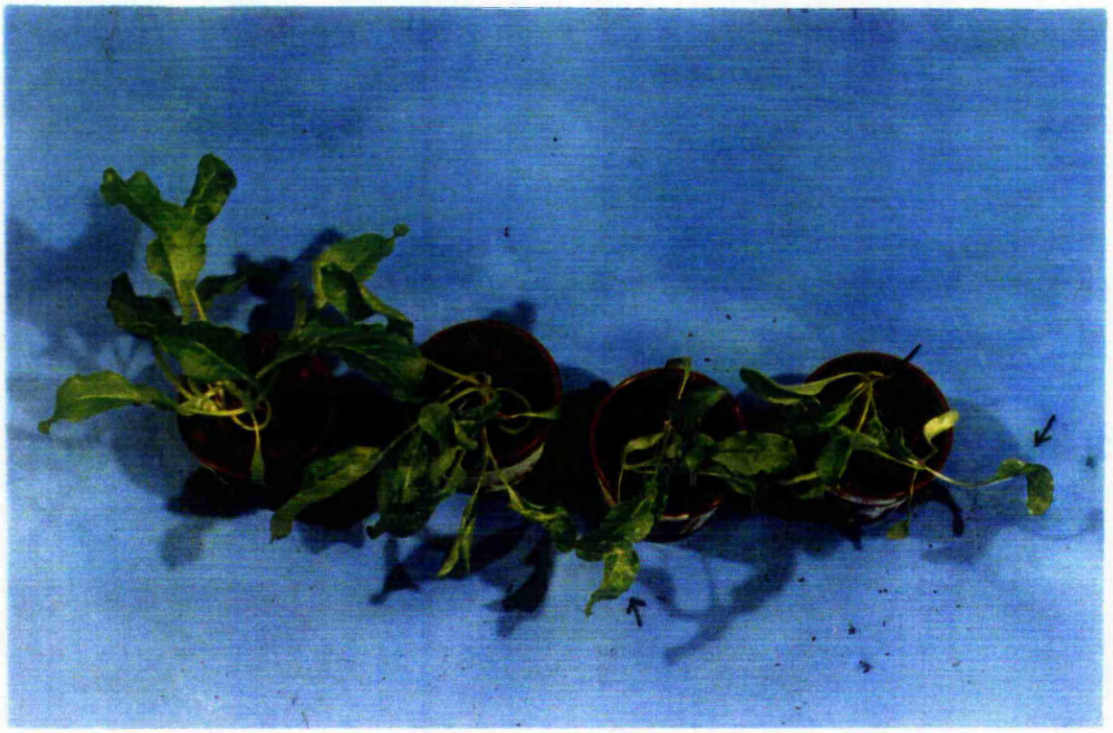


Figure 3.3 Effects of different concentrations of phenmedipham on the shoot dry weights of 3 sugarbeet cultivars. Values are means, where n = 4 and bars represent 2 standard errors. Different letters represent significant differences ($p<0.05$) detected using DMRT. Statistical analyses are presented in Apeendix 1.6.

Plate 3.6 Effects of phenmedipham (0, 0.57, 1.14 and 2.28 kg AI ha^{-1}, left to right respectively) on sugarbeet cv. Saxon, 13 d after treatment. Note: chlorotic/necrotic areas on leaves (right; arrow). Plants become smaller after treatment with 1.14 and 2.28 kg AI ha^{-1} herbicide.



0 0.57 1.14 2.28 kg AI ha^{-1}

growth in two spring barley cultivars (Figures 3.1 and 3.2). Barley is reported to be moderately tolerant to diclofop-methyl, although not as tolerant as wheat, which rapidly detoxifies the herbicide (Wu & Santelmann, 1976). Diclofop-methyl is hydrolysed to diclofop acid in all species and in wheat tolerance is due to aryl-hydroxylation followed by conjugation to an aryl glucoside (Boldt & Putman, 1981). In the present study cultivars were affected differently by treatment with diclofop-methyl - two showed significant reductions in shoot dry weight, whilst the others were not affected at all. Differences in the tolerances and susceptibilities of Australian spring barley cultivars have been noted in response to the application of 0.56 and 1.68 kg AI ha^{-1} diclofop-methyl (Lemerle *et al*, 1986). Susceptibility to the herbicide also seems to depend on the environmental conditions at the time of spraying and during the growing season, with high soil moisture and low temperatures increasing the phytotoxicity of diclofop-methyl (Dortenzio & Norris, 1980).

Diclofop-methyl injury did not appear on the leaves of spring barley until approximately 3 d after treatment at which time inter-veinal and leaf margin chlorosis appeared in association with scorched leaf tips (Plates 3.1 and 3.2). Contact damage evident as brown spots was only found on the leaves present at the time of spraying (Plate 3.1). Later effects included retarded and stunted growth (Plate 3.2). Diclofop-methyl and its metabolites have limited translocation and thus chlorosis of the leaves of susceptible species may not kill the plant. However, if this is combined with treatment to the meristematic region, then growth reductions will occur and may ultimately result in plant death. Tolerant species may be similarly affected, although to a lesser extent allowing the plant to recover. Recovery of a crop also depends on the timing of the herbicide application. For example, if sprayed at the 2-3 leaf stage, the plant is more likely to recover than if application occurs at the 2 tiller stage (M°Mullan, 1993). Therefore, plants would be likely to recover from the damage described in this study and would be expected not to show any reductions in yield.

Diclofop-methyl also produced visible injury on the sugarbeet cultivars although no reductions in shoot dry weight were apparent (Table 3.6). The tolerance of the crop may be due to either inactivation of the herbicide in the leaves as occurs in wheat (Donald & Shimabukuro, 1980) or diclofop-methyl may be unable to bind to the site of action, as in soybean (Hoppe, 1985).

Mecoprop-p is widely used for the control of broad-leaved and grass weeds in cereals although it has been shown to affect plant root growth in some crops (Skuterud,

1975; Greaves & Sargent, 1986). In the present study, reductions of 25 % in the shoot dry weight of Nugget occurred in response to the application of field-rate mecoprop-p (Table 3.4). There is very little information about the effects of mecoprop-p on barley, although in other studies on winter wheat, leaf area and shoot fresh weight were reduced by 32 and 47 % respectively in response to 10 kg AI ha^{-1} (7 x field-rate; Whipps & Greaves, 1986). Injury similar to that in the present study was observed in the form of slight scorching of wheat leaves leading to chlorosis of the leaf tips 15 d after treatment (Plate 3.4; Whipps & Greaves, 1986).

Clopyralid had no effect on either spring barley or sugarbeet (Tables 3.1 and 3.4) and no visible injury symptoms were observed on any plants. Sugarbeet has previously been shown to be tolerant to clopyralid and selectivity between the crop and susceptible weeds is not explained by differences in uptake, movement or metabolism (Thompson & Cobb, 1986; Wilson, 1995). Barley has been demonstrated to be tolerant to clopyralid at rates up to 0.9 kg AI ha^{-1} (recommended field-rate = 0.07 kg AI ha^{-1}) with no injury or yield reductions observed 2 weeks after application (O'Sullivan & Kossatz, 1984).

Phenmedipham is the most widely used herbicide in sugarbeet and has been shown to injure the crop under normal application conditions (Cantwell & Norris, 1973; Hendrick, 1973; Preston & Biscoe, 1982; Proctor, 1993). In the present study, field-rate phenmedipham reduced the shoot dry weights (33-55 % of untreated control) of all 3 sugarbeet cultivars (Figure 3.3). Similar reductions in plant dry weight (37 % of untreated control) were observed in a controlled environment study conducted under high (95 %) and low (50 %) relative humidity (Preston & Biscoe, 1982). When compared to field-grown plants treated in the same manner, smaller reductions (26 %) in plant dry weight were found (Preston & Biscoe, 1982). Indications of recovery, even from weight reductions of 45 % due to phenmedipham treatment, were observed 7 weeks after herbicide application in field-grown plants. Recovery within 3-7 weeks of phenmedipham treatment has also been observed in other studies (Hendrick, 1973; Schweizer, 1974). Hence, glasshouse-grown plants might have been expected to recover from the injury observed, had growth been allowed to continue.

The observed symptoms of injury due to phenmedipham (Plate 3.6) have been described previously as chlorotic areas around initial spray contact injury which appear within 2 h of treatment under high temperatures and 2-3 d at low temperature (Preston & Biscoe, 1982). Product label information recommends that phenmedipham is not sprayed during periods of sunny weather with temperatures above 21°C, as such conditions may

increase the damage to the plants (Anon, 1992b).

The use of fenpropimorph indicated that chemical control of any pests or diseases was not feasible, due to interactions occurring with the herbicide in certain cultivars (Table 3.2). Interactions may have also occurred in later experiments with ozone, since another fungicide, benomyl, has been shown to reduce the effect of ozone on *Phaseolus vulgaris* L. (Pell, 1976). Further studies with spring barley could only be conducted under conditions with little or no powdery mildew present.

3.5 CONCLUSIONS

Clopyralid exerted no significant effects on either spring barley or sugarbeet. Diclofop-methyl had no effect on the spring barley cvs. Tyne and Nugget, whilst decreases in shoot dry weight were observed in Sherpa and Corgi. Treatment with fenpropimorph to control powdery mildew produced varied results dependent on the cultivar. Mecoprop-p significantly ($p = 0.043$) reduced the weights of the barley cultivar Nugget. Phenmedipham significantly reduced the shoot dry weights of all 3 sugarbeet cvs., but there was no effect of application of diclofop-methyl.

Glasshouse-grown plants did not seem to be as sensitive to treatment with herbicides as expected. The incidence of powdery mildew within the glasshouse gave cause for concern about the use of spring barley in later studies. This was compounded by the fact that the fungicide used to control the disease influenced the effect of the herbicides on the crop.

Results from this preliminary study allowed a choice of which crops and herbicide combinations to use in the study. Sugarbeet cvs. Saxon and Celt and spring barley cvs. Sherpa and Corgi were chosen. It was also decided to use all 3 herbicides on sugarbeet and spring barley. Spring barley would continue to be used unless powdery mildew infection interfered, in which case the crop would be withdrawn from the study. In addition to barley and sugarbeet, two cultivars of spring oilseed rape, Starlight and Galaxy, were also investigated.

CHAPTER 4 - INTERACTIONS BETWEEN OZONE POLLUTION AND HERBICIDES

4.1 INTRODUCTION

The impact of herbicides on plants may be influenced by environmental conditions and *vice versa*. Product label information generally outlines the most unfavourable conditions for application. For example, several products are advised not to be applied when conditions are bright and sunny with temperatures above 21°C (Anon, 1992b), or the expectation of a frost or rainfall in the following 24-48 hours (Anon, 1992a). Testing during the pre-registration period also determines which products can be successfully tank-mixed. Air pollution effects are not considered during the testing period, although they may influence the magnitude of response to the herbicide.

A series of studies have been conducted, mainly on economically important crops in USA and Canada, employing herbicides most frequently used in these situations (Chapter 1, Section 1.5). The outcome of exposure to the combination of herbicides and ozone has been found to depend on several factors including; the timing of application of the herbicide relative to the occurrence of ozone episodes; the sensitivity of the species to both the herbicide and the pollutant; the concentration of the pollutant during the episode; and the meteorological conditions before, during and after the application of ozone/herbicide.

Following the preliminary study (Chapter 3), the sugarbeet cvs. Saxon and Celt and spring barley cvs. Sherpa and Corgi were used in further experiments to determine the nature of any interactions between ozone and herbicides. Two spring oilseed rape cvs. were also chosen for study. Starlight is currently one of the most widely used oilseed rape cvs. and Galaxy has recently been introduced onto the NIAB listings (NIAB, 1994).

Very few studies have been carried out in Europe to determine the potential for interactions in northern European crops. It was necessary to determine the responses of the crops and cvs. to an ozone episode. A concentration of 100 nl l^{-1} for 7 h d^{-1} for 2 d was chosen to represent an episode which could occur in the UK. The control of the exposure system did not allow the experimental exposure to exactly simulate an natural episode. Initial experiments also had to give an indication of the interactions between ozone pollution and the herbicide, using analysis of growth parameters. This study also facilitated the nature of interactions to investigate in more detail.

4.2 MATERIALS AND METHODS

Spring barley (cvs. Sherpa and Corgi), spring oilseed rape (*Brassica napus* L. cvs. Galaxy and Starlight) and sugarbeet (cvs. Saxon and Celt) were sown and grown-up as detailed in Chapter 2. At the 2-3 leaf stage, plants were sprayed with one of the following herbicides at field rate: barley - diclofop-methyl; oilseed rape - diclofop-methyl, clopyralid or metazachlor; sugarbeet - phenmedipham. Three days later, the plants were exposed to 100 nl l^{-1} ozone for 7 h d^{-1} for 2 d (Chapter 2, section 2.4, procedure (i)). Sugarbeet plants were also exposed to the same concentration of ozone followed by treatment with field rate phenmedipham, diclofop-methyl or clopyralid (Chapter 2, section 2.4, procedure (ii)).

Seven days after the final treatment, visible injury was assessed according to the method used in Chapter 3 section 3.2. Where plants were treated with both ozone and a herbicide, injury was noted as total injury. Shoots were excised at soil level and leaf area was measured. Shoot dry weight was determined by drying the tissue in an oven at 80°C until the weight was constant (2-3 d). Experiments were repeated 2-4 times for each cultivar and herbicide combination.

The mean of 4 pots in a chamber was treated as a replicate, with 2 replicates per experiment, repeated 2-3 times. To discover there were any significant differences between treatments, a two-way ANOVA test (Unistat v.4) was conducted. If the null hypothesis was rejected (i.e. significant differences occurred between treatments at the $p<0.05$ level), further analysis was conducted using Duncan's Multiple Range Test (DMRT). All statistical differences are quoted at the 5% level.

4.3 RESULTS

4.3.1 General Comments

Symptoms of ozone injury differed slightly dependent on the species. In barley, injury usually occurred at the tips or edges of the leaf. Areas of the leaf lamina turned necrotic within 3-4 d after the end of ozone exposure (Plate 4.1). Oilseed rape developed chlorotic, mottled areas (Plate 4.2c). Ozone injury on sugarbeet leaves appeared as a fine stipple of chlorotic flecks 1-2 mm in length (Plate 4.3a and b). In all species, injury appeared first on the oldest leaves of the plant.

Injury resulting from application of herbicides was the same as described in Chapter 3. Clopyralid and metazachlor produced no visible effects on spring oilseed rape and

Plate 4.1 Ozone injury on spring barley cv. Sherpa, 7 d after treatment.
Note: chlorotic areas between veins (arrowed)

Control Ozone

Plate 4.2 Injury symptoms on oilseed rape cv. Starlight, 7 d after ozone exposure.
a) control; b) diclofop-methyl alone; c) ozone alone; d) clopyralid and ozone;
e) metazachlor and ozone.
Note: chlorotic lesions on c) and e) due to ozone (arrowed).

Plate 4.3 Effects of ozone on sugarbeet cv. Saxon, 10 d after ozone exposure.
a) area of leaf affected - note white flecks on leaf (arrowed). Bar = 1 cm.
b) ozone injury (x 10 mag). White areas are injury due to ozone. Silver sheen = light reflecting off leaf hairs.

a)

b)

diclofop-methyl injury appeared as small circular areas of chlorosis indicative of contact damage.

4.3.2 Spring Barley

Treatment with ozone alone or diclofop-methyl alone did not significantly alter the shoot dry weight of Sherpa (Figure 4.1). In Corgi, ozone alone decreased shoot dry weight (Appendix 2.1), but there was no effect other treatments. There were no significant interactions between diclofop-methyl and ozone in either cultivar (Appendix 2.1). Experiments with the remaining herbicides were not performed due to *Erysiphe graminis* infection of the plants.

4.3.3 Spring Oilseed Rape

The amount of visible injury due to individual treatments on cvs. Galaxy and Starlight is shown in Table 4.1 (Appendix 2.2.1). Ozone alone and diclofop-methyl alone produced similar amounts of damage on both cvs. The shoot dry weights of both cvs. were unaffected by exposure to ozone (Figure 4.2 and Appendices 2.2, 2.3, and 2.4).

4.3.3.1 Diclofop-methyl

Diclofop-methyl, and diclofop-methyl followed by ozone had no significant effects on either cv. (Plate 4.2b; Appendix 2.2). No significant interactions occurred between diclofop-methyl and ozone in either cultivar.

4.3.3.2 Metazachlor

Metazachlor did not significantly affect either cv.. Shoot dry weights of both cvs. were not affected after treatment with metazachlor followed by ozone (Plate 4.2e, Appendix 2.3).

4.3.3.3 Clopyralid

Clopyralid alone had no effect on either cultivar (Figure 4.2). Treatment with clopyralid followed by ozone did not alter the shoot dry weights of cv. Galaxy with respect to the controls (Plate 4.2d). However, a significant interaction was indicated by ANOVA ($p = 0.044$; Appendix 2.4). Since both ozone alone and clopyralid alone, stimulated shoot dry weights non-significantly, this response indicated antagonism (Appendix 2.4 and 2.4.1). No interaction occurred between clopyralid and ozone in cv. Starlight.

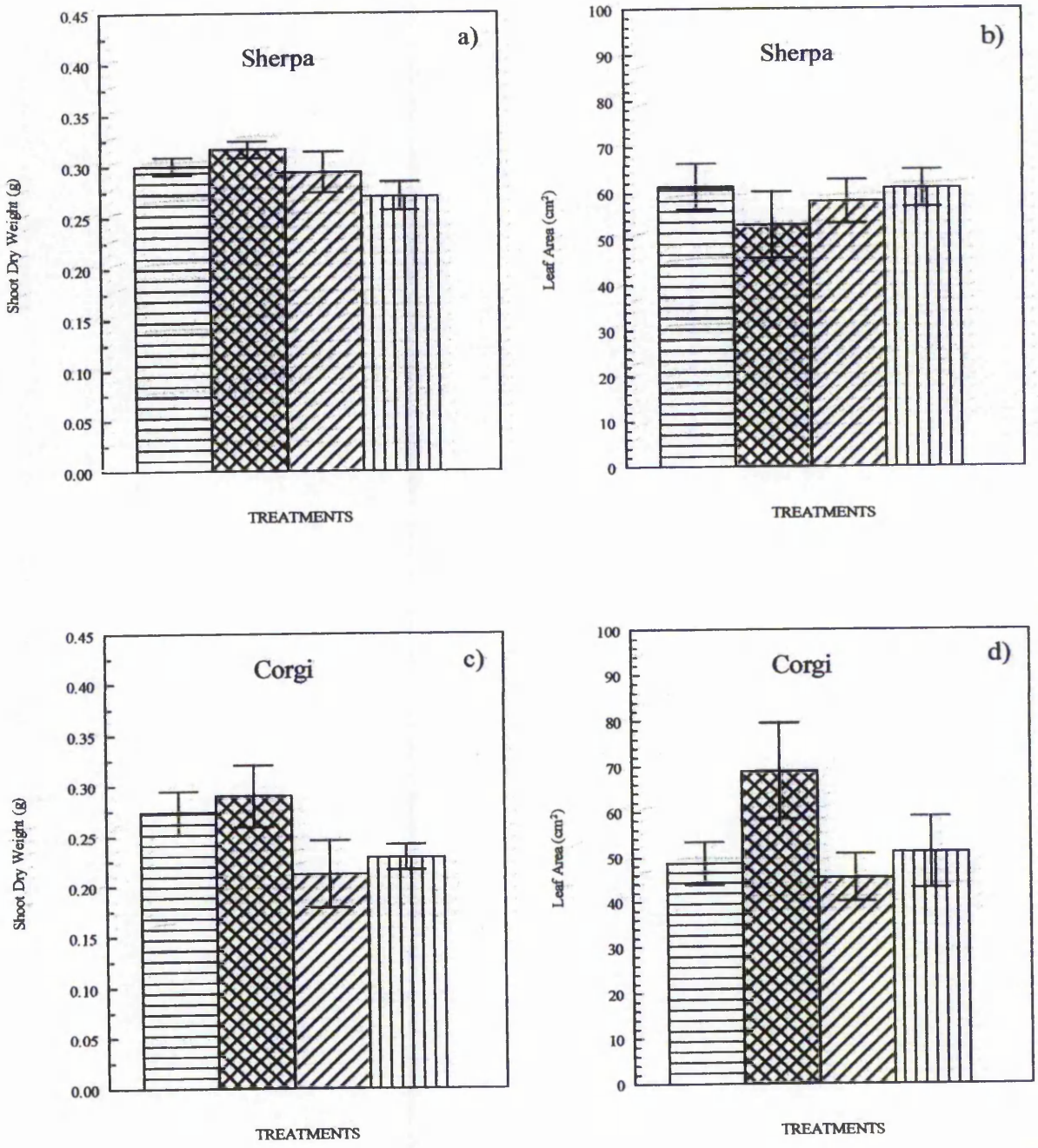


Figure 4.1 Effects of diclofop-methyl (0.95 kg AI ha^{-1}) and/or ozone (100 nl l^{-1}, 7 h d^{-1}, 2 d) on the shoot dry weight (a & c) and leaf area (b & d) of spring barley cvs. Sherpa and Corgi Values are means, where n = 4 and bars represent 2 standard errors. Statistical analyses are presented in Appendix 2.1.

KEY: control; ozone alone; herbicide alone; herbicide and ozone;

Table 4.1 Effects of diclofop-methyl and/or ozone on the appearance and extent of visible injury on spring oilseed rape cvs Galaxy and Starlight, 7 d after the end of exposure to ozone. Values are raw data means, where n = 4 and different letters after each mean represent a significant difference between values in the same column (DMRT, $p<0.05$, Appendix 2.2.1).

Treatment	Visible injury score (0-100)	
	Galaxy	Starlight
Control	0	0
Diclofop-methyl alone	16.3 a	10.9 a
Ozone alone	35.9 b	39.3 b
Ozone and diclofop-methyl	40.7 b	47.4 b

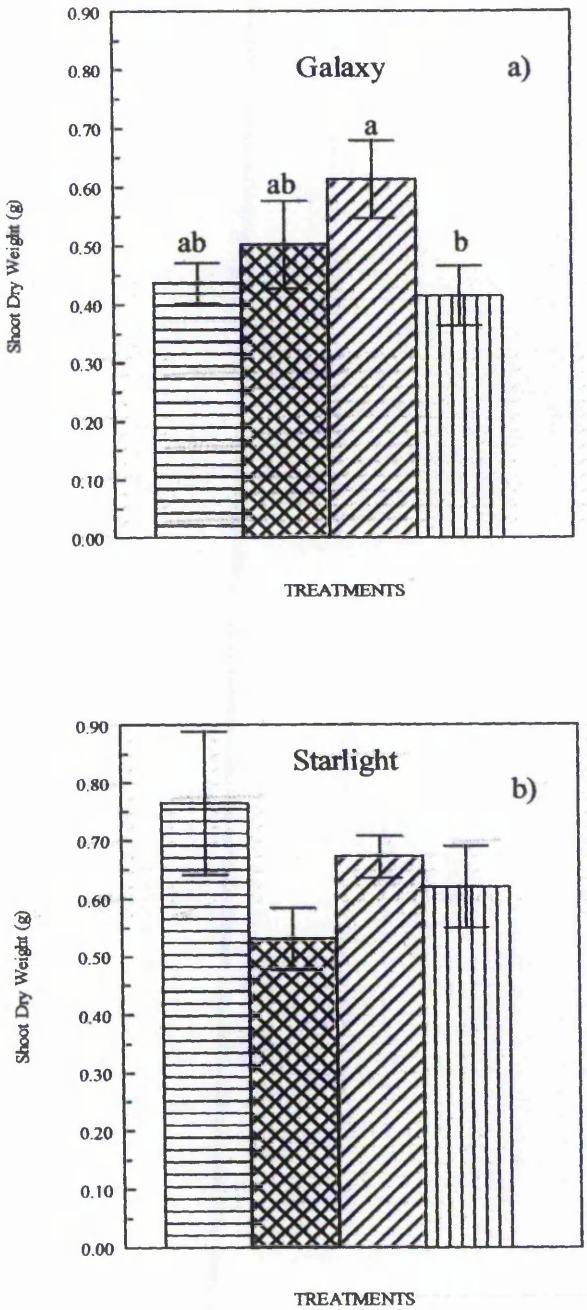


Figure 4.2 Effects of clopyralid (0.10 kg AI ha^{-1}) and/or ozone (100 nl l^{-1}, 7 h d^{-1}, 2 d) on shoot dry weight of spring oilseed rape cvs. Galaxy and Starlight. Values are means, where n = 4 and bars represent 2 standard errors. Letters indicate significant differences between means using DMRT ($p<0.05\%$). Statistical analyses are presented in Appendix 2.4.

KEY: control; ozone alone; herbicide alone; herbicide and ozone;

4.3.4 Sugarbeet

When treated using procedure (i), exposure to ozone of sugarbeet cv. Saxon altered leaf area 7 days after treatment, although this effect was not evident 14 d after treatment (Figure 4.3a & c, Appendix 2.5). Exposure to ozone reduced shoot dry weight, 7 and 14 d after the end of treatment (Figure 4.3b & d, Appendix 2.6). Further experiments using procedure (ii), showed that exposure to ozone had no consistent effect on shoot dry weight or leaf area of either cultivar (Figures 4.4, 4.5, and 4.6; Appendices 2.8, 2.9 2.10 and 2.11).

Plants exposed to ozone had a small amount of injury 7 d after the end of exposure, although this had approximately doubled 14 d after treatment (Table 4.2, Appendix 2.7). Phenmedipham damage was similar in extent to that resulting from ozone (Plate 4.4a and b). When both treatments were applied, the visible damage was not different from the expected additive value.

4.3.4.1 Phenmedipham

a) Procedure (i) Phenmedipham had no effect on leaf area 7 d after treatment, although 14 d after treatment a reduction of 16 % was observed (Figure 4.3c). Treatment with phenmedipham followed by exposure to ozone resulted in reductions in leaf area of 29 and 13 %, 7 and 14 d, respectively (Figure 4.3 a & c; Appendix 2.5 $p=0.012$ and $p=0.084$, respectively). Phenmedipham also significantly reduced shoot dry weight by 14 and 28 %, 7 and 14 d after treatment, respectively (Figure 4.3b & d; Appendix 2.6). Decreases in shoot dry weight of 30 and 32 % were observed 7 and 14 d after treatment, respectively, when plants were treated with phenmedipham followed by exposure to ozone (Appendix 2.6). Seven days after treatment, the interaction was synergistic (Appendix 2.6, $p=0.037$), whilst 14 d after treatment, an antagonistic interaction was observed in shoot dry weight (Appendix 2.6, $p=0.026$).

b) Procedure (ii) Phenmedipham alone decreased leaf area by 38 and 41 % Saxon and Celt, respectively, (Figures 4.4a & c; Appendices 2.8 and 2.10). Shoot dry weight was also reduced in Saxon and Celt by 36 and 48 % respectively; (Figures 4.4b & d; Appendices 2.9 and 2.11). Treatment with ozone followed by phenmedipham resulted in a significant reduction in both parameters in both cvs. (Appendices 2.8, 2.9, 2.10 and 2.11). When two-way ANOVA tests were conducted, leaf areas of both cvs. and shoot dry weight of Celt indicated significant interactions which were antagonistic (Appendix 2.8.1 Saxon leaf area, $p=0.046$; Appendix 2.9.1 Celt leaf area, $p=0.004$; Appendix 2.11 Celt

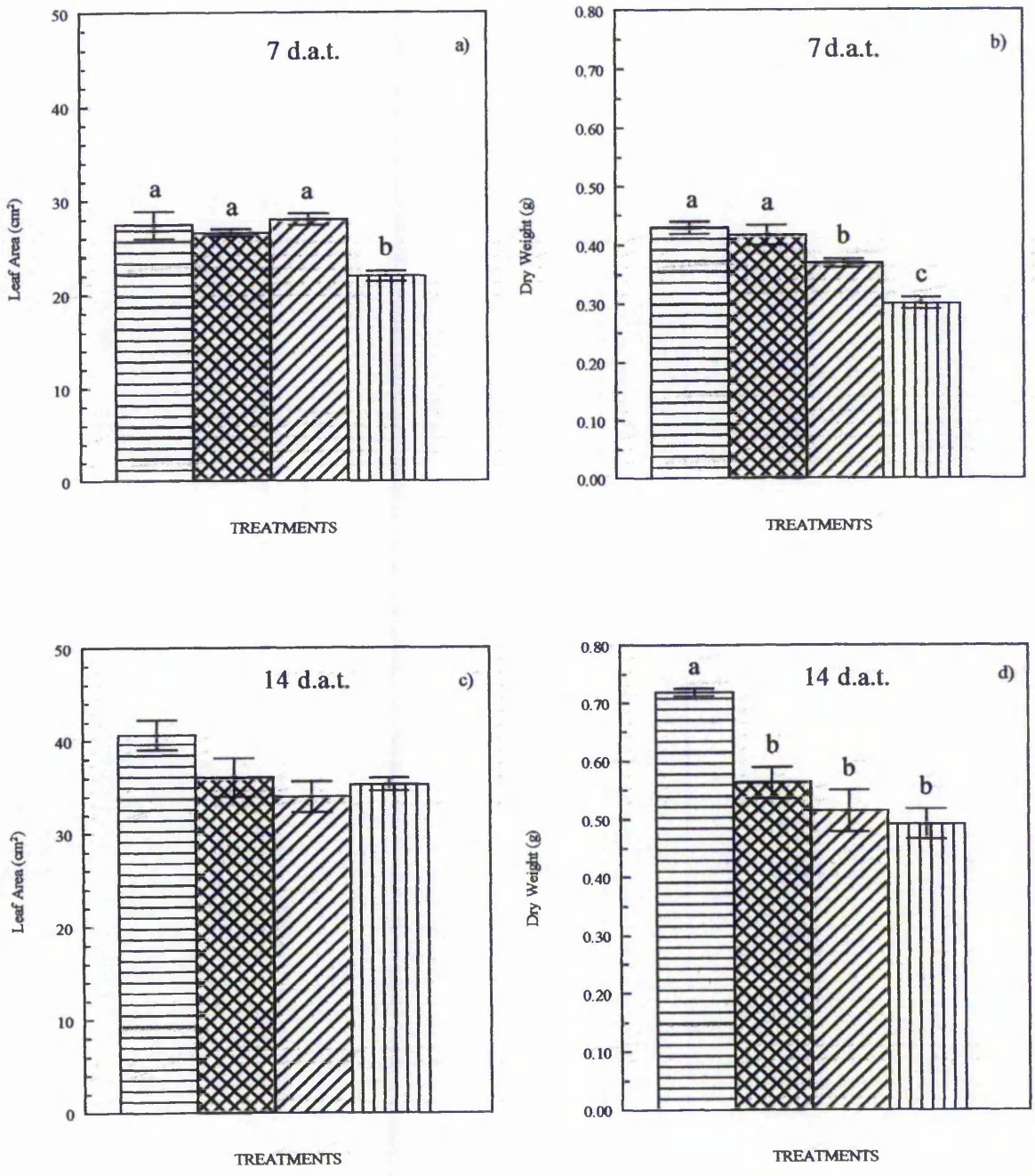


Figure 4.3 Effects of phenmedipham (1.14 kg AI ha^{-1}) and/or ozone (100 nl l^{-1}, 7 h d^{-1}, 2 d) applied using procedure (i) on sugarbeet cv. Saxon leaf area (a & c) and dry weight (b & d), 7 and 14 d after treatment. Values are means, where n = 4 and bars represent 2 standard errors. Different letters indicate significant diffences using DMRT ($p<0.05\%$). Statistical analyses are presented in Appendices 2.5, 2.6, and 2.7.

KEY: control; ozone alone; herbicide alone; herbicide and ozone;

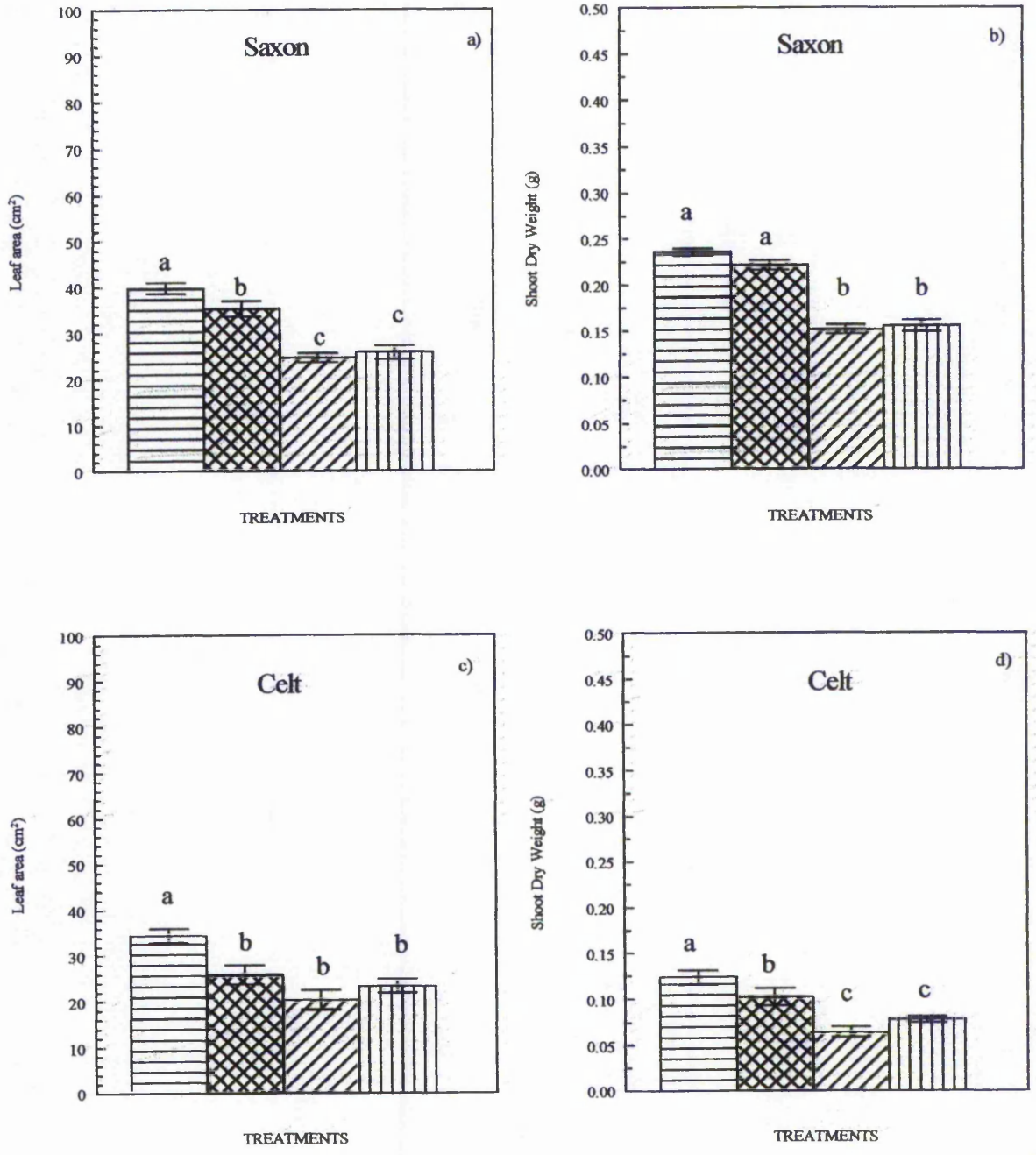


Figure 4.4 Effects of ozone (100 nl l^{-1}, 7 h d^{-1}, 2 d) and/or phenmedipham (1.14 kg AI ha^{-1}) applied using procedure (ii) on sugarbeet cv. Saxon and Celt, leaf area (a & c) and dry weight (b & d), 7 after treatment. Values are means, where n = 4 and bars represent 2 standard errors. Different letters indicate significant differences between the means using DMRT ($p<0.05\%$). Statistical analyses are presented in Appendices 2.8, 2.9, 2.10 and 2.11.

KEY: control; ozone alone; herbicide alone; herbicide and ozone;

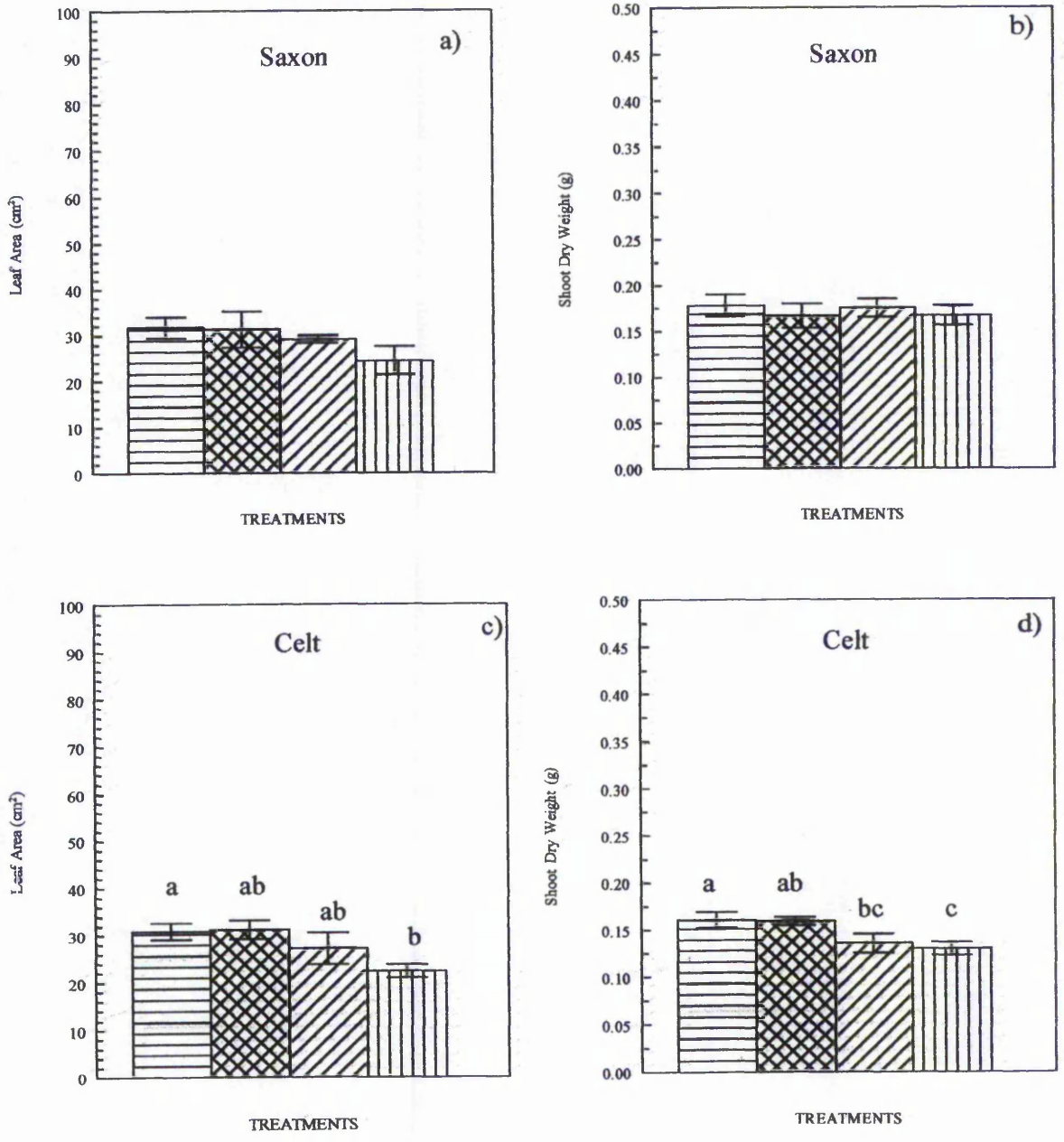


Figure 4.5 Effects of ozone (100 nl l^{-1}, 7 h d^{-1}, 2 d) and/or diclofop-methyl (1.14 kg AI ha^{-1}) applied using procedure (ii) on sugarbeet cvs. Saxon and Celt, leaf area (a & c) and shoot dry weight (b & d). Values are means, where n = 4 and bars represent 2 standard errors. Different letters represent significant differences between the means using DMRT ($p<0.05\%$). Statistical analyses are presented in Appendix 2.12.

KEY: control; ozone alone; herbicide alone; herbicide and ozone;

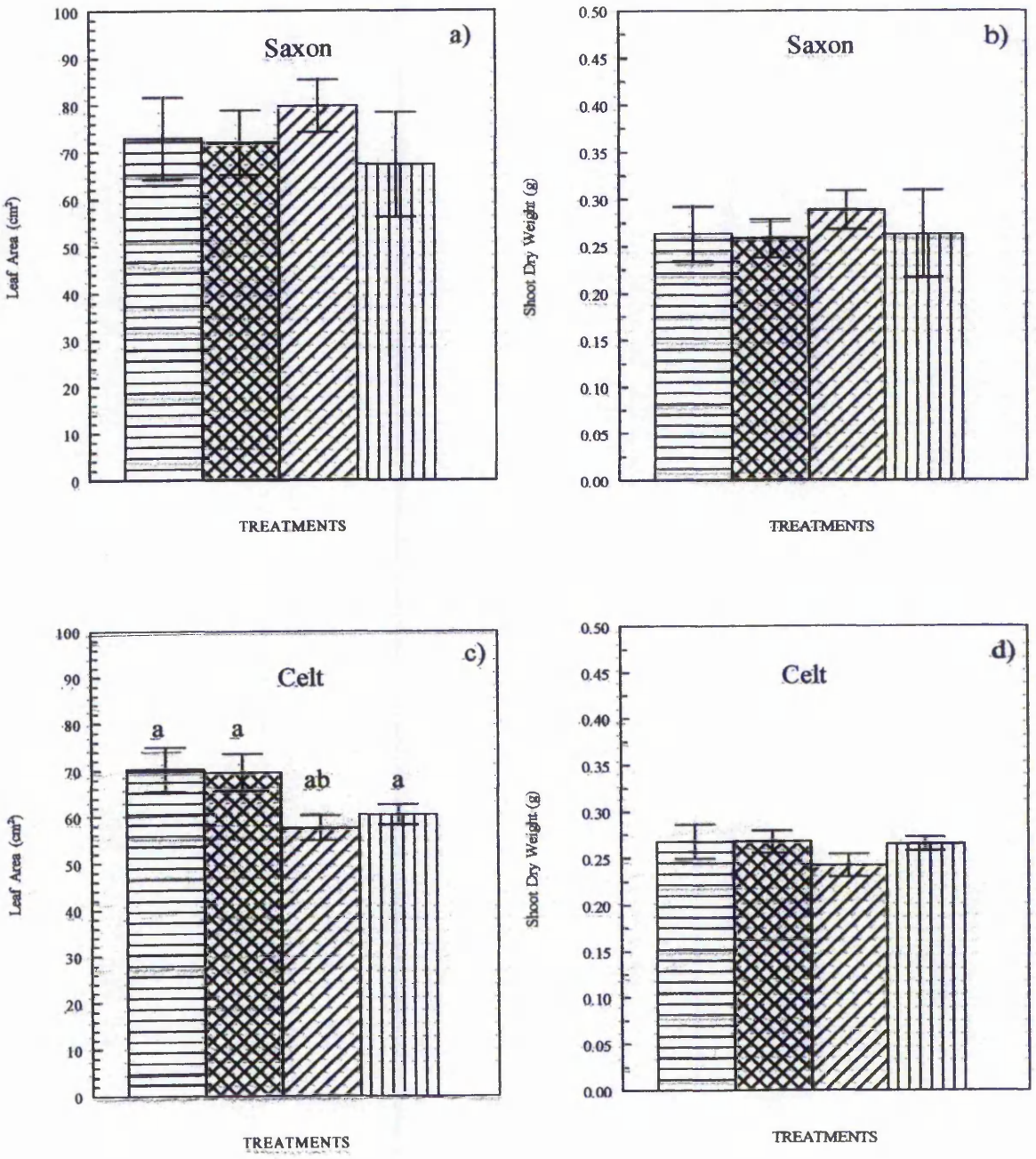


Figure 4.6 Effects of ozone (100 nl l^{-1}, 7 h d^{-1}, 2 d) and/or clopyralid (0.10 kg AI ha^{-1}) applied using procedure (ii) on sugarbeet cvs. Saxon and Celt, leaf area (a & c) and shoot dry weight (b & d). Values are means, where n = 4 and bars represent 2 standard errors. Different letters represent significant differences between means using DMRT ($p<0.05\%$). Statistical analyses are presented in Appendix 2.13.

KEY: control; ozone alone; herbicide alone; herbicide and ozone;

Table 4.2 Effects of phenmedipham and/or ozone on the appearance and extent of visible injury on sugarbeet cv Saxon, 7 d and 14 d after the end of exposure to ozone. Values are means, where n = 4 and different letters after each mean represent a significant difference between values in the same column (DMRT, $p<0.05$, Appendix 2.7).

Treatment	Visible injury score (0-100)	
	7 days	14 days
Control	0	0
Phenmedipham alone	9.5 a	12.1 a
Ozone alone	20.9 a	19.6 a
Ozone and phenmedipham	29.5 b	58.9 b

Plate 4.4 Effects of phenmedipham and ozone on sugarbeet cv. Saxon.
a) phenmedipham injury, bar = 1 cm.
b) phenmedipham injury (x 10 mag) note: pitted areas - contact injury.
c) ozone and phenmedipham injury on leaf.

shoot dry weight, p=0.018), whilst shoot dry weight of Saxon showed an additive response (Appendix 2.10).

4.3.4.2 Diclofop-methyl

Diclofop-methyl significantly reduced shoot dry weight of Celt (Figure 4.5; Appendix 2.12), whilst no significant effect was observed in either parameter in Saxon or leaf area of Celt. Similarly exposure to ozone followed by treatment with the herbicide did not have a significant effect on the shoot dry weight of Celt (Appendix 2.12).

4.3.4.3 Clopyralid

Clopyralid alone reduced leaf area of cv. Celt significantly (Figure 4.6). All interactions were non-significant (Appendix 2.13).

4.4 DISCUSSION

The objectives of these experiments were to:

i) determine the responses of spring oilseed rape to particular herbicides;

ii) establish the effects of ozone pollution on certain crops and cvs.;

iii) show interactions between ozone pollution and the herbicides; and

iv) facilitate the choice of an interaction for further study.

Due to the large variation in growth encountered during this study, none of the herbicides had any significant effect on spring oilseed rape cvs. (Figure 4.2). In previous studies, effects of clopyralid were only observed at high application rates (O'Sullivan *et al*, 1985). In the UK, the recommended rate of 0.1 kg AI ha^{-1} would not be expected to produce any damaging effect on the crop. The ability of oilseed rape to tolerate high application rates of clopyralid may be due to effects at the site of clopyralid action within the plant (Hall & Van den Born, 1988).

Diclofop-methyl produced visible injury on oilseed rape, although this could be attributed to spray contact. There has been no published work on the effects of diclofop-methyl specifically on oilseed rape. However, other studies have indicated most dicotyledonous plants are tolerant of the herbicide (Hoppe, 1985; Wright & Shimabukuro, 1987). Few studies have been conducted using metazachlor on oilseed rape, although those that have indicate tolerance to metazachlor at rates up to 1.8 kg AI ha^{-1} (Stormonth & Woodroffe, 1982).

Another of the objectives was to determine the effects of ozone on the crops and cvs. used in the study. Injury due to ozone pollution has been described as a scattered distribution of roughly symmetrical chlorotic flecks developing between veins (Wellburn,

1994). This type of injury was noted in all 3 of the crops used in the present study.

Exposure of barley to ozone resulted in visible injury symptoms on the oldest leaves, but had no effect on shoot dry weight or leaf area in either cv.. The literature indicates that barley is considerably less sensitive to ozone pollution than most other cereals, including wheat, oat and rye, in its ability to withstand acute and chronic doses of the pollutant at critical periods during the growth of the crop (Adaros *et al*, 1991a). However, injury and growth reductions have been noted after exposure to relatively high ozone concentrations (Sechler & Davis, 1964; Ashmore & Önal, 1984). Several studies conducted in open-top chambers over periods of 36-140 d, at various concentrations (21-111 nl l^{-1} 7/8 h seasonal mean), resulted in no effects on grain yield and quality at concentrations below a 7 h seasonal mean of 60 nl l^{-1} (Temple *et al*, 1985; Adaros *et al*, 1991a; Pleijel *et al*, 1992). In the present study, spring barley was infested by powdery mildew and consequently was ruled out of further study.

Exposure to ozone produced no effects in oilseed rape (Figure 4.2). In a previous study, an increasing effect of ozone was observed on most growth and yield parameters as concentrations increased (Adaros *et al*, 1991a). Ozone injury found on oilseed rape was described as a colour change to green-violet and brown (Adaros *et al*, 1991a). This conflicts with observations from the present study where injury was seen as a chlorotic stipple on the oldest expanded leaves (Plate 4.2). Other studies with *Brassica* sp. (*B. rapa*) have observed similar damage to that described here on the oldest leaves as a result of exposure to ozone (80 nl l^{-1} 7 h seasonal mean; Heagle *et al*, 1985).

When sugarbeet was exposed to ozone approximately 25 d after sowing, shoot dry weights were decreased 7 and 14 d after the end of exposure (Figure 4.3). However, when plants were exposed to ozone 17 d after sowing, there were no significant effects on shoot dry weight in Saxon (Figures 4.4, 4.5 and 4.6). In Celt, shoot dry weight was reduced in one set of experiments, but this was not consistent with other experiments (Figures 4.4, 4.5 and 4.6). These results may indicate a leaf age effect on ozone damage, since the leaves exposed at 25 d would be expanded to a greater amount. This effect, where the older the leaves at exposure, the more susceptible the plant to alterations in physiological and biochemical processes, has been observed in previous studies for some species, such as wheat (Nie *et al*, 1993; Bender *et al*, 1994) and oat (Myhre *et al*, 1988). However, not all species become more susceptible as the leaves age. For example, *Plantago major* (Reiling & Davison, 1994) and soybean (Reich *et al*, 1986) are equally susceptible at all leaf ages. Further work would need to be conducted to confirm a leaf age effect of ozone sensitivity

in sugarbeet.

Previous studies on garden beet (*Beta vulgaris*) have shown that injury symptoms consisted of a fine stipple which became necrotic toward the end of a 5 week exposure period to 200 nl l^{-1} (1-3 h d^{-1}; Ogata & Maas, 1973). This type of damage was observed in the present study in response to a much lower concentration applied over a shorter time period. Quantitative analysis of long-term exposure of garden beet to ozone (>1 h d^{-1}) resulted in a reduction of 50 % in the shoot dry weight, which was much greater than those observed in the present study. If plants become more susceptible as the tissue ages, then long-term exposure would be expected to reduce shoot weights to a greater extent.

The interactions observed in the present study were varied. Antagonistic interactions in shoot dry weight occurred between clopyralid and ozone in OSR cv. Galaxy, and ozone and phenmedipham in sugarbeet cvs. Saxon and Celt. A transient synergistic interaction was also observed in Saxon 7 d after treatment with phenmedipham followed by exposure to ozone. The remaining experiments all revealed additive interactions between the herbicides and ozone pollution.

Previous studies on interactions between ozone and herbicides have been conducted on crops grown mostly in the USA and Canada. There has been no previous work on interactions in shoot dry weight on oilseed rape. When treated with clopyralid and ozone, oilseed rape gave an indication of cv. differences which have been observed in other species in response to combinations of different herbicides and ozone (e.g. tobacco; Carney *et al*, 1973). Starlight responded in an additive manner to clopyralid followed by ozone, whilst Galaxy responded antagonistically to the same treatments.

Clopyralid and ozone may both lead to the production of ethylene within the plant (Mehlhorn & Wellburn, 1987; Thompson & Cobb, 1986). Ethylene production has been shown to be increased after treatment with clopyralid only in susceptible species, such as *Matricaria perforata* Merat. (Thompson, 1989). Similarly, Ozone is also thought to induce the production of stress ethylene in susceptible plants as a consequence of the formation of active oxygen species (Elstner *et al*, 1985). In peas (*Pisum sativum* L.), a 7 h exposure to 100 nl l^{-1} ozone, resulted in a doubling of the amount of ethylene produced and severe necrosis of the leaves (Mehlhorn & Wellburn, 1987). A study of ozone sensitive and ozone tolerant clones/cvs./populations of 6 different species found that all the sensitive clones/cvs. /populations produced more ethylene when exposed to ozone, whilst those tolerant to ozone reduced or kept emissions of ethylene at the same level (Wellburn & Wellburn, 1996). Plants exposed to two or more treatments may or may not

produce greater amounts of ethylene. For example, *Avena sativa* L. exposed to 294 nl l^{-1} ozone for 3 h either prior to or following a 1 h treatment of simulated acid rain (pH 2.8-5.6) did not show any increase in ethylene production over that observed when plants were only exposed to ozone (Pell & Puente, 1986). Whilst, Mehlhorn & Wellburn (1987) noted that exposures to nitric oxide or nitrogen dioxide (150 nl l^{-1}) increased the amount of ethylene production in peas and predisposed the plants to ozone injury.

Future work might include a study of the production of ethylene from oilseed rape cvs. treated with clopyralid and/or ozone, to test the hypothesis that after treatment with clopyralid, plants are more susceptible to ozone episodes due to alterations in the rate of ethylene formation.

In the present study, application of ozone followed by phenmedipham resulted in antagonistic effects on the growth of two cvs. of sugarbeet (Figure 4.4). Previous studies on the interactions between herbicides that inhibit photosynthesis and ozone have similarly indicated antagonism, although these interactions were dependent on the concentration of ozone and species used (Phatak & Proctor, 1976; Mersie *et al*, 1990). In a study of the interactions between ozone and the photosynthetic inhibitor metribuzin on tomato, the nature of the interaction depended on the cv., exposure period (1 or 3 h), ozone concentration (75, 150 or 300 nl l^{-1}), metribuzin rate (0.28 or 0.56 kg AI ha^{-1}) and light intensity prior to treatment (Phatak & Proctor, 1976). Four cvs. showed synergistic interactions after 1 h exposure to ozone, whilst high PPFD before ozone exposure (75 nl l^{-1}) and application of metribuzin (0.56 kg AI ha^{-1}) resulted in antagonistic effects. In another study, conducted on maize (*Zea mays* L.), the interaction between atrazine and ozone produced varying results dependent on the concentration of ozone used (Mersie *et al*, 1990). Soil treatment with atrazine (2.5 or 3.5 kg AI ha^{-1}) prior to exposure to 200 nl l^{-1} ozone resulting in additive effects in seedling maize. However, when the ozone concentration was increased to 300 nl l^{-1}, the response was antagonistic for dry weight only. This would indicate that experiments need to be clearly defined and easily reproducible to give a reliable account of the interaction which occurs for a particular set of conditions.

The present study suggested that the relative timing of the treatments is also important in determining the direction of the interaction between ozone and phenmedipham. This has also been observed in previous studies (Hatzios & Yang, 1983). For example, treatment of velvetleaf (*Abutilon theophrasti* Medic.) with chlorsulfuron (0.06 or 0.12 kg AI ha^{-1}) prior to exposure to ozone (200 nl l^{-1}) resulted in an antagonistic

response (Hatzios & Yang, 1983). However, when the treatments were reversed, the treatments were additive. Alterations in the interactions in velvetleaf were also shown when the herbicide was PP009 (fluazifop-butyl). When treated with herbicide first the interaction was additive, whilst exposure to ozone prior to application of fluazifop-butyl resulted in a synergistic interaction. The relative timing of the two treatments determined which treatment exerted an effect on the other. For example, in the above interaction in velvetleaf between ozone and fluazifop-butyl, ozone may alter the hydrolysis of fluazifop-butyl to its active form, which would not occur in the normally tolerant velvetleaf. This may render the plant sensitive to the herbicide, resulting in a synergistic response. Further explanations for these interactions may include the fact that ozone alters the metabolism of the herbicide, as observed in the interaction between ozone and diphenamid in tomato and pepper (Hodgson *et al*, 1973; Hodgson & Hoffer, 1977).

4.5 CONCLUSIONS

Shoot dry weight of oilseed rape was not affected by any of the herbicides and only diclofop-methyl induced visible injury symptoms. Damage caused by diclofop-methyl was in the form of round chlorotic areas, indicative of contact injury. Ozone did not have any consistent effect on either cv. of oilseed rape. Similarly, barley was not affected by exposure to ozone. Sugarbeet was only affected when the plants were older (25 d after sowing) at the time of exposure. When the plants were younger (17 d after sowing), neither cv. was consistently affected by exposure to ozone.

The interactions observed in the present study were varied. Antagonistic interactions in shoot dry weight occurred between clopyralid and ozone in OSR cv. Galaxy, and ozone and phenmedipham in sugarbeet cvs. Saxon and Celt. In sugarbeet cv Saxon the treatment with phenmedipham followed by ozone resulted in a synergistic interaction 7 d after the end of exposure and an antagonistic interaction 14 d after treatment. The remaining experiments all revealed additive interactions between the herbicides and ozone pollution.

Difficulties encountered with the use of spring barley, namely in the species susceptibility to powdery mildew, caused the crop to be disregarded from further study. Similarly problems arose with spring oilseed rape, due to the lower leaves being particularly brittle and easily snapped.

CHAPTER 5 - A PHYSIOLOGICAL STUDY OF THE INTERACTION BETWEEN OZONE AND PHENMEDIPHAM IN SUGARBEET

5.1 INTRODUCTION

Phenmedipham is known to interfere with photosynthesis in both sensitive and tolerant species, through the binding of the herbicide to the D_1 protein in photosystem II (Cobb, 1992). Photosynthetic parameters of sensitive plants are also affected by ozone (Balaguer *et al*, 1995; Salam & Soja, 1995). The RuBisCO content of the leaf seems to be directly affected by ozone-induced premature senescence, since no effect has been shown on RuBisCO synthesis (Nie *et al*, 1993). Stomatal conductance has also been reported to both increase and decrease due to ozone (Guzy & Heath, 1993), although it is believed that stomatal closure may result from increases in internal carbon dioxide concentration due to reduced photosynthesis (Reiling & Davison, 1994).

Ozone is also known to increase the permeability of membranes in susceptible plants, measured by ^{86}Rb fluxes (Evans & Ting, 1973). When ozone enters the leaf *via* the stomata, it is thought to dissolve rapidly in the apoplast and be converted into active oxygen species such as superoxide, hydroxyl radicals and H_2O_2 at the plasma membrane (Heath, 1994b). The formation of such species prior to symptom appearance has been demonstrated in *Pisum sativum* L. and *Phaseolus vulgaris* L. using electron spin resonance (Mehlhorn *et al*, 1990). The changes observed in leakage are thought to be due to disruptions in the plasma membrane (Heath & Castillo, 1988) and the inhibition of pumps and transporters (Dominy & Heath, 1985). Herbicides that inhibit photosynthesis such as phenmedipham, can also affect membrane leakage through the production of active oxygen species (Halliwell, 1991). This occurs by inhibition of the light reactions of photosynthesis due to the binding of a herbicide to the D_1 protein in photosystem II. The resulting excess excitation energy is eventually transferred to oxygen resulting in the generation of singlet oxygen and other toxic species.

The aim of this study was to determine the effect of exposing sugarbeet cv. Saxon to ozone followed 3 d later by phenmedipham treatment, on physiological processes including photosynthetic parameters, membrane leakage and loss of ions from cells.

5.2 MATERIALS AND METHODS

5.2.1 Growth and Treatment of Plants

Sugarbeet (*Beta vulgaris* cv. Saxon) was sown in 7 cm diameter pots as described in Chapter 2, section 2.1. The pots were initially maintained in the glasshouse at 22°C and 14h daylength (natural light supplemented with sodium halide lamps during the winter). At the young seedling stage (10 d after sowing) the plants were thinned to two per pot and the pots transferred to a growth cabinet (Fitotron, Sanyo) at 21°C day, 10°C night, 50% relative humidity and 200 μmol photons m^{-2} s^{-1} PAR, 14 h daylength. Plants were treated 21 d after sowing according to procedure (ii) in Chapter 2 section 2.4, i.e. 100 nl l^{-1} ozone for 2 d, followed 3 d later by phenmedipham at 1.14 kg AI ha^{-1}. The 7 h mean ozone concentrations and climatic conditions during the exposure period are presented in Table 2.1 (Chapter 2). Plants were returned to the growth cabinet after exposure to ozone or filtered air, and after the application of phenmedipham.

5.2.2 Photosynthesis

An Infra-Red Gas Analyser (IRGA; LCA4, Analytical Development Company, Hoddeston, UK) was used to measure carbon dioxide and water exchange by a leaf enclosed within a portable leaf chamber.

An open system was utilised in which ambient air (357 ppm CO_2) was passed through the chamber on a continuous basis. Air was taken from a nearby sample point outdoors, at a height of 4-5 m above ground level, to reduce the influence of the operator. The IRGA was set up to record various parameters, including reference and sample carbon dioxide concentration (μmol mol^{-1}), photosynthetically active radiation incident on the chamber (μmol photons m^{-2} s^{-1}), chamber, air and leaf surface temperature (°C), mass flow of air per unit of leaf area (mol m^{-2} s^{-1}) and reference and sample water vapour concentration to and from the chamber (mmol mol^{-1}), 3 min after the leaf was placed in the leaf chamber. From these parameters, photosynthetic rate (μmol CO_2 m^{-2} s^{-1}) stomatal conductance (mol H_2O m^{-2} s^{-1}) and transpiration rate (mmol H_2O m^{-2} s^{-1}) were calculated and recorded.

Readings were taken at approximately the same time each day (around 1500 h) within the growth cabinet, except those readings taken before and after exposure to ozone (days -4 and -3 respectively) which were taken at 0900 h and 1630 h respectively within

the exposure chambers. Two readings were obtained from the oldest two leaves of each plant, using 8 plants per treatment. Plants were handled carefully to reduce the amount of mechanical damage sustained in the duration of the experiment.

5.2.3 Chlorophyll Determination

A modified method (based on Arnon, 1949) was used to assay for the photosynthetic pigments within the oldest two leaves, 7 d after treatment with the herbicide. Leaf tissue was weighed and ground to a powder in liquid nitrogen. Two cm^3 of 80% aq. (v/v) acetone and a small amount of magnesium carbonate (0.1 g) were added to the tissue after it was placed in a polythene vial. These vials were sealed and placed on a tray, covered with a black plastic bag to prevent photo-oxidation and stored at 4°C for 2-3 d. The samples (leaf powder and 80% acetone) were ground in a pestle and mortar with a small amount of acid washed sand and the resulting slurry centrifuged (MSE Chilspin, Fisons, Loughborough, UK) at 3000 g for 10 min at 4°C. The supernatant was decanted, and the pellet re-extracted with 2 cm^3 of acetone as necessary, until the pellet was colourless. After pooling and mixing the supernatants for each sample and subsequent re-extractions, the absorbance was read at wavelengths of 710 (turbidity), 663, 645 and 470 nm using a spectrophotometer (S505, Perkin Elmer, Beaconsfield, UK). Calculations of chlorophyll content were based on the equations for 80% acetone of Lichtenhaler & Wellburn (1983).

5.2.4 Electrolyte Leakage

At daily intervals after exposure to ozone and treatment with phenmedipham, samples of tissue were taken for analysis of electrolyte leakage. Strips of similar size (approximately 3 cm^2) were cut from the first and second leaves, avoiding the major veins and edges of the leaves. These were placed in deionised water for 1 h to remove any debris from the cut surfaces. The water was then decanted and the tissue was carefully dried and weighed. Twenty cm^3 of deionised water was added to the tissue to give 0.07-0.15 g tissue cm^{-3} solution. The flask containing the tissue was placed in an illuminated (180 μmole m^{-2} s^{-1}), shaking waterbath at 20°C. Readings were taken 24 h after the final deionised water had been added to the strips. A flow-through electrode (glass flow cell, K = 1, Labtech Instruments, Wrexham, UK) was connected to a digital conductivity meter (PTI-18, F.S.A. Laboratory Supplies, Loughborough, UK) calibrated with 2.5 mol m^{-3} potassium chloride. The solution surrounding the tissue was pumped into the electrode

using a peristaltic pump (flow rate 10 cm^3 min^{-1}; P-1, Pharmacia) for approximately 4 min until a steady reading was obtained. Deionised water was used to rinse the electrode between readings. The total electrolyte content of the tissue was attained following repeated (3 times) freezing the tissue in liquid nitrogen and allowing it to defrost. Final readings were taken 24 h after defrosting. Results were expressed as $_{\mu}$Siemens cm^{-1} g^{-1} tissue. Samples (1.5 cm^3) were stored at -20°C for later analysis of the ion content of the electrolyte.

5.2.5 Ion Chromatography

An ion chromatograph (DX-100, Dionex, Camberley, UK) with an autosampler (Dionex), controlled using AI-450 software, was used to determine the ionic composition of the leachate. Samples (0.5 cm^3) were injected through an Ionpac CG12 guard column (Dionex) onto an Ionpac CS12 ion exchange column (Dionex) using 20 mol m^{-3} methane sulphonic acid as the eluent, over a total running time of 10 min, with a flow rate of 1.5 cm^3 min^{-1}. Cation concentrations were calculated using the standard curves generated by injecting 0, 2, 5, 10, 25 and 50 ppm of each ion onto the column (Appendix 3, Figure 1). Cations analysed were lithium, sodium, potassium, ammonium, magnesium and calcium. Anion contents were determined by injecting the sample of leachate through an AG12a guard column (Dionex) onto an AS12 ion exchange column (Dionex) using carbonate bicarbonate (2.7 mol m^{-3} disodium carbonate and 0.3 mol m^{-3} sodium bicarbonate) as the eluent and 25 mol m^{-3} sulphuric acid as the regenerant. Anion concentrations were calculated using the standard curves generated by injecting 0, 2, 5, 10, 25 and 50 ppm of each ion onto the column (Appendix 3, Figure 2). The anions analysed were fluoride, chloride, nitrate, phosphate and sulphate.

Linear regression was performed on the results from the standard curves, with zero intercepts stated to produce the equation:

peak area = m *x* ion concentration

where, m = x-coefficient

From the x-coefficient, results from samples were calculated by:

$$\frac{\text{peak area}}{\text{x- coefficient}} = \text{concentration (ppm)}$$

5.2.6 Electron Microscopy

5.2.6.1 Chemicals

Disodium hydrogen orthophosphate ($Na_2HPO_4.2H_2O$; BDH, Lutterworth, Leicestershire); absolute ethanol (Fisons); glutaraldehyde (Agar Scientific, Stansted, Essex); lead nitrate (BDH); osmium tetroxide (OsO_4; Agar Scientific); sodium citrate (BDH); sodium dihydrogen orthophosphate ($NaH_2PO_4.2H_2O$; BDH); Spurr's resin (Agar Scientific); uranyl acetate (Agar Scientific).

5.2.6.2 Reagents

100 mol m^{-3} and 50 mol m^{-3} sodium phosphate buffer (Soresens) Solutions of the same molarity of disodium hydrogen orthophosphate and sodium dihydrogen orthophosphate were mixed to obtain a buffer of pH 7.0.

3% (v/v) Soresens buffered glutaraldehyde (GDA): 100 mol m^{-3} phosphate buffer (10 cm^3); deionised water (7.6 cm^3) and 25% (v/v) GDA (2.4 cm^3).

2% (w/v) osmium tetroxide (OsO_4): OsO_4 (250 mg) and 50 mol m^{-3} phosphate buffer (12.5 cm^3). Prepared at least 24 h prior to use to ensure that it had completely dissolved in the buffer and was stored at 4°C in the dark.

Ethanol dehydration series: 25 %, 50 %, 75 %, 90% and 100% (v/v) absolute ethanol. Dilutions were made using deionised water.

Spurr's low viscosity resin (Spurr, 1969): Vinyl cyclohexane dioxide (ERL 4206; 10.0 cm^3); polypropylene glycol (Der 736; 6.0 cm^3); nonenyl succinic anhydride (NSA; 26.0 cm^3) and dimethylaminoethanol (S-1; 0.4 cm^3). 25 % (v/v) and 50 % (v/v) concentrations of Spurr's resin were made using absolute ethanol.

Uranyl acetate: A saturated solution was prepared in a 50:50 mixture of 70 % (aq) ethanol and deionised water and stored at 4°C.

Reynolds lead citrate: Lead nitrate (1.33 g); sodium citrate (1.76 g); deionised water (30.0 cm^3) and 1000 mol m^{-3} NaOH (8.0 cm^3). Lead nitrate and sodium citrate were shaken vigorously for 1 min then at intervals for 30 min. NaOH was added to give clear solution and made up to 50 cm^3 with deionised water and stored at 4°C.

5.2.6.3 Embedding

Three and 7 d after phenmedipham treatment, tissue samples were embedded for ultrastructural observation (Figure 5.1). Pasteur pipettes were used to prevent damage

1. Cut a 2-3 mm^2 section from each of 8 plants per treatment and place in 3 cm^3 50 mol m^{-3} sodium phosphate buffer.

↓

2. Remove buffer with a Pasteur pipette and add 1 cm^3 3 % (v/v) GDA and rotate for 2 h.

↓

3. Remove GDA and wash tissue with 50 mol m^{-3} phosphate buffer for 3 x 5 min.

↓

4. Replace buffer with 1 cm^3 osmium tetroxide and rotate for 1.5 h.

↓

5. Wash tissue with deionised water for 2 x 5 min.

↓

6. Dehydrate in an ethanol series:

   25 % (5 min)
   50 % (10 min)
   75 % (10 min)
   90 % (15 min)
   100 % (5 min)
   fresh 100 % (20 min)

↓

7. Remove ethanol and replace with 1 cm^3 25 % (v/v) Spurrs resin and rotate for 20 min.

↓

8. Replace with 1 cm^3 50 % (v/v) Spurrs resin and rotate overnight.

↓

9. Remove 50 % and add 100 % (v/v) Spurrs resin. Rotate for 3 h.

↓

10. Place section in embedding mould (Agar Scientific) and fill with fresh 100 % (v/v) Spurrs resin.

↓

11. Polymerise the resin at 70°C for 9 h.

Figure 5.1 Flow scheme of tissue preparation for electron microscopy (Benton, 1994).

occurring whilst transferring the tissue.

5.2.6.4 Tissue Sectioning and Staining

For electron microscopy, approximately 90 nm thick sections were cut using an ultra-microtome (OMu-2, Reichart, Austria). Chloroform was used to stretch the sections, which were mounted on 100 mesh carbon coated copper grids (Agar Scientific). To stain the sections for electron microscopy, droplets of uranyl acetate were placed onto parafilm in a petri dish and the grids inverted onto the stain. The grids were washed in distilled water after 30 min and then placed onto droplets of lead citrate for 30 min.

5.2.6.5 Ultrastructural Examination

Five blocks from each treatment were sectioned and examined using a transmission electron microscope (Joel 2010 TEM, Tokyo, Japan). Cell structure was observed with particular interest in the integrity of cellular membranes and chloroplasts. For each treatment, the number of starch grains per 100 chloroplasts in 5 blocks was counted. Photographs were taken on Kodak Electron Imagefilm (SO-163) and printed on Ilford Ilfospeed photographic paper.

5.2.7 Statistical Analysis

All experiments consisted of 4 treatments; namely control, ozone alone, phenmedipham alone, and ozone followed by phenmedipham. For each treatment 4 pots, each containing 2 plants, were used. Photosynthesis studies and membrane leakage studies were performed 4 times with 2 replicates in each experiment (n=8), whilst chlorophyll determinations were performed twice with 2 replicates in each experiment (n=4). Statistical analysis of all experiments was conducted using Duncan's Multiple Range Test, whenever an ANOVA had proved significant. Different letters on the tables indicate significant differences between treatments at the 5 % level.

5.3 RESULTS

5.3.1 Gas Exchange

Placing the control plants in the exposure chambers resulted in an increases in photosynthesis (day -3; Figure 5.2) and a decrease in stomatal conductance (Figure 5.3). However, the control plants recovered by day -1, 22 h after returning them to the glasshouse after exposure (day -2).

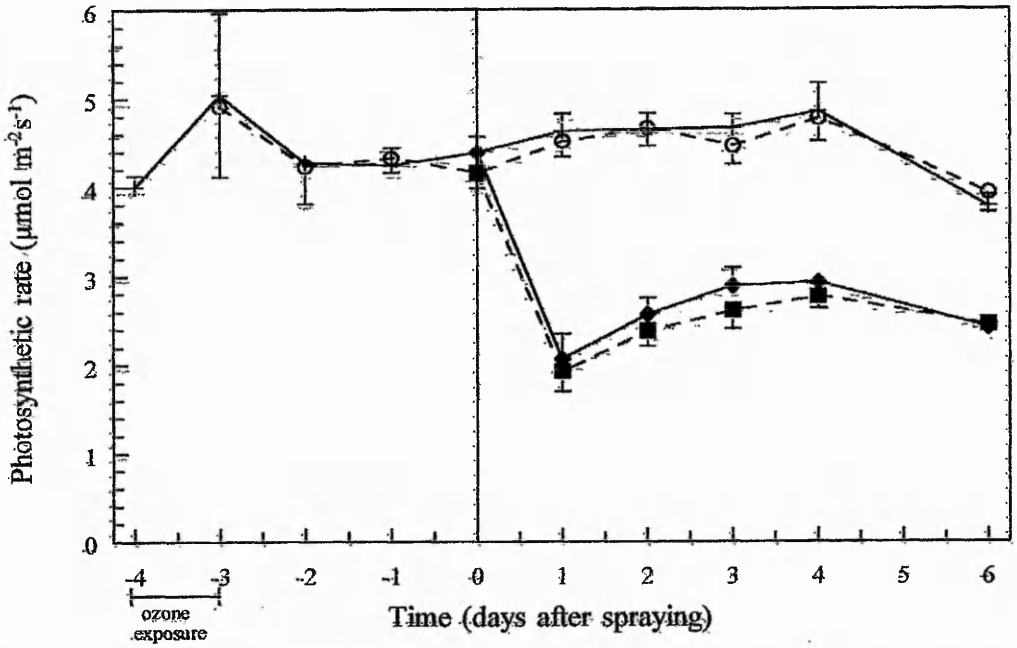


Figure 5.2 Effects of ozone and/or phenmedipham on photosynthetic rate of sugarbeet cv. Saxon. Values are means ± SE, where n = 4-10. Statistical analyses are presented in Appendices 3.1 and 3.2.
Key: control (+); ozone alone (O); phenmedipham alone (◆); ozone and phenmedipham (■)

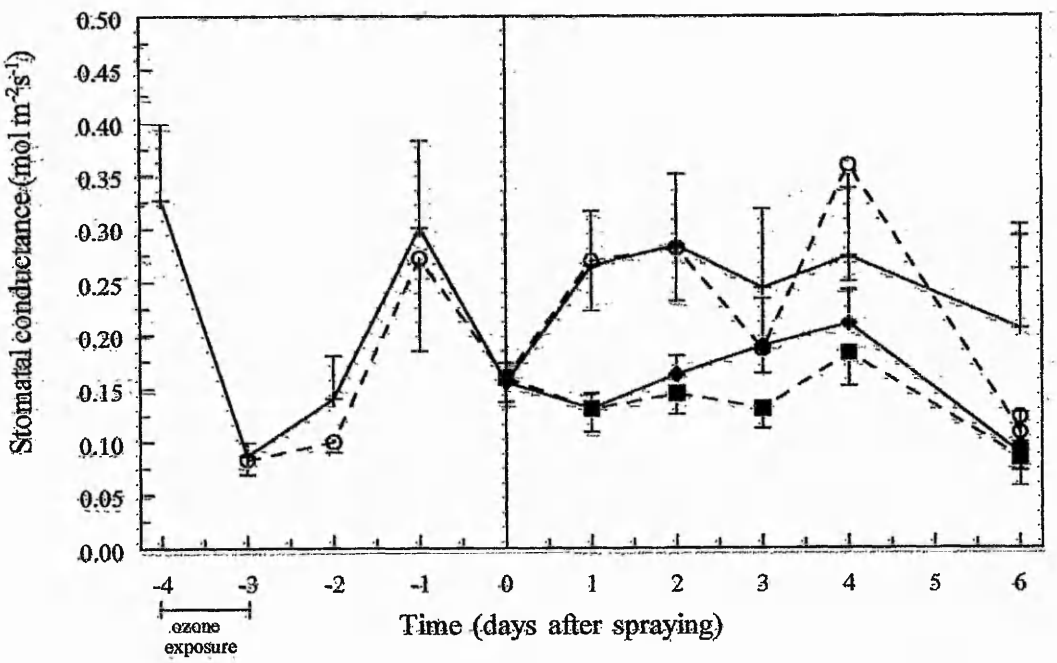


Figure 5.3 Effects of ozone and/or phenmedipham on stomatal conductance of sugarbeet cv. Saxon. Values are means ± SE, where n = 4-10. Statistical analyses are presented in Appendices 3.3 and 3.4.
Key: control (+); ozone alone (O); phenmedipham alone (◆); ozone and phenmedipham (■)

The rate of photosynthesis and stomatal conductance were unaffected by a short term ozone exposure (Figures 5.2 and 5.3; Appendices 3.1 and 3.3). However, plants treated with phenmedipham, or ozone followed by phenmedipham, showed a rapid and significant decrease (56 and 58 % respectively) in photosynthetic rate 1 d after spraying ($p<0.001$; Appendix 3.2). Subsequently a slight recovery was noted but rates had not returned to control values 6 d after spraying. Similarly, stomatal conductance was decreased by 49 and 49 % 1 d after spraying for plants treated with phenmedipham alone and ozone and phenmedipham, respectively (Figure 5.3; $p<0.001$; Appendix 3.4).

5.3.2 Pigment Determinations

In the previous Chapter it was noted that the leaves of plants treated with ozone developed chlorotic lesions 1-2 mm in length, 2 to 3 d after exposure (Plate 4.3), whilst phenmedipham characteristically induced round chlorotic spots of 5-10 mm diameter, 2 to 4 d after spraying (Plate 4.4). Both phenmedipham and ozone injury occurred only on leaves that were present at the time of treatment. To quantify this response, total chlorophyll *a* and *b* and total xanthrophyll and carotenoid contents of these leaves were determined. Total chlorophyll content was reduced by ozone 1 d after the end of exposure, although 10 d later (day 7) chlorophyll content had recovered to pre-exposure levels (Table 5.1). Pigment contents were significantly ($p<0.001$) reduced following treatment with phenmedipham (Appendix 3.5). When treated with both ozone and phenmedipham, chlorophyll contents were intermediate between those treated with either ozone alone and phenmedipham alone. These results were mirrored by the total xanthrophyll and carotenoid contents, with similar responses to all 3 treatments, although the interactions were not significant ($p>0.05$) for total chlorophyll or total xanthrophyll and carotenoids (Appendices 3.5 and 3.6).

5.3.3 Membrane Leakage

Plants treated with phenmedipham and ozone followed by phenmedipham showed an increase (277 % and 222 % respectively) in membrane leakage reaching a maximum 2 d after herbicide treatment (Figure 5.4; Appendix 3.7). Conversely, leakage was unaffected by exposure to ozone. Plants treated with ozone and phenmedipham had significantly ($p<0.05$) lower membrane leakage than those treated with phenmedipham alone 2 and 5 d after treatment (Appendix 3.7). The interactions were significant and were synergistic (Appendix 3.7).

Table 5.1 Effects of ozone and/or phenmedipham on the total chlorophyll and total carotenoid content on a fresh weight basis of sugarbeet cv. Saxon 1 d after the end of ozone exposure (d -2) and 7 d after herbicide treatment. Values are means, where n=4. Expected values are calculated as described in Chapter 2, section 2.5. Different letters indicate significant differences at the 5% level according to Duncan's Multiple Range Test.

	Total chlorophyll ($\mu g\ g^{-1}$)		Total carotenoids ($\mu g\ g^{-1}$)	
	day - 2	day 7	day -2	day 7
Control	697.2±86.4	771.9 a	201.9±23.1	113.2 a
Ozone	522.1±73.6	809.0 a	140.9±27.9	121.6 a
Phenmedipham	-	526.4 b	-	86.2 b
Ozone and phenmedipham	-	628.1 c	-	96.3 b

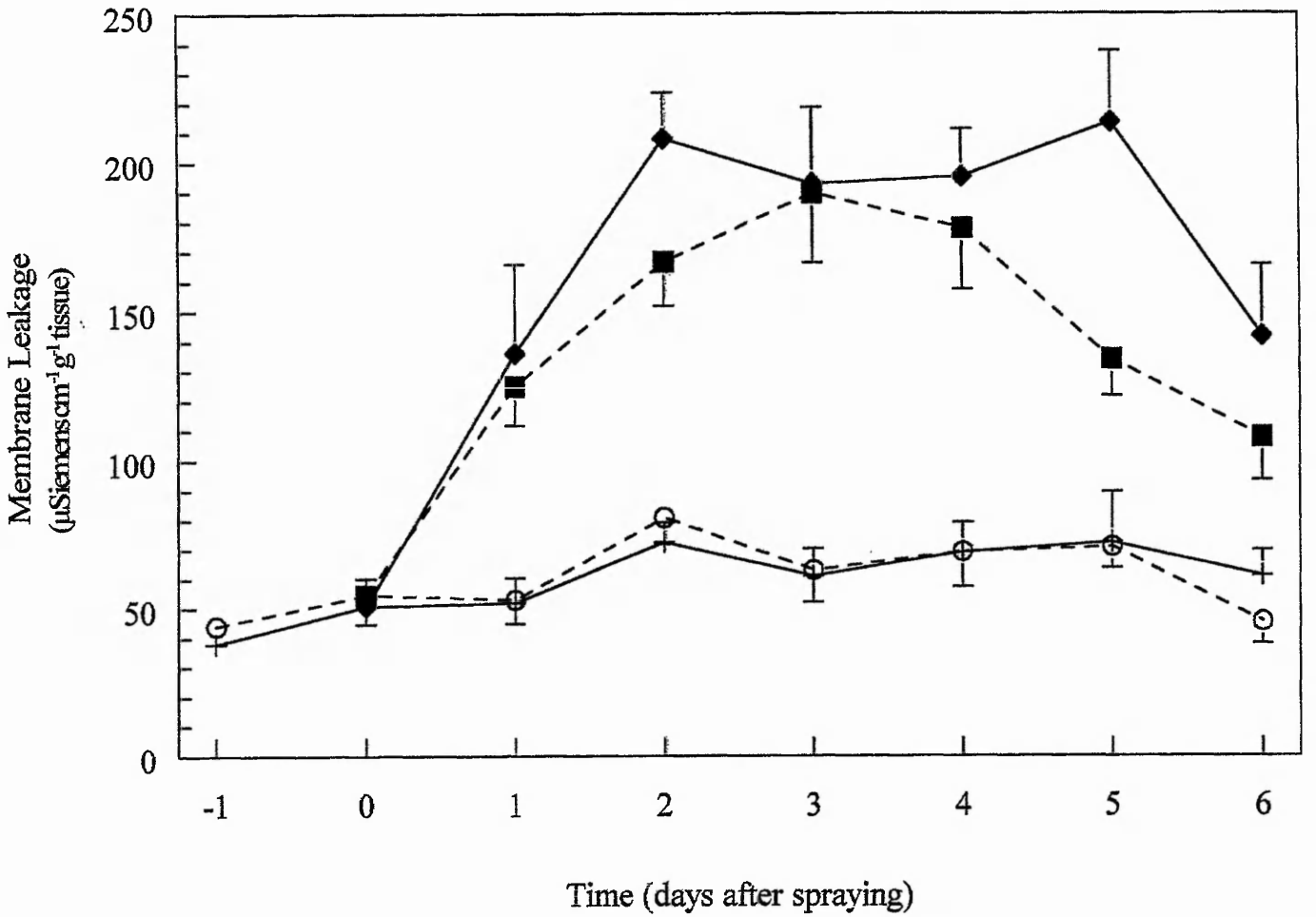


Figure 5.4 Effects of ozone and/or phenmedipham on the membrane leakage of sugarbeet cv. Saxon.
Values are means ± SE, where n = 6-12. statistical analyses are presented in Appendix 3.11.
Key: control (+); ozone alone (O); phenmedipham alone (◆); ozone and phenmedipham (■).

5.3.4 Ion Chromatography

Exposure to ozone had no effect on the leakage of the ions measured.

5.3.4.1 Cations:

Treatment with phenmedipham (alone and with ozone) increased the leakage of sodium (630 and 550 % of control, respectively; Figure 5.5; Appendix 3.8) and potassium (520 and 530 % of control, respectively; Figure 5.6; Appendix 3.9). Similar effects were observed with magnesium (525 and 430 % of control, respectively; Figure 5.7; Appendix 3.10). Measurement of ammonium content showed large variations, with no detectable ammonium in several replicates (Figure 5.8). Thus, it was felt that no analysis could be carried out on the data. Effects on calcium and lithium were not consistent nor significant.

5.3.4.2 Anions:

Leakage of fluoride and chloride ions was not affected significantly by any treatment (data not presented). Analysis of the nitrate content of the leachate showed large increases in response to treatment with phenmedipham alone and with ozone followed by phenmedipham (551 and 507 % of control, day 3, respectively; Figure 5.9; Appendix 3.11). Phosphate loss was increased by application of the herbicide (444 and 1013 % of control, day 3, respectively; Figure 5.10; Appendix 3.12).

5.3.5 Electron Microscopy

Ozone had no effect on the number of starch grains per chloroplast of sugarbeet 6 or 10 d after the end of exposure (3 and 7 d after herbicide treatment; Table 5.2; Appendix 3.13). Phenmedipham reduced the number of starch grains within the chloroplasts at both times. Exposure to ozone followed by application of phenmedipham resulted in a faster reduction in the number of starch grains than phenmedipham alone after 3 d, with some recovery after 7 d.

No ultrastructural effects of ozone were observed 6 or 10 d after the end of exposure (Plates not presented). When treated with phenmedipham, plants generally had more plastoglobuli within the chloroplasts and there was evidence of some damage to the tonoplast. However, there were no significant increases in thylakoid appression (Table 5.3). Effects of ozone followed by phenmedipham included an increase in the amount of thylakoid appression in all the sections examined, 3 and 7 d after herbicide treatment (Table 5.3; Appendix 3.14).

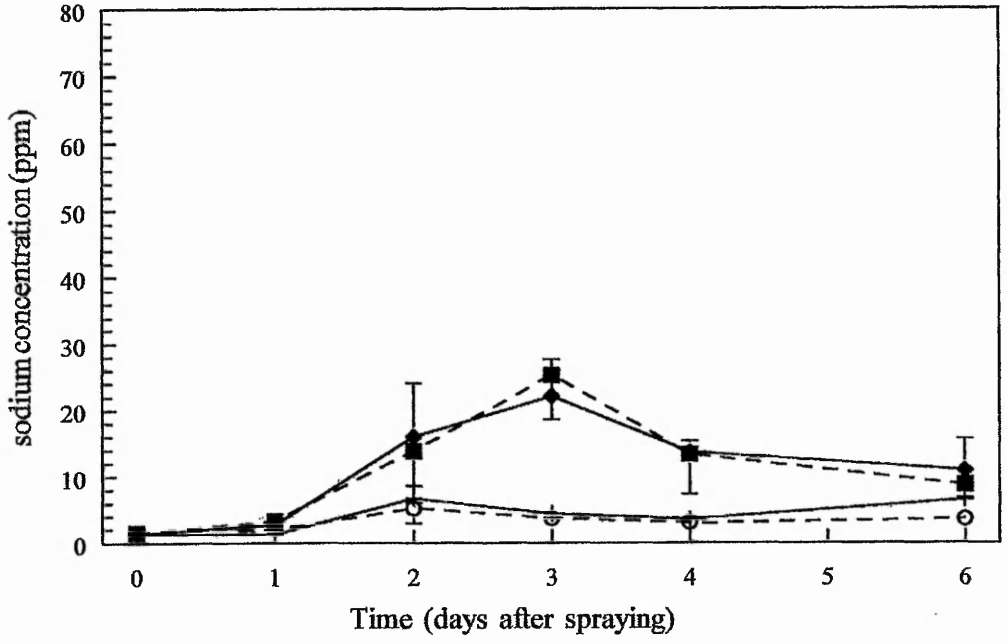


Figure 5.5 Effects of ozone and/or phenmedipham on the leakage of sodium ions from sugarbeet cv. Saxon. Values are means, where n=6 and bars represent SE.
Statistical analyses are presented in Appendix 3.12.
Key: control (+); ozone alone (O); phenmedipham alone (◆);
ozone and phenmedipham (■).

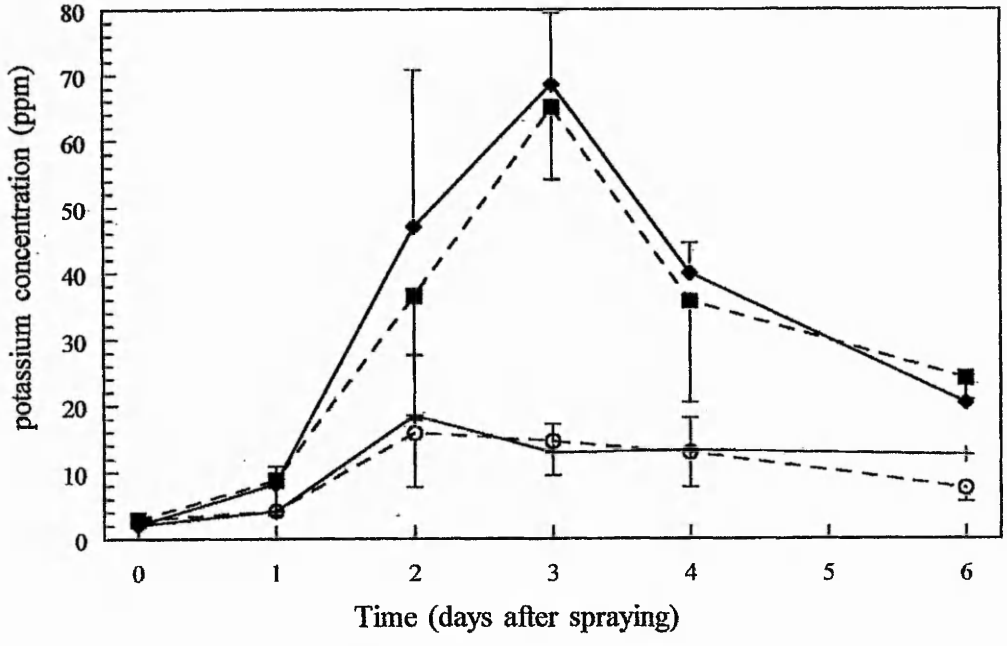


Figure 5.6 Effects of ozone and/or phenmedipham on the leakage of potassium ions from sugarbeet cv. Saxon. Values are means, where n=6 and bars represent SE.
Statistical analyses are presented in Appendix 3.13.
Key: control (+); ozone alone (O); phenmedipham alone (◆);
ozone and phenmedipham (■).

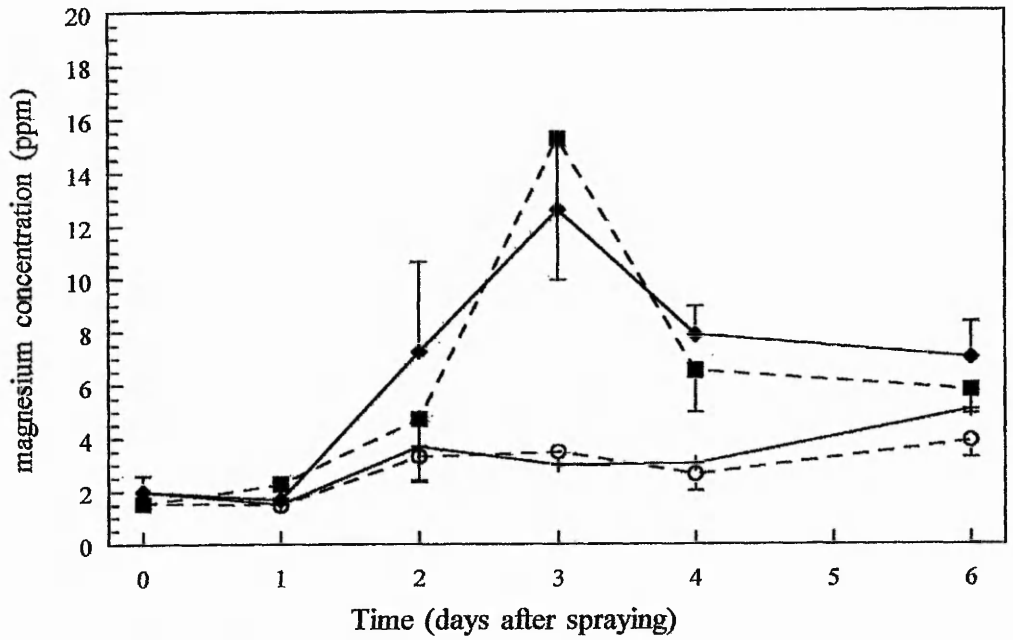


Figure 5.7 Effects of ozone and/or phenmedipham on the leakage of magnesium ions from sugarbeet cv. Saxon. Values are means, where n=6 and bars represent SE.
Statistical analyses are presented in Appendix 3.14.
Key: control (+); ozone alone (O); phenmedipham alone (◆);
ozone and phenmedipham (■).

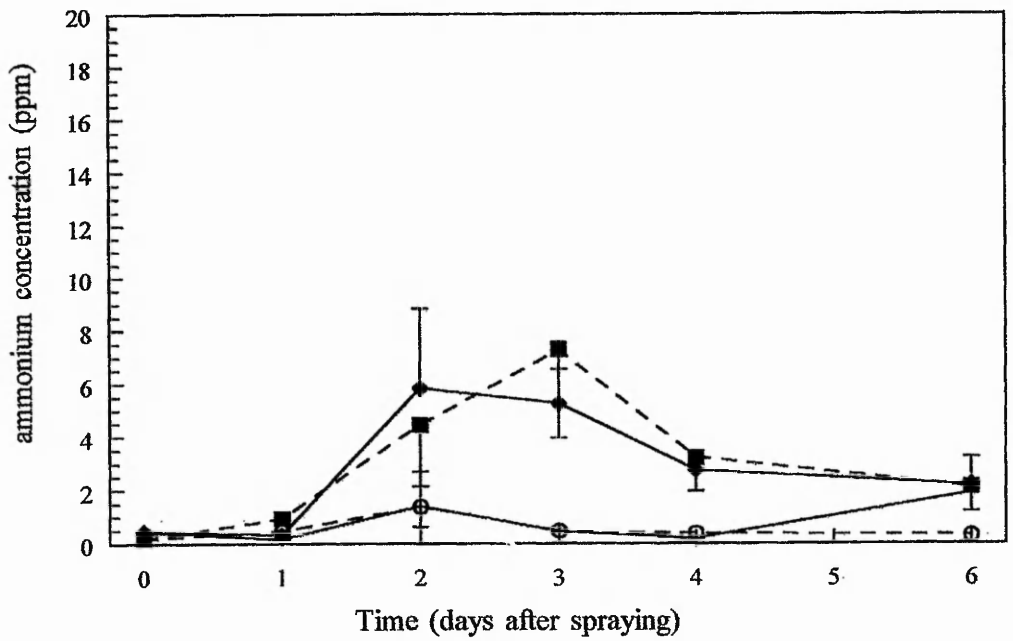


Figure 5.8 Effects of ozone and/or phenmedipham on the leakage of ammonium ions from sugarbeet cv. Saxon. Values are means, where n=6 and bars represent SE.
Key: control (+); ozone alone (O); phenmedipham alone (◆);
ozone and phenmedipham (■).

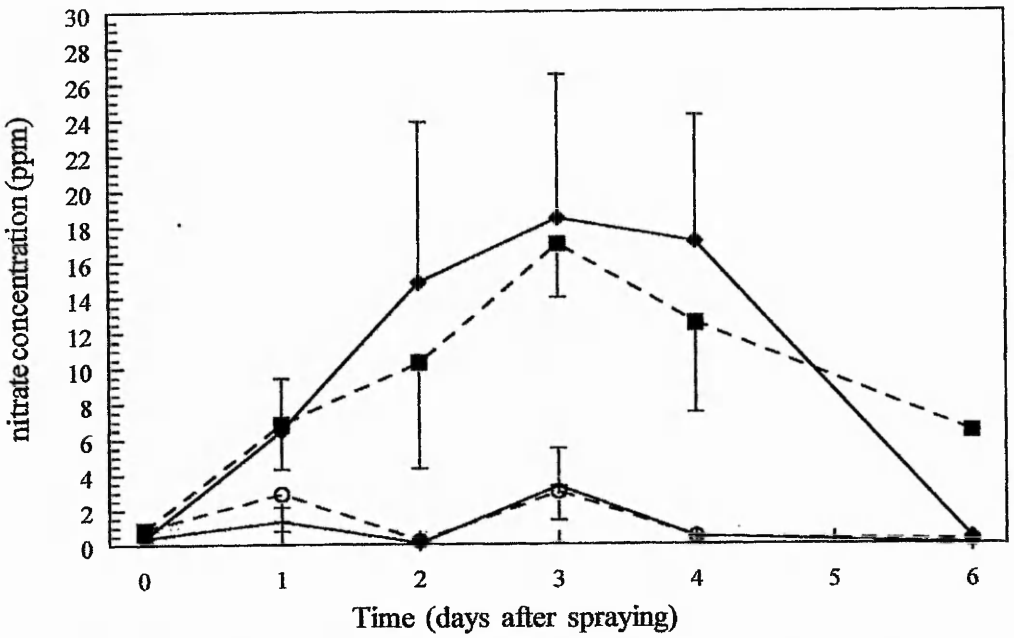


Figure 5.9 Effects of ozone and/or phenmedipham on the leakage of nitrate ions from sugarbeet cv. Saxon. Values are means, where n=6 and bars represent SE. Statistical analyses are presented in Appendix 3.15.

Key: control (+); ozone alone (O); phenmedipham alone (◆); ozone and phenmedipham (■).

Table 5.2 Effects of ozone and/or phenmedipham on the number of starch grains per 100 sugarbeet chloroplasts. Values are means ± s.e., where n = 5 (Appendix 3.15). Different letters indicate significant differences between the means at the 5% level according to Duncan's Multiple Range Test.

Days after Herbicide Treatment	Number of starch grains per 100 chloroplasts			
	Control	Ozone	Phenmedipham	Ozone and Phenmedipham
3	187.0 ± 13.4 a	176.8 ± 5.5 a	119.4 ± 8.3 b	60.0 ± 16.4 c
7	203.4 ± 27.2 a	190.2 ± 14.9 a	58.6 ± 8.2 c	118.4 ± 10.4 b

Table 5.3 Effects of ozone and/or phenmedipham on thylakoid appression in sugarbeet chloroplasts. Values are means number of thylakoids per granum ± s.e., where the number of granum per chloroplast = 10 and the number of chloroplasts examined = 5 (Appendix 3.16). Different letters indicate significant differences between the means at the 5% level according to Duncan's Multiple Range Test.

Days after Herbicide Treatment	Thylakoid appression Number of thylakoids per granum			
	Control	Ozone	Phenmedipham	Ozone and Phenmedipham
3	4.64 ± 0.10 ab	5.06 ± 0.20 bc	4.92 ± 0.21 ab	6.78 ± 0.18 d
7	5.18 ± 0.04 bc	4.32 ± 0.42 a	5.68 ± 0.13 c	7.40 ± 0.29 d

5.4 DISCUSSION

The aim of this study was to determine if there were interactive effects of ozone pollution and the herbicide phenmedipham on physiological parameters in sugarbeet cv. Saxon. This study initially focused on photosynthesis, as both ozone and phenmedipham have been reported to decrease CO_2 uptake in susceptible plants (Hendrick *et al*, 1974; Guzy & Heath, 1993).

In the present study, ozone had no significant effect on leaf photosynthetic rates, or stomatal conductance (Figures 5.2 and 5.3), although chlorophyll content was decreased immediately following ozone exposure (Table 5.1). Short-term studies on susceptible species tend to show reductions in photosynthetic rate prior to the appearance of visible symptoms of injury (Forberg *et al*, 1987; Myhre *et al*, 1988; Guzy & Heath, 1993). However, recovery may occur within a few hours of acute exposure to non-injurious concentrations (Miller, 1988). Reductions in photosynthesis of sugarbeet may have occurred during the course of exposure, however, measurements were not made until after the exposure, by which time the plants may have recovered. A study of alterations on photosynthesis during exposure would help elucidate these effects, but was not possible due to Health and Safety Regulations.

Previous studies have determined sensitivity to ozone by ozone-induced chlorophyll loss and/or inhibition of photosynthesis (Guzy & Heath, 1993). Observed losses of chlorophyll may be due to decreases in the amount of carotenoids protecting the chlorophyll from photo-oxidative damage (Demmig-Adams & Adams, 1996). Since sugarbeet only showed a transient reduction in chlorophyll content and no persistent effects on the rate of leaf photosynthesis, it can be concluded that sugarbeet is tolerant to the ozone concentrations used in this study. Effects on growth were transient when exposed 17 d after sowing and not consistent when 25 d old at exposure (Figures 4.3 & 4.4)

The primary site of phenmedipham damage is the chloroplast where it blocks photosynthetic electron transport (Cobb, 1992). This was observed in this study as a 50 % reduction in photosynthetic rate with incomplete recovery after 7 d (Figure 5.2). Similar effects on photosynthesis in response to 1 kg AI ha^{-1} phenmedipham have been shown in sugarbeet (55 % reduction) with greater reductions occurring at increasing temperatures (20-35°C; Arndt & Kotter, 1968). Inhibition rates were similar in sugarbeet and

susceptible species (Arndt & Kotter, 1968; Voss *et al*, 1984). However, photosynthesis in sugarbeet usually returned to control values within 10 d of the herbicide treatment (Voss *et al*, 1984; Prodoehl *et al*, 1992). No previous work has been published on the effects of phenmedipham on stomatal conductance. In this study, stomatal conductance of plants treated with phenmedipham decreased, with recovery occurring 4 d after herbicide treatment. This may be due to the herbicide decreasing the photosynthetic rate causing an increase in the sub-stomatal carbon dioxide concentration, which would result in the closure of the stomata.

Membrane leakage was unaffected by exposure to ozone. Conversely, plants treated with phenmedipham and ozone followed by phenmedipham showed an increase in membrane leakage reaching a maximum 2 d after herbicide treatment (Figure 5.4). Other studies have shown that in susceptible plants, potassium and ^{86}Rb (acting as a tracer for potassium) fluxes across membranes increase after treatment with ozone (Evans & Ting, 1973; Chimiklis & Heath, 1975; M°Kersie *et al*, 1982).

Large effects on the membrane leakage of sugarbeet resulted from treatment with phenmedipham. Increases in leakage occurred before chlorosis developed on the leaves, although contact injury as a result of spraying was evident a few hours after herbicide application. The blocking of electron transport by phenmedipham leads to a build up in excitation energy which on transfer to other molecules leads to the production of active species including singlet oxygen, hydrogen peroxide, superoxide and hydroxyl radicals. These can damage membranes through lipid peroxidation and oxidation of sulphydryl groups of proteins. Chloroplast membranes are probably the first to be affected due to their close proximity to the thylakoid membrane and the production of free radicals. In the present study, membrane leakage was first detected **x** hours after application of phenmedipham.

Total anion and cation pool sizes were not determined due to problems with the ion chromatograph resulting in the loss of a significant number of samples. Although exposure to ozone had no effect on the leakage of any of the ions analysed, treatment with phenmedipham (alone and with ozone) increased the concentrations of sodium (Figure 5.5), potassium (Figure 5.6), nitrate (Figure 5.9) and phosphate (Figure 5.10) in the leachate. Since these ions are primarily stored in the vacuole the large increases detected in the leachate suggests damage to the tonoplast membrane or the series of ports, carriers and channels which actively transport the ions across membranes. Other studies of the effects

on ion loss from ozone- or herbicide-treated plant tissue have shown increases in potassium fluxes (Harris & Dodge, 1972; Chimiklis & Heath, 1975; McKersie *et al*, 1982; Heath & Castillo, 1988), but have not studied fluxes of sodium, nitrate and phosphate.

Application of phenmedipham resulted in an increase in magnesium ion concentration in the leachate (Figure 5.7). Magnesium acts as a metal activator for most enzymes that use ATP or other nucleoside di- or tri-phosphate as a substrate. This cation is found mainly in the chloroplast and mitochondrion and would be expected to decrease in concentration in the chloroplast after treatment with phenmedipham, due to the primary effect of the herbicide on this organelle. The observed increase in magnesium concentration in the leachate of phenmedipham-treated plants is likely to have represented a loss of integrity of the chloroplast envelope.

Ammonium ions are produced in mitochondria during photorespiration by the conversion of two glycine molecules to one serine (Sarojini & Oliver, 1983). Since NH_4^+ is toxic, it is rapidly incorporated into glutamine with glutamate by glutamine synthetase (Givan, 1979). The observed increase in NH_4^+ concentration following treatment with phenmedipham may thus indicate either a breakdown in mitochondrial membranes or an alteration of the activity of the detoxifying enzymes of this cation. Phenmedipham may have also increased the production of ammonium ions through an increased rate of photorespiration. A previous study recorded an increase in the activity of glutamate synthase and reductions in the activities of glutamine synthetase and glutamate dehydrogenase in a sugarbeet suspension culture (Zelmer & Günther, 1988). Future work could study glutamate dehydrogenase, glutamine synthetase and glutamate synthase activities in whole plants.

Sugarbeet exposed to ozone followed by treatment with phenmedipham responded in a similar way to plants treated with the herbicide alone in most of the physiological studies conducted. However, increases in permeability were significantly less than those for phenmedipham treated plants 5 d after treatment, providing evidence of an interaction. Plants also showed an increase in thylakoid appression, a symptom associated with sub-lethal doses of herbicide, although this was not observed in tissue treated with phenmedipham alone.

5.5 CONCLUSION

Ozone had very little effect on the physiological parameters studied, confirming that sugarbeet is relatively tolerant to ozone at the seedling stage. Small effects were observed on chlorophyll content immediately after exposure, although no reduction in photosynthetic rate was seen. Similarly, ozone did not induce any alterations in the leakage of membranes or the loss of any electrolytes. In contrast, phenmedipham induced large reductions in the rate of photosynthesis, stomatal conductance, and chlorophyll content, whilst membrane leakage and the loss of various ions were increased.

Alterations in photosynthetic parameters as a result of exposure to ozone followed by application of phenmedipham did not differ from those seen in response to phenmedipham alone. Total chlorophyll and carotenoid contents of tissue treated with both ozone and phenmedipham were intermediate between contents in plants treated with ozone alone and phenmedipham alone. Studies of membrane leakage provided some indication of an antagonistic interaction at certain times.

CHAPTER 6 - A BIOCHEMICAL STUDY OF THE INTERACTION BETWEEN OZONE AND PHENMEDIPHAM IN SUGARBEET

6.1 INTRODUCTION

Additional active oxygen species may be produced in response to adverse environmental conditions, including the air pollutants sulphur dioxide, nitrogen dioxide and ozone (Grimes *et al*, 1983; Mehlhorn *et al*, 1987; Kanofsky & Sima, 1991); excess light (Critchley, 1988); chilling (Wise & Naylor, 1987); water deficit (Smirnoff, 1993) and herbicides (Halliwell, 1991) - especially inhibitors of photosystem II and carotenoid biosynthesis, and redox-active herbicides such as paraquat and diquat (Shaaltiel *et al*, 1988).

When ozone enters the leaf *via* the stomata it is thought to dissolve rapidly in extra-cellular water and be converted into active oxygen species, such as superoxide, hydroxyl radicals and H_2O_2 (Heath, 1994a). The formation of such species prior to symptom appearance has been demonstrated in *Pisum sativum* L. and *Phaseolus vulgaris* L. using electron spin resonance (Mehlhorn *et al*, 1990). Similarly, when the light reactions of photosynthesis are inhibited by the binding of a herbicide to the D_1 protein in photosystem II, the resulting excess excitation energy is eventually transferred to oxygen resulting in the generation of singlet oxygen and other toxic species. Although the production of active oxygen species has been implicated in the mechanisms of action of both ozone and the herbicide paraquat, studies on transgenic plants have shown no cross-tolerance to ozone in varieties tolerant to paraquat or vice versa (Shaaltiel *et al*, 1988;Wellburn *et al*, 1998). Regardless of the cause of formation of active oxygen species, the net effect is membrane damage through the oxidation of unsaturated fatty acids or specific enzyme sites (Halliwell & Gutteridge, 1989). Protein (enzyme and non-enzyme) and nucleic acid damage may also occur, resulting in impaired function and altered metabolism (Monk *et al*, 1989).

Plants contain several enzymatic and non-enzymatic protective systems to combat these potentially damaging oxygen species, including scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT) and non-specific peroxidases (GPOD). Damage is also prevented by the antioxidant actions of ascorbic acid (Vitamin C), reduced glutathione (GSH), ∝-tocopherol (Vitamin E) and carotenoids (Kangasjärvi *et*

al, 1994). A change in the activity of these protective systems could provide an explanation for the observed interactions between ozone and the photosynthetic inhibitor herbicide, phenmedipham.

The aims were to establish the activities of several antioxidant enzymes and compounds in sugarbeet; and to determine the time-course of changes in the antioxidant defence systems of plants treated with ozone alone, phenmedipham alone or ozone followed by phenmedipham.

6.2 MATERIALS AND METHODS

6.2.1 Growth and Treatment of Plants

Sugarbeet cv. Saxon was sown as described in Chapter 2, section 2.1. The pots were initially maintained in the glasshouse. At the young seedling stage (14 d after sowing) the plants were thinned to 2 per pot and transferred to a growth cabinet (Fitotron, Sanyo; Chapter 2, section 2.1). When the plants reached the 2-3 leaf stage (21 d after sowing) they were exposed to either 100 nl l^{-1} of ozone or filtered-air for 7h d^{-1} on 2 consecutive days in a closed system according to procedure (ii) in Chapter 2, section 2.4. The 7 h mean ozone concentration and climatic conditions during the exposure period are presented in Table 2.1. Three days after the end of exposure, the plants were treated with phenmedipham (1.14 kg AI ha^{-1}) or distilled water, as described in Chapter 2, section 2.3.

6.2.2 Chemicals

All chemicals were supplied by Sigma Chemicals, Poole, UK except where stated otherwise. K_2HPO_4 (dipotassium orthophosphate; BDH), K_2PO_4 (potassium dihydrogen orthophosphate; BDH), DTPA (diethylenetriamine penta acetic acid), PVPP (polyvinyl polypyrrolidine), ascorbate, NADH, NADPH, ascorbate oxidase, GSSG (oxidised glutathione), L-methionine, NBT (nitroblue tetrazolium), Triton-X-100, riboflavin, guaiacol, CDNB (1-chloro-2,4-dinitrobenzene).

6.2.3 Extraction Procedure

Approximately 1g of leaf tissue was frozen in liquid nitrogen and ground to a fine powder using a pestle and mortar. This powder was transferred with a spatula to a centrifuge tube (50 cm^3) containing potassium phosphate buffer pH 7.0 (4.25 cm^3; 100

mol m^{-3}), ascorbate (0.50 cm^3; 100 mol m^{-3}), DTPA (0.25 cm^3; 100 mol m^{-3}) and PVPP (0.2 g) according to Hull (1992). The mixture was homogenised (Ultra Turrax) at high speed for 20s and the homogenate centrifuged at 20000 x g for 10 min at 4°C. A 2.5 cm^3 aliquot of supernatant was desalted through a Sephadex G-25 PD-10 column (Pharmacia) and the remainder placed in a polythene tube, frozen in liquid nitrogen and stored at -20°C until required for total glutathione assays.

6.2.4 Assays

The following enzymes were assayed from the same extraction: ascorbate peroxidase (APX; EC 1.11.1.11); monodehydroascorbate reductase (MDHAR; EC 1.6.5.4); glutathione reductase (GR; EC 1.6.4.2); superoxide dismutase (SOD; EC 1.15.1.1); catalase (CAT; EC 1.11.1.6) and guaiacol peroxidase (GPOD; EC 1.11.1.7). Ascorbate peroxidase, monodehydroascorbate reductase and glutathione reductase were assayed immediately after extraction to prevent decay of activity (Hull, 1992). The remaining enzymes were assayed on extracts which had been stored at -20°C, after previous work had demonstrated no loss of activity after freezing (Hull, 1992). Assays were conducted in a reaction volume of 1 cm^3 apart from SOD (3 cm^3). In each case, an extract volume of 0.05cm^3 was used with the exception of SOD in which the extract volume used was variable depending on the activity of the enzyme.

6.2.4.1 Ascorbate Peroxidase

Ascorbate peroxidase was assayed according to the method of Nakano & Asada (1981) using a reaction mixture containing potassium phosphate buffer pH 7.0 (0.85 cm^3; 100 mol m^{-3}) with DTPA (0.2 mol m^{-3}), ascorbate (0.05 cm^3; 10 mol m^{-3}) and hydrogen peroxide (0.05 cm^3; 5 mol m^{-3}). The oxidation of ascorbate by hydrogen peroxide to monodehydroascorbate was followed by determining the change in absorbance at 290 nm (Figure 6.1). Background activity was checked prior to adding hydrogen peroxide.

6.2.4.2 Monodehydroascorbate Reductase

A modification of the method of Hossain *et al* (1984) was used to assay for monodehydroascorbate reductase. The oxidation of NADH, determined from the decrease in absorbance at 340 nm, was measured in a reaction mixture containing

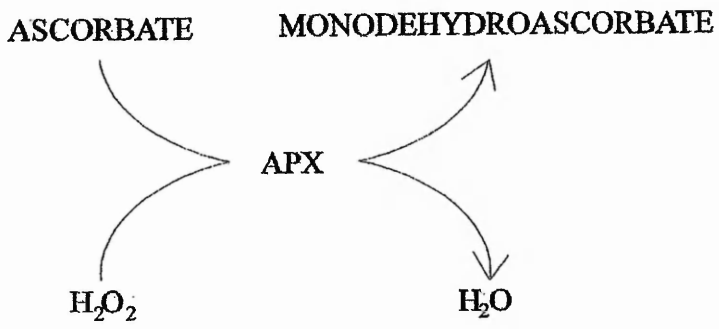


Figure 6.1 Assay for ascorbate peroxidase

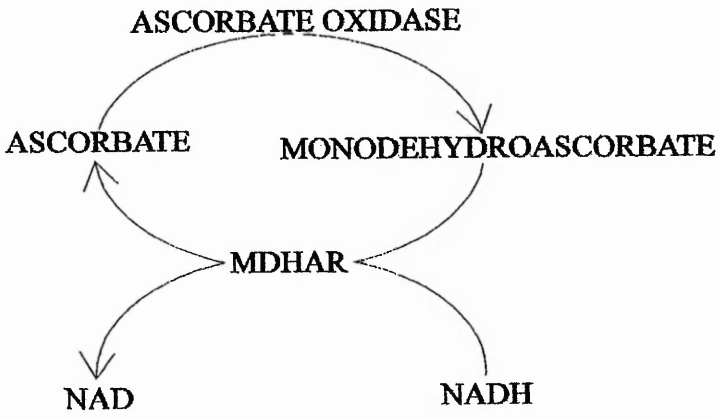


Figure 6.2 Assay for monodehydroascorbate reductase

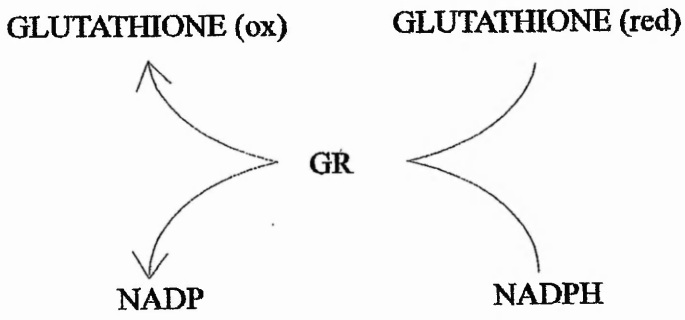


Figure 6.3 Assay for glutathione reductase

potassium phosphate buffer pH 7.8 (0.8 cm^3; 100 mol m^{-3}) with DTPA (0.2 mol m^{-3}), ascorbate (0.05 cm^3; 10 mol m^{-3}), NADH (0.05 cm^3; 3 mol m^{-3}) and ascorbate oxidase (0.05 cm^3; 0.2 units 50 cm^{-3}; Figure 6.2). For every set of samples analysed a check of the ascorbate oxidase activity was carried out. The reaction mixture was as before with the ascorbate oxidase replaced by 0.05 cm^3 potassium phosphate buffer. If a doubling of the rate of reaction was not observed then the ascorbate oxidase required changing.

6.2.4.3 Glutathione Reductase

Glutathione reductase was measured spectrophotometrically at 340 nm, using a reaction mixture containing potassium phosphate buffer pH 7.8 (0.8cm^3; 100 mol m^{-3}) with DTPA (0.2 mol m^{-3}), GSSG (0.05 cm^3; 10 mol m^{-3}), NADPH (0.05 cm^3; 3 mol m^{-3}; modified from Schaedle & Bassham, 1977). The reaction was based on the oxidation of NADPH (Figure 6.3). Background activity of other enzymes using NADPH was checked by replacing the GSSG with 0.05 cm^3 potassium phosphate buffer.

6.2.4.4 Superoxide Dismutase

Superoxide dismutase was assayed according to the competitive inhibition method of Beyer & Fridovich (1987; Figure 6.4). Solution 'A' consisted of potassium phosphate buffer pH 7.8 (16 cm^3; 50 mol m^{-3}) containing DTPA (0.2 mol m^{-3}), L-methionine (2 cm^3; 10 mol m^{-3}), NBT (1.4 cm^3; 57 mmol m^{-3}), Triton-X-100 (1 cm^3; 1%(v/v)) and riboflavin (0.4 cm^3; 1.13 mmol m^{-3}). The spectrophotometer was zeroed against a blank (0.5 cm^3 solution 'A' + 0.5 cm^3 phosphate buffer) at 560 nm. A sample volume of 0.5 cm^3 (x cm^3 sample + (0.5 - x) cm^3 buffer) was added to 0.5 cm^3 of solution 'A' and each sample was read in turn to give an initial reading. The cuvettes were placed in front of a 125W fluorescent light for 5 min to activate the riboflavin, which oxidises methionine producing a semiquinone. The riboflavin semiquinone reduces O_2 to $O_2^{\cdot-}$, which reduces the NBT to an insoluble purple formazan compound (Figure 6.5). After this time the absorbance was re-measured at 560 nm. The difference between the t = 0 reading and the t = 5 min reading was calculated and the percentage of the control was determined. The sample volume x was adjusted until the percentage of control was 34%. One unit of SOD is defined as the quantity which gives exactly one half of the maximum inhibition of the superoxide dependant reduction of NBT at the recommended pH of 7.8 (Beyer & Fridovich, 1987). As only 65-70% of the reduction of NBT is inhibited by high levels

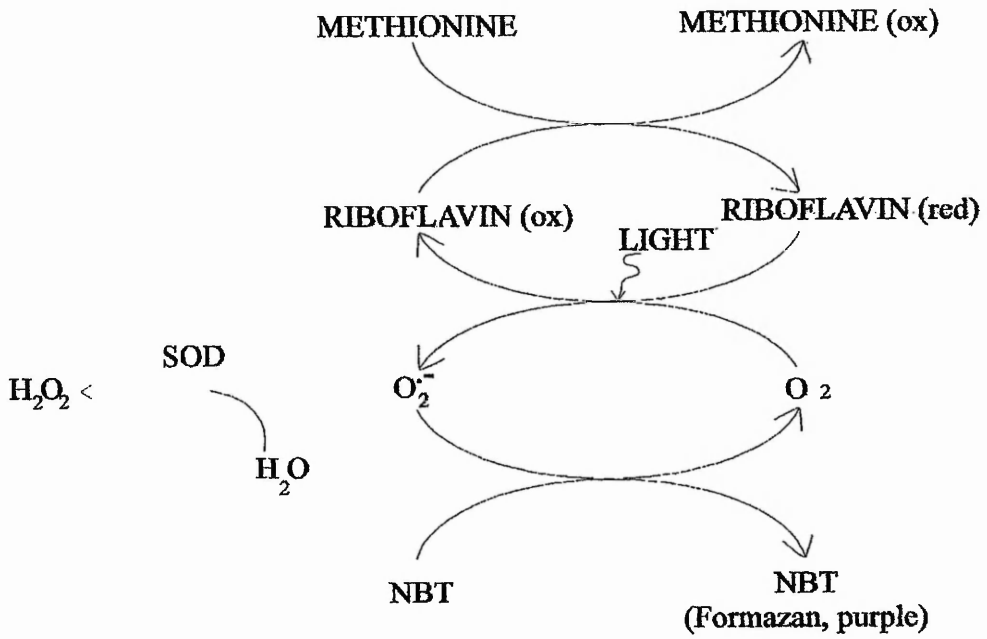


Figure 6.4 Assay of superoxide dismutase. A photochemical method is used to produce superoxide *in situ*. Riboflavin is activated by a photon of light from a fluorescent tube (gives an excess of blue light). In its excited state, riboflavin oxidises the electron donor methionine. Riboflavin is reduced to a semiquinone which reduces O_2 to $O_2^{\cdot-}$ which in turn reduces nitroblue tetrazolium to an insoluble purple formazan compound. SOD competes for $O_2^{\cdot-}$ inhibiting the production of formazan. Activity of SOD is negatively correlated to colour change (Beyer & Fridovich, 1987).

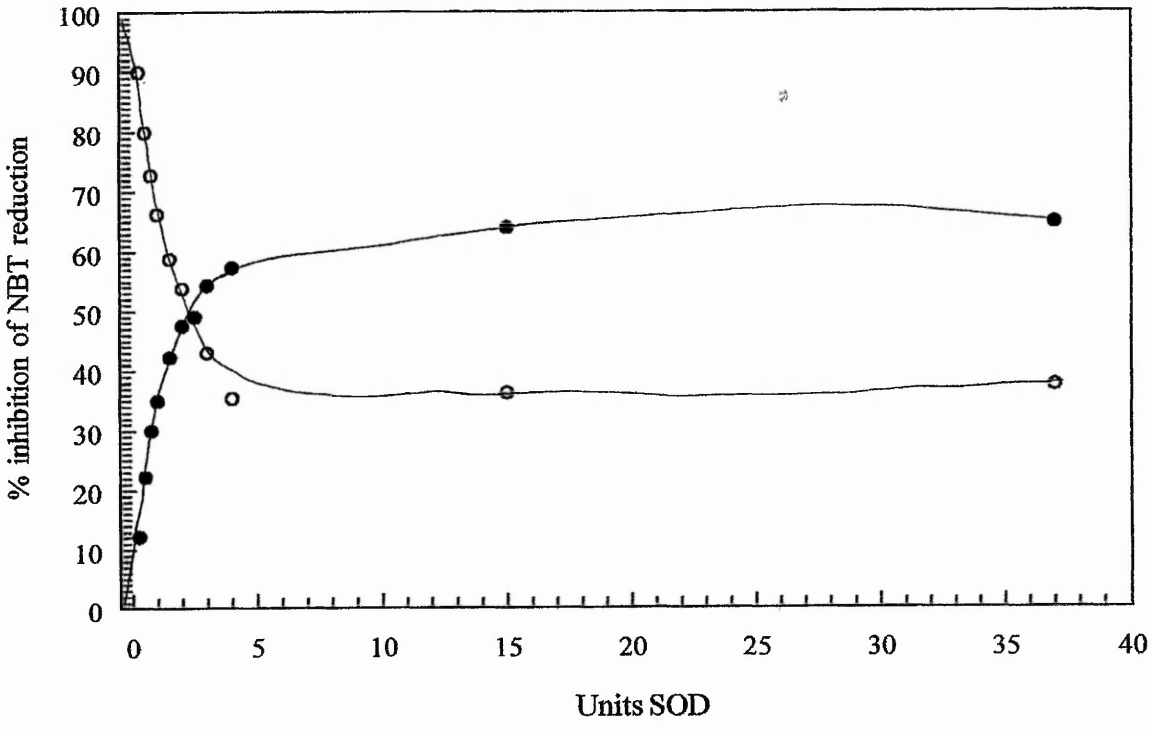


Figure 6.5 Calculation of SOD activity (Hull, 1992). Key: maximum SOD inhibition (●); $\%V_{max}$ = 100 - (% inhibition of NBT reduction rate) (O).

of SOD, 30-35% is therefore independent of SOD. Hence the volume of the sample required in the assay is dependant on the activity of the SOD within the sample (Figure 6.5).

6.2.4.5 Catalase

Catalase was assayed using potassium phosphate buffer at pH 7.0 (0.948 cm^3; 100 mol m^{-3}) and hydrogen peroxide (0.002 cm^3; 0.05% (v/v)). The assay was based on the decrease in absorbance at 240 nm due to the reduction of hydrogen peroxide by catalase. Catalase activity was calculated as ΔA_{240} g^{-1} fresh weight min^{-1}.

6.2.4.6 Guaiacol Peroxidase

Guaiacol peroxidase was assayed according to the method of Horsman & Wellburn (1975). This was based on the reduction of hydrogen peroxide and the oxidation of guaiacol, an artificial electron donor by guaiacol peroxidase. A reaction mixture in potassium phosphate buffer pH 6.0 (0.848 cm^3; 100 mol m^{-3}) containing DTPA (0.2 mol m^{-3}), hydrogen peroxide (0.002 cm^3, 0.05% (v/v)) and guaiacol (0.1 cm^3; 100 mol m^{-3}) was assayed for the change in absorbance at a wavelength of 470 nm and activity calculated.

6.2.4.7 Protein

The protein content of each sample was measured in triplicate using a modified Bradford (1976) method. All enzyme concentrations were expressed on a protein basis. A representative standard curve produced using BSA is shown in Figure 6.6.

6.2.4.8 Glutathione S-Transferase

Aliquots of the sample extraction were used to determine glutathione *S*-transferase (GST) activity using a modified method of Habig & Jakoby (1981). The reaction of CDNB in ethanol (0.1 cm^3; 10 mol m^{-3}) with potassium phosphate buffer pH 6.5 (0.75 cm^3; 100 mol m^{-3}) containing DTPA (0.2 mol m^{-3}) was monitored at 340 nm. Activity was calculated using the extinction coefficient of the conjugate. (9.6 mM^{-1} cm^{-1})

6.2.4.9 Total Glutathione

Tissue was extracted as detailed for antioxidant enzymes, with the exception that the supernatant was not passed through a desalting column. Total glutathione content

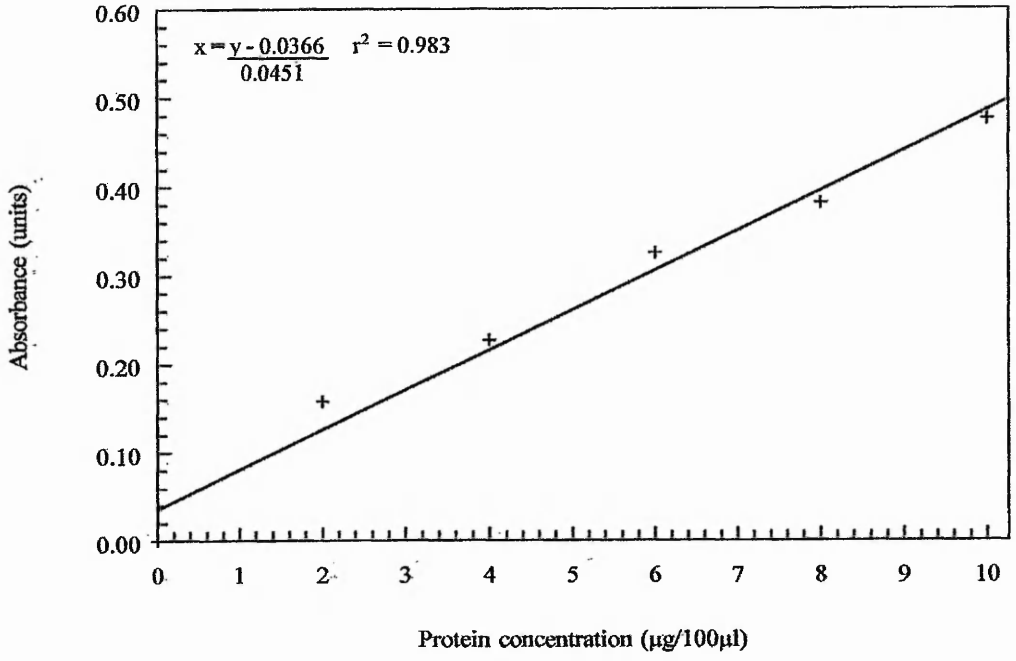


Figure 6.6 Protein standard curve using BSA. Values are means, where n=3.

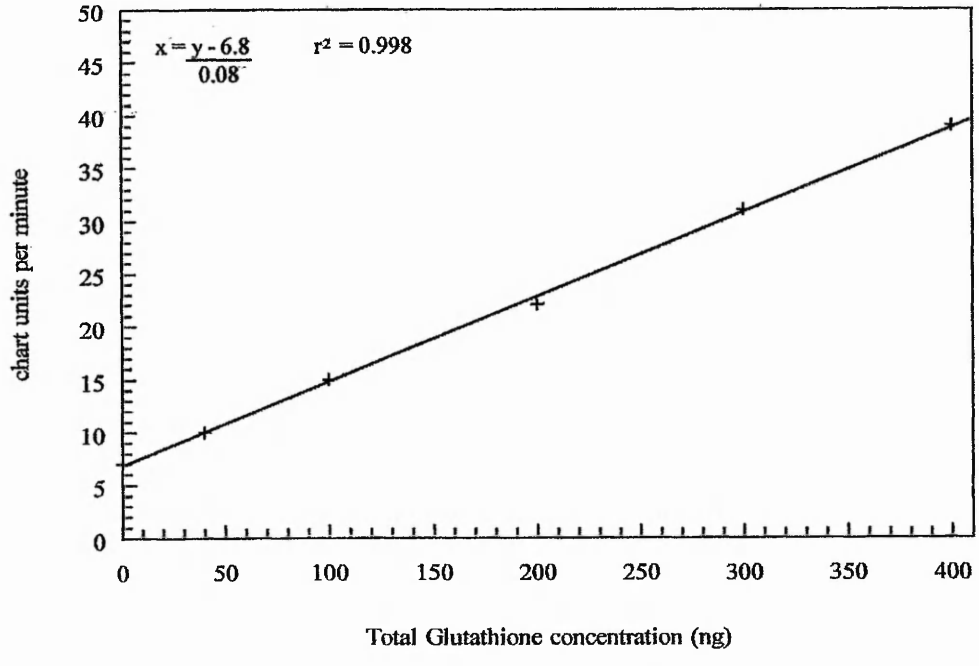


Figure 6.7 Total glutathione standard curve using GSH. Values are means, where n=3.

was assayed according to the method of Griffith (1980). An aliquot (1 cm^3) of extract was added to potassium phosphate buffer pH 7.5 (1.5 cm^3; 500 mol m^{-3}), mixed well and termed solution C. The reduction in absorbance measured at 412 nm in a reaction mixture containing potassium phosphate buffer pH 7.5 (0.5 cm^3; 500 mol m^{-3}) containing DTPA (5 mol m^{-3}), DTNB (0.2 cm^3; 6 mol m^{-3}), GR (1 unit), solution C (0.1 cm^3) and NADPH (0.1 cm^3; 2 mol m^{-3}) was used to determine the total glutathione content of the sample. A standard calibration curve was calculated using GSH to give a range from 0 to 400 ng GSH cm^{-3} (Figure 6.7). Background values were subtracted from all data.

6.2.5 Statistical Analysis

All experiments were performed on 2 replicates of 4 treatments namely; control, ozone alone, phenmedipham alone, and ozone followed by phenmedipham. For each treatment, a replicate comprised pooled fully expanded leaves harvested from 2 plants from each of 2 pots. The number of plants was limited by practical constraints. Enzyme experiments were repeated twice. Statistical analysis of all experiments was conducted using two-way ANOVA followed where significant with Duncan's Multiple Range Test (DMRT) and significant differences at the 5% level determined.

6.3 RESULTS

The specific activities of active oxygen scavenging enzymes were determined following exposure to ozone and/or phenmedipham treatment. With the exceptions noted, enzyme activities expressed on a protein basis showed no significant interactions. For this reason, all figures and statistical analyses expressed on a protein basis are presented in Appendix 5 (Table A5.1; Figures A5.1-A5.7).

6.3.1 Effects of exposure to ozone

One and 2 d after the end of the exposure to ozone (2 and 1 d before treatment with phenmedipham, respectively), the activities of the enzymes on both a fresh weight and protein basis were similar to those of the untreated plants (Table 6.1, Appendix 4.1; Appendix 5.1, Table A5.1). An exception to this was APX expressed on a protein basis, which had increased above the control values (Appendix 5, Table A5.1; Appendix 5.1.2).

Exposure to ozone did not affect the amount of protein in the plant throughout the

Table 6.1 Effects of ozone on the activities, on a fresh weight basis, of several antioxidant enzymes in sugarbeet 1 and 2 days after the end of exposure. Values are means ± standard error, where n=4. No significant differences were noted between the control and ozone treatment either 1 or 2 days after exposure. Statistical analyses are presented in Appendix 4.1.

	1 day after ozone exposure		2 days after ozone exposure	
	Control	Ozone	Control	Ozone
Protein (mg g^{-1} fresh weight)	12.56 ± 0.32	10.80 ± 1.02	10.47 ± 0.94	11.35 ± 0.32
Superoxide dismutase (units SOD g^{-1} fresh weight)	3612.5 ± 173.6	4001.9 ± 229.4	4996.3 ± 247.4	4192.5 ± 240.1
Ascorbate peroxidase (nmol g^{-1} fresh weight min^{-1})	55.07 ± 3.50	65.15 ± 4.33	61.09 ± 2.64	80.40 ± 11.17
Monodehydroascorbate reductase (nmol g^{-1} fresh weight min^{-1})	3.94 ± 0.79	4.11 ± 0.89	4.54 ± 0.18	4.93 ± 0.67
Glutathione reductase (nmol g^{-1} fresh weight min^{-1})	3.16 ± 0.10	3.78 ± 0.37	3.61 ± 0.19	4.64 ± 0.48
Catalase (ΔA_{450} g^{-1} fresh weight min^{-1})	0.896 ± 0.137	0.731 ± 0.049	0.551 ± 0.082	0.555 ± 0.051
Guaiacol peroxidase (nmol g^{-1} fresh weight min^{-1})	2.30 ± 0.40	1.99 ± 0.23	2.18 ± 0.14	2.83 ± 0.51
Glutathione transferase (nmol g^{-1} fresh weight min^{-1})	1.59 ± 0.10	1.94 ± 0.16	1.55 ± 0.13	2.032 ± 0.23

experimental period (Table 6.1, Appendix 5.1; Figure 6.8; Appendix 4.2). When calculated on a fresh weight basis, the activity of SOD was decreased by exposure to ozone alone 3 d after exposure (d 0; Figure 6.9; Appendix 4.3), whilst elevations in activities were apparent 3 (0 d after spraying; MDHAR, Figure 6.11; and GPOD, Figure 6.14) and 5 (2 d after spraying; APX, Figure 6.10; MDHAR, Figure 6.11; GR, Figure 6.12; CAT, Figure 6.13; GPOD, Figure 6.14; and GST, Figure 6.15) days after the end of exposure (Appendices 4.4, 4.5, 4.6, 4.7, 4.8 and 4.9, respectively). Activity of GPOD remained elevated until the end of the experiment (Figure 6.14). Total glutathione content was decreased until 5 d after the end of exposure (d3, Figure 6.16; Appendix 4.10).

6.3.2 Effects of treatment with phenmedipham

Application of phenmedipham alone altered the protein content on d 2, but not on other days after spraying (Figure 6.8; Appendix 4.2). SOD (Figure 6.9; Appendix 4.3) activity was decreased by treatment with phenmedipham alone on d2. However, other chloroplastic enzymes, such as APX and GR, showed increased activity relative to controls on d2 (Figures 6.10 and 6.12; Appendices 4.4 and 4.6). GR activity remained elevated for the duration of the experiment in response to phenmedipham (Figure 6.12; Appendix 4.6). Activities of the enzymes CAT, GPOD and GST were elevated by varying amounts. CAT activity was initially lower 1 d after herbicide treatment, but had increased at 3 d (Figure 6.13; Appendix 4.7), whilst that of GPOD was elevated 2 d after treatment and remained high until 6 d after application of the herbicide (Figure 6.14; Appendix 4.8). GST activity also increased 1 d after treatment (Figure 6.15; Appendix 4.9). Total glutathione content was significantly decreased 1 and 2 d after treatment with phenmedipham (Figure 6.16; Appendix 4.10).

6.3.3 Effects of exposure to ozone followed by treatment with phenmedipham

Treatment with ozone followed by phenmedipham did not affect protein contents until 4 d after herbicide application, when they were reduced (Figure 6.8; Appendix 4.2). One d after herbicide treatment MDHAR, GR, GPOD and GST activities were elevated (Figures 6.11, 6.12, 6.14 and 6.15, respectively; Appendices 4.5, 4.6, 4.8 and 4.9, respectively). The activity of all of the enzymes, except SOD, were elevated 2 d after herbicide treatment, whilst the total glutathione content was reduced. Three d after herbicide application the activities of GR, CAT, GPOD, and GST remained elevated.

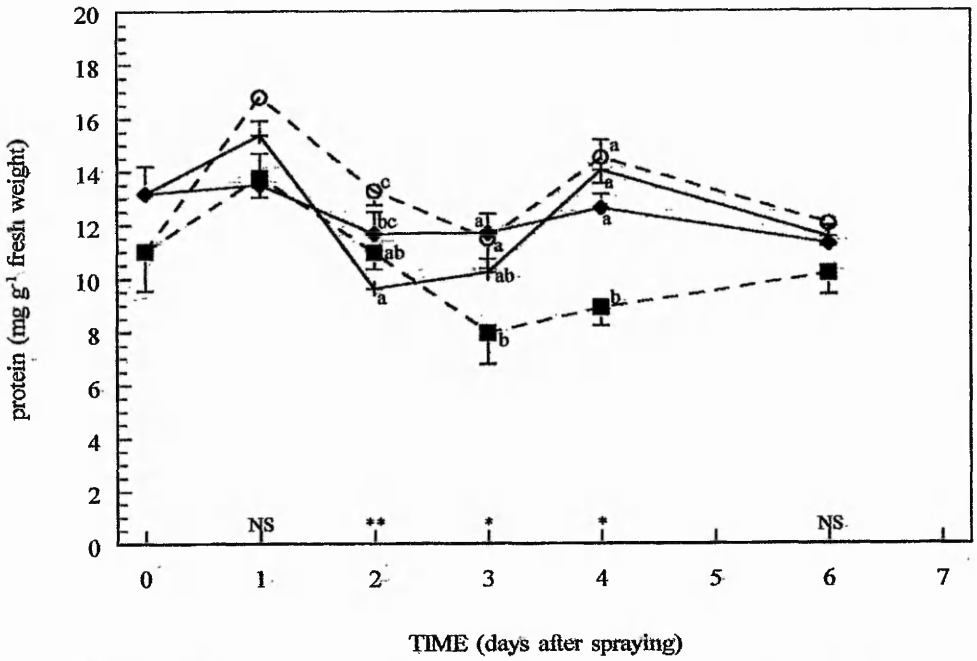


Figure 6.8 Effects of ozone and/or phenmedipham on protein content in sugarbeet cv. Saxon. Values are means ± standard error, where n = 4. Statistical analyses are presented in Appendix 4.2.

Different letters indicate significance within that day. The significance of interactions between ozone and phenmedipham on a particular day is indicated by * ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$) and NS - non-significant.

Key: control (+); ozone alone (O); phenmedipham alone (◆); ozone and phenmedipham (■).

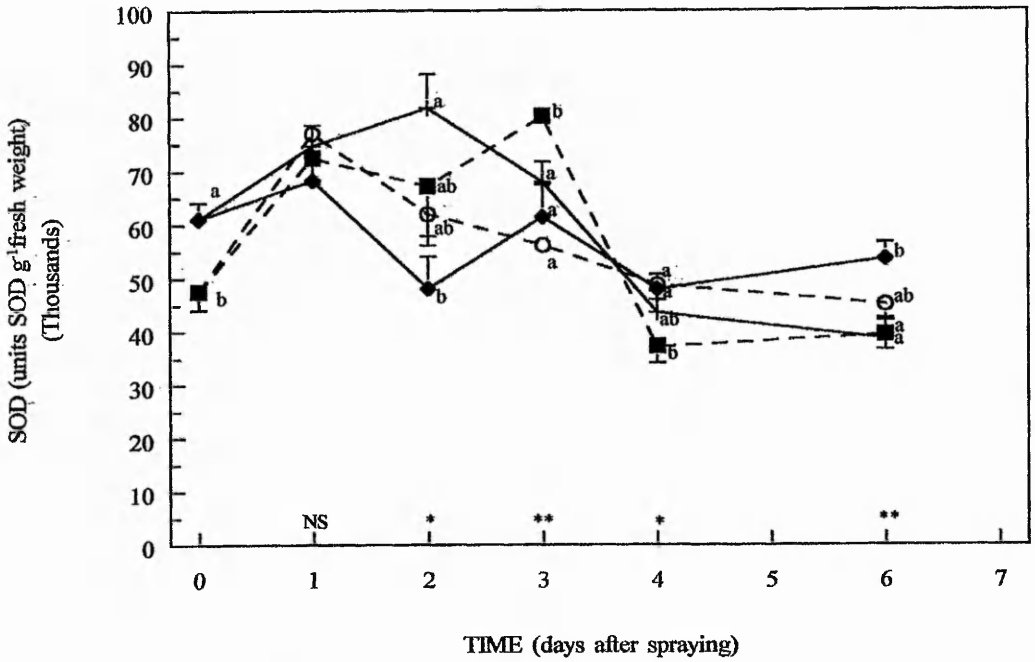


Figure 6.9 Effects of ozone and/or phenmedipham on superoxide dismutase activity in sugarbeet cv. Saxon. For key and statistical analysis see Figure 6.8 and Appendix 4.3.

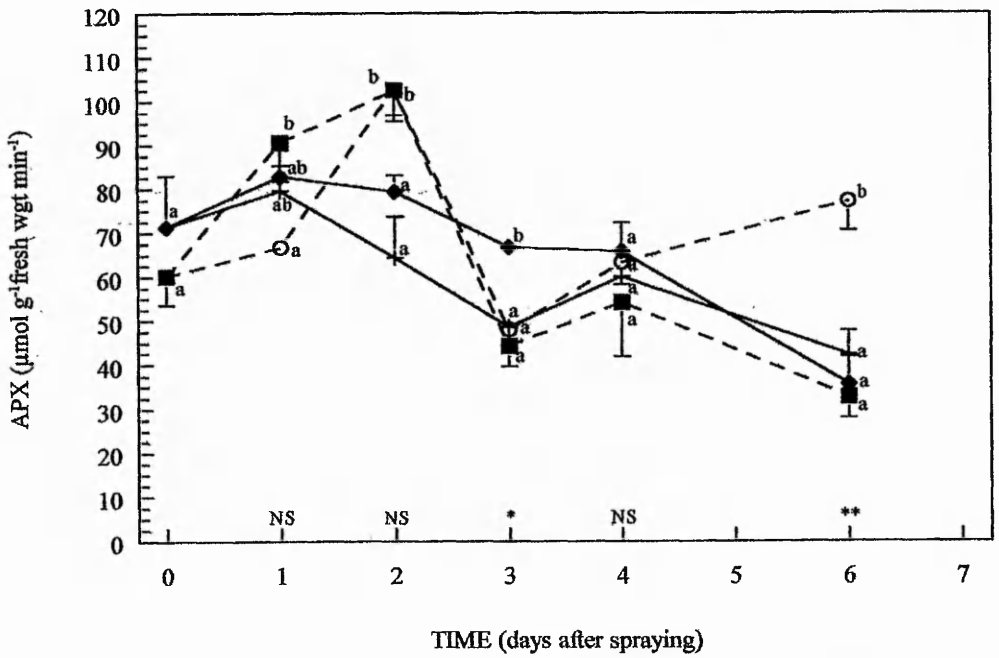


Figure 6.10 Effects of ozone and/or phenmedipham on ascorbate peroxidase activity i sugarbeet cv. Saxon. For key and statistical analysis see Figure 6.8 and Appendix 4.4.

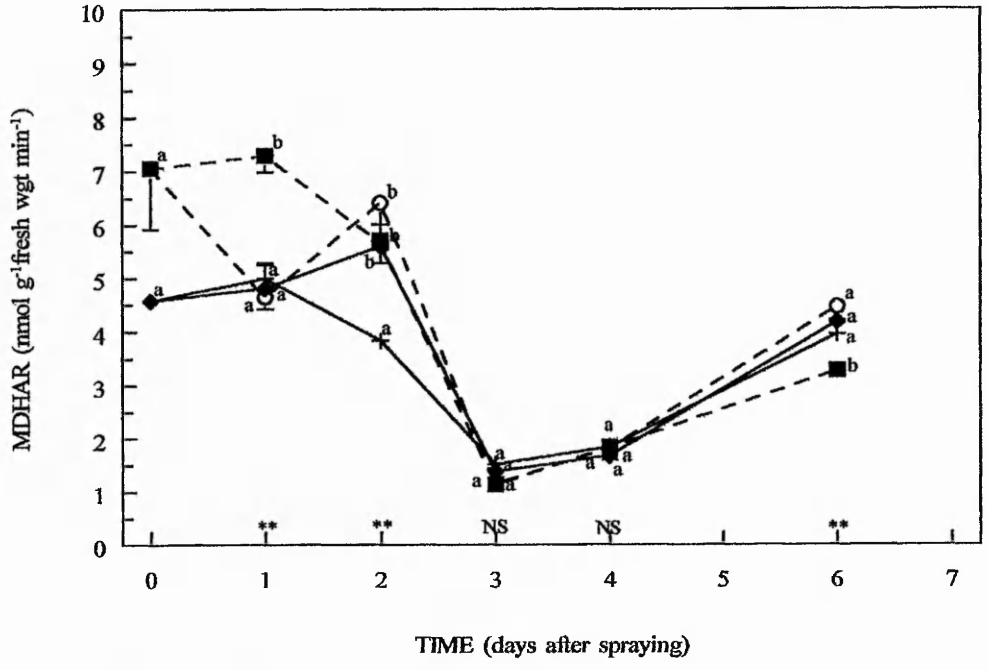


Figure 6.11 Effects of ozone and/or phenmedipham on monodehydroascorbate reductase in sugarbeet cv. Saxon. For key and statistical analysis see Figure 6.8 and Appnedix 4.5.

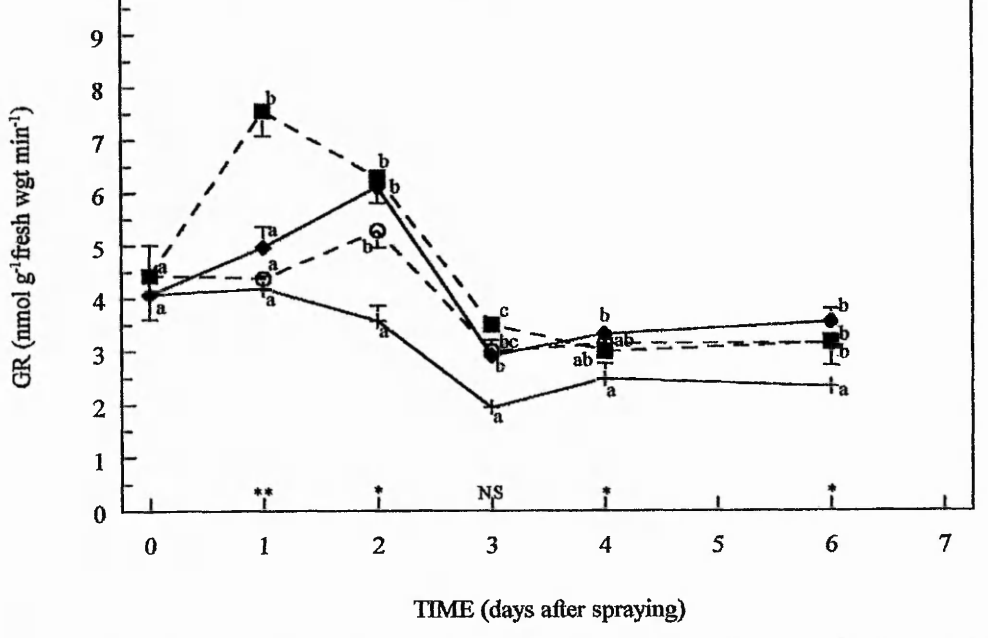


Figure 6.12 Effects of ozone and/or phenmedipham on glutathione reductase activity in sugarbeet cv. Saxon. For key and statistical analysis see Figure 6.8 and Appendix 4.6.

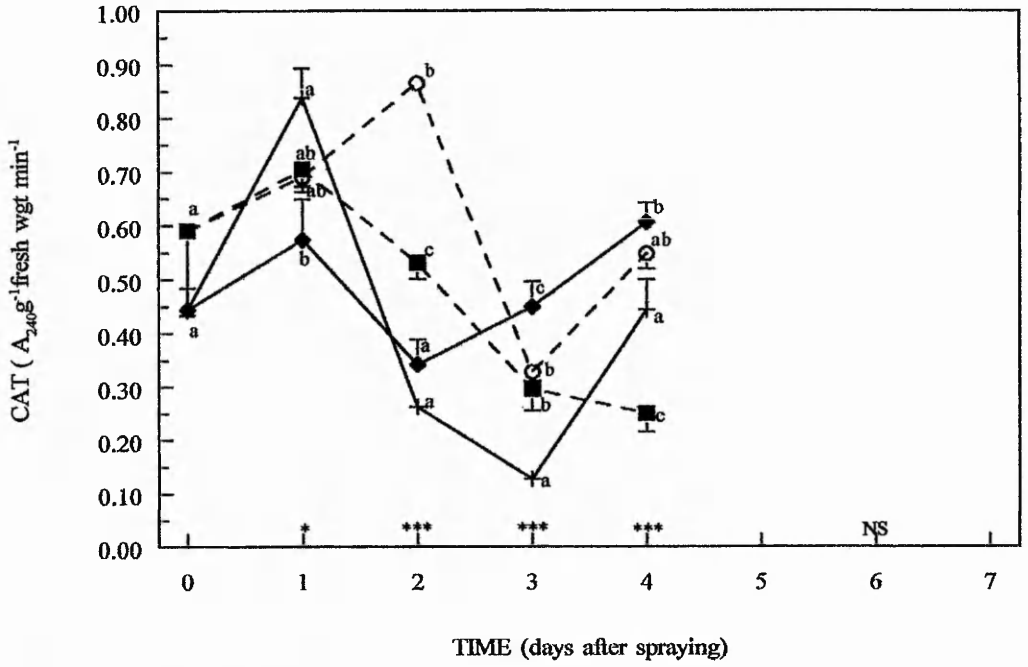


Figure 6.13 Effects of ozone and/or phenmedipham on catalase activity in sugarbeet cv. Saxon. For key and statistical analysis see Figure 6.8 and Appendix 4.7.

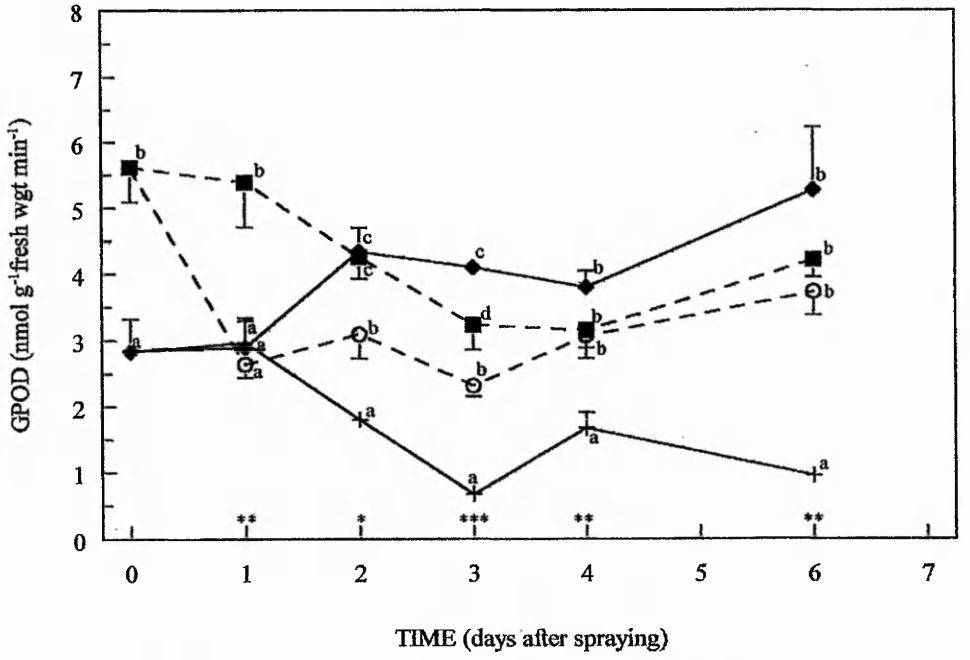


Figure 6.14 Effects of ozone and/or phenmedipham on guaiacol peroxidase activity in sugarbeet cv. Saxon. For key and statistical analysis see Figure 6.8 and Appendix 4.8.

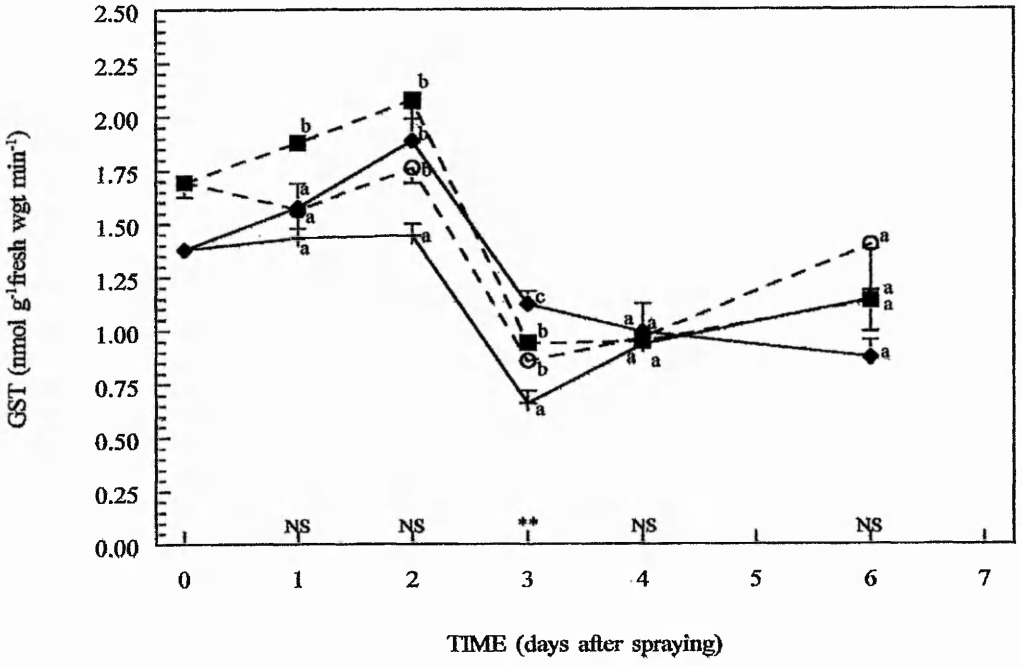


Figure 6.15 Effects of ozone and/or phenmedipham on glutathione transferase activity in sugarbeet cv Saxon. For key and statistical analysis see Figure 6.8 and Appendix 4.9.

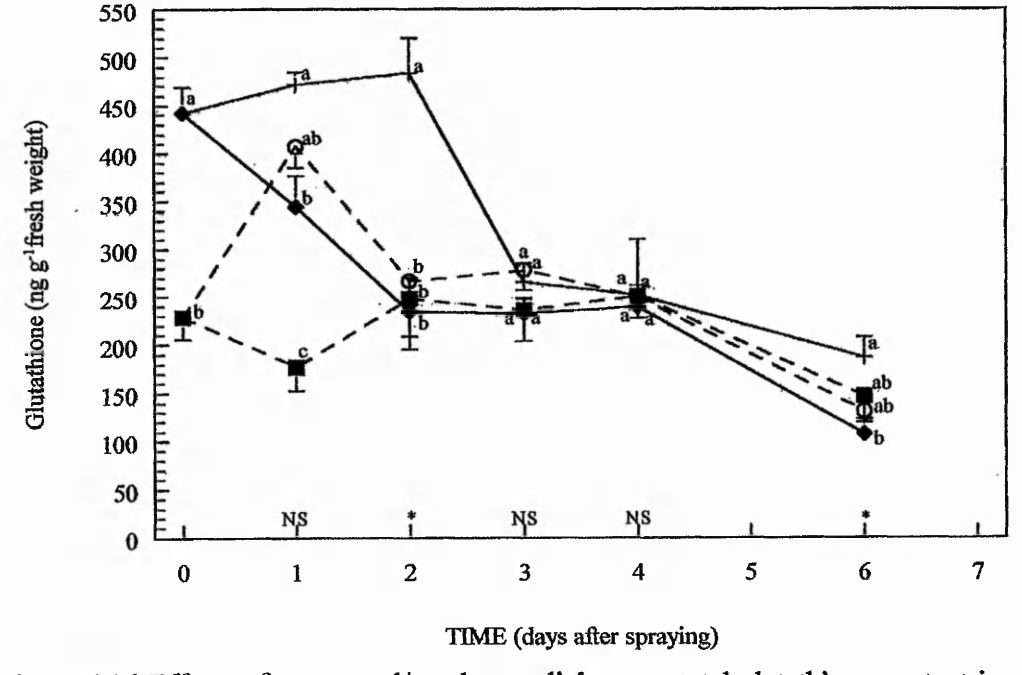


Figure 6.16 Effects of ozone and/or phenmedipham on total glutathione content in sugarbeet cv Saxon. For key and statistical analysis see Figure 6.8 and Appendix 4.10.

6.3.4 Interactions

When plants were exposed to ozone and then treated with phenmedipham, activities of some of the enzymes indicated a significantly greater than expected response at some time during the experimental period. MDHAR, GR and GPOD activities all showed a greater than additive response 1 d after spraying (Figures 6.11, 6.12 and 6.14, respectively; Appendices 4.5, 4.6, and 4.8, respectively; p=0.001, 0.004 and 0.008, respectively), whilst the activity of CAT showed a less than additive response for the duration of the experiment (Figure 6.13; Appendix 4.7; p=0.018, <0.001, 0.001, <0.001 for d 2, 3, and 4, respectively). Activities of MDHAR, GR, CAT and GPOD exhibited a less than additive interaction on day 2 (Figures 6.11, 6.12, 6.13 and 6.14, respectively; Appendices 4.5, 4.6, 4.7 and 4.8, respectively; p=0.009, 0.047, <0.001, and 0.047, respectively). Similarly, a less than additive interaction was also noted in total glutathione content 2 d after herbicide treatment (Figure 6.16; Appendix 4.10; p=0.005), whilst protein contents of plants exposed to ozone followed by treatment with phenmedipham were lower than expected 2, 3 and 4 d after herbicide application (Figure 6.8; Appendix 4.2; p=0.004, 0.020 and 0.031, respectively). A greater than additive interaction was observed in SOD activity 2, 3 and 4 d after treatment with phenmedipham (Figure 6.9; Appendix 4.3; p=0.021, 0.002, 0.030, respectively). GR, CAT and GST activities were reduced below expected values 4 d after herbicide treatment (Figure 6.12, 6.13 and 6.15, respectively; Appendices 4.6, 4.7 and 4.9, respectively; p=0.012, <0.001 and 0.003, respectively).

6.4 DISCUSSION

The aim of this study was to determine combined effects of ozone and phenmedipham on cellular free radical scavenging enzyme activity in sugarbeet. Emphasis was placed on a time-course of the changes in activities of the antioxidant defence system. The discussion of the responses of the antioxidant enzymes overlaps with other chapters in this thesis and is addressed in greater detail in the general discussion (Chapter 7, section 7.2).

6.4.1 Ozone

Ozone and herbicide tolerance have been linked with changes in antioxidant defence systems (Halliwell, 1991; Guzy and Heath, 1993). The primary effect of ozone

is on the plasma membrane (Luwe *et al*, 1993) and therefore ozone would be expected to increase the activity of extracellular and cytoplasmic scavengers rather than chloroplastic antioxidants. A previous study investigating the effects of ozone on the accumulation of mRNAs in *Arabidopsis thaliana* noted increased levels of several cytosolic antioxidant isozymes whilst the levels of chloroplastic isozymes were decreased (Conklin & Last, 1995).

There was an no significant increase in total cellular GST activity in sugarbeet in response to ozone exposure. Previous studies in more sensitive species have shown increases in GST activity, for example in barley (Price *et al*, 1990). In 2 similar studies on *Arabidopsis thaliana* a 26 fold increase in GST mRNA was observed 3h after ozone exposure (Sharma & Davis, 1994) and a more recent study showed the response of GST mRNA to be very rapid (2-fold increase in 30 min; Conklin & Last, 1995). The amount of GST mRNA remained high until the end of exposure to ozone, although 24 h after exposure, levels had almost returned to initial concentrations. Clearly induction of GST is an important response to ozone in sensitive species, where it may play a role in catalysing the detoxification of lipid peroxides, conjugating glutathione with hydrophobic electrophiles. GST may also act as a peroxidase against free fatty acyl hydroperoxides (Price *et al*, 1990). Reductions in total glutathione content also occurred 3 and 5 d after the end of exposure to ozone (48 and 45 % decreases, respectively) which could lead to the conclusion that GST was conjugating glutathione rather than acting as a peroxidase. Glutathione contents were not determined for the 2 d after the end of exposure, although it may be expected that the contents would be reduced.

Peroxidase enzymes in the intercellular space and those bound to cell walls are usually assayed using the non-specific electron donor guaiacol. In this study, induction of GPOD activity by ozone was not significant until 3 d after exposure, but was still an important response to ozone, attaining a maximum 6 d after exposure (245 % increase). The increase in GPOD activity indicates that hydrogen peroxide was produced during exposure to ozone. APX, MDHAR and CAT activities were also elevated 5 d after exposure (2d after herbicide treatment) and after the appearance of visible injury, suggesting a prolonged increase in H_2O_2. Since no increases were observed in the activity of SOD, it would seem that superoxide was either not produced or other

scavengers were reacting with it to produce hydrogen peroxide (e.g. ascorbic acid, glutathione or ferrodoxin). Catalase has been used in previous studies as an additional extra-chloroplastic marker, although in the present study it showed a delayed response to ozone, 5 and 6 d after exposure. Published findings on the response of CAT to ozone show a high variability that is dependent on the ozone concentration (Sharma & Davis, 1994; Fangmeier *et al*, 1994). Consequently the role of CAT in detecting ozone resistance is not clear. Catalase is contained mainly within peroxisomes, although other isozymes exist in the mitochondria and cytosol (Scandalios, 1993). The late response of catalase may indicate that other scavengers were overwhelmed, since visible injury due to ozone occurred prior to the increase in activity.

Increases in the activity of GR may be in response to the redox state of glutathione within the cell, although this occurred 5 d after exposure. Non-enzymic reduction of DHA to ascorbate involves reduced glutathione becoming oxidised. GR reduces this back to GSH (Figure 1.9). This may be in response to the reaction of ascorbate with superoxide radicals and hydrogen peroxide.

6.4.2 Phenmedipham

The primary site of phenmedipham damage is the chloroplast where it blocks photosynthetic electron transport (Cobb, 1992). Only SOD activity indicated a decrease in activity in response to treatment with phenmedipham, again suggesting either very little superoxide was produced or the radicals were detoxified by other scavengers. GR activity was increased 1 day after treatment and remained elevated until the end of the experiment. Activity of GST increased for days 2 and 3, whilst total glutathione contents were reduced for d 1 and 2. Increases in GR activity occurred prior to the elevation of APX activity suggesting an effect on the ratio of GSH to GSSG, i.e. an increase in the amount of GSSG stimulating reductase activity. This suggested alteration in glutathione ratio may arise from the non-enzymatic scavenging of superoxide radicals. Observations in sugarbeet indicated a 50 % reduction in the total glutathione content of leaves 2 d after treatment with phenmedipham. Phenmedipham is not thought to be conjugated to glutathione, although another thiocarbamate herbicide, EPTC, is metabolised in this manner (Carringer *et al*, 1978). Sugarbeet detoxifies phenmedipham via hydroxylation and monoglycosylation (Davies *et al*, 1990).

Two days after treatment with phenmedipham the activity of GPOD was elevated

and remained so until the end of the experiment, showing the largest elevation of any enzyme. CAT activities were also significantly increased 3 d after herbicide treatment. These two factors would suggest that the production of hydroxyl radicals from hydrogen peroxide after crossing chloroplast membranes was increased, resulting in highly localised cellular damage.

6.4.3 Ozone followed by phenmedipham

In plants exposed to ozone prior to application of phenmedipham, exposure to ozone had already decreased SOD activity and glutathione content whilst increasing the activities of both GPOD and MDHAR. One day after herbicide treatment activities of MDHAR, GR, GPOD and GST were significantly elevated. By 2 d after phenmedipham treatment, activities of all the enzymes except SOD were significantly increased. Previous studies have linked ozone tolerance with elevated GR activity within the chloroplast (Price *et al*, 1990), whilst others have noted increased cytosolic CuZn-SOD activity to raise tolerance and elevated chloroplastic SOD activity was linked with the development of injury (Pitcher & Zilinskas, 1996). However, another study correlated an increase in cytosolic SOD in peas with the onset of injury rather than as a defensive response (Doulis & Alscher, 1996). Further work to determine the activities of individual isozymes of the major antioxidant enzymes (SOD, APX and GR) is required to clarify exactly where major responses are occurring.

The observed interactions varied depending on the enzyme and time after treatment. Greater than additive responses were observed for MDHAR, GR and GPOD. These may have helped to confer the ability to detoxify the active oxygen species. However, these responses were reversed 2 d after treatment with phenmedipham. These observations are consistent with the hypothesis that initial treatment with ozone induced antioxidant activity, so that when the plants were subjected to an additional oxidative stress by phenmedipham, antioxidant status was already elevated and more able to detoxify the active oxygen species produced. These findings will be related to other findings in chapter 7.

6.5 CONCLUSION

Ozone increased the activities MDHAR and GPOD, 3 d after exposure, in sugarbeet, consistent with the tolerance of this crop to ozone pollution. Phenmedipham

initially elevated the activities of MDHAR, GR, GPOD and GST and decreased the content of glutathione (GSH and GSSG). Alterations were also observed in the activities of APX and the cytosolic enzyme, CAT.

When exposed to ozone prior to application of phenmedipham, activities of all enzymes, except SOD, were elevated 2 d after herbicide treatment. Furthermore, some of the enzymes (MDHAR, GR and GPOD) had activities which showed a greater that additive response 1d after herbicide treatment. This response was reversed 2d after phenmedipham treatment for these enzymes, and SOD and CAT. Protein contents were also lower than expected between d 2 and 4, whilst GR, CAT and GPOD exhibited a similar response 4d after herbicide application. Since physiological effects were not greater in plants treated with ozone and phenmedipham, this might suggest that ozone was increasing the titre of the enzymes sufficiently, to lead to an increased tolerance to phenmedipham damage.

CHAPTER 7 - GENERAL DISCUSSION

7.1 SELECTION OF HERBICIDE AND CROPS FOR FURTHER STUDY: SUMMARY OF RESULTS

Preliminary experiments focused on the effects of early post-emergence herbicides on 3 spring sown crops. Clopyralid exerted no effects on any of the crops. Diclofop-methyl had no effect on spring barley cvs Tyne and Nugget, whilst decreases in shoot dry weight were induced in Sherpa and Corgi. Application of diclofop-methyl had no significant effect on shoot dry weight of sugarbeet cvs Saxon, Amethyst and Celt. Shoot dry weight of oilseed rape cvs Galaxy and Starlight was not affected by diclofop-methyl. On all crops, damage caused by diclofop-methyl comprised round chlorotic areas, indicative of contact injury. Mecoprop-p reduced the weight of spring barley cv Nugget whilst no consistent effects were observed on the remaining cultivars, although results were affected to varying degrees by treatment with fenpropimorph to reduce powdery mildew infection on plants. A significant interaction between fenpropimorph and mecoprop-p was observed in Sherpa. Injury symptoms due to mecoprop-p consisted of chlorotic lesions on sprayed leaves similar to those resulting from the application of diclofop-methyl. Metazachlor produced no injury or reductions in growth in oilseed rape, whilst phenmedipham reduced shoot dry weights of all 3 sugarbeet cvs. Phenmedipham symptoms comprised chlorotic spots that merged to form large areas covering 20-40 % of sprayed leaves.

The effects of ozone on injury and growth of all crops were generally slight compared to those resulting from herbicides. Visible injury due to ozone consisted of chlorotic flecks, 1-2 mm in length occurring between the veins of leaves present at the time of exposure. Ozone did not have any consistent effect on injury or growth of oilseed rape cvs. Starlight and Galaxy. Similarly, barley was not affected by exposure to ozone. Sugarbeet only developed growth reductions when the plants were older (25 d after sowing) at the time of exposure. When plants were younger (17 d after sowing), neither cultivar was consistently affected by exposure to a simulated ozone episode.

Interactions were studied by treating plants with field rate herbicide followed 3 d later by exposure to 100 nl l^{-1} ozone for 2 d or reversing the treatments (i.e. ozone followed by herbicide). The only significant interactions which occurred were between clopyralid and ozone in OSR cv Galaxy, and ozone and phenmedipham in sugarbeet cvs

Saxon and Celt which were antagonistic (treatments applied in the order stated). Practical problems with spring oilseed rape, due to lower leaves being particularly brittle and easily snapped, meant that this species was unsuitable for a detailed study of the interactions between clopyralid and ozone. Instead, the interaction between ozone and phenmedipham in sugarbeet cv. Saxon was chosen.

7.2 EFFECTS OF OZONE POLLUTION ON SUGARBEET

The present study indicated that ozone has little persistent physiological effect on sugarbeet cv. Saxon. Previous studies were conducted with very high concentrations of ozone, which would not be expected to occur in the UK. For example, Ogata & Maas (1973) used *Beta vulgaris* (garden beet) for growth studies and showed greater effects on the growth of roots than of the shoots at 200 nl l^{-1} for 1-3 h d^{-1} for 5 weeks.

Sugarbeet was injured by 100 nl l^{-1} ozone for 2 d, in the present study but this did not result in a persistent decrease in photosynthesis or shoot dry weights (Table 7.1). This contrasts with a previous study at higher ozone concentrations where photosynthesis was observed to decline by 20 and 51 % in *Beta vulgaris* in response to a 90 min exposure to 650 and 900 nl l^{-1} ozone respectively, with only small amounts of visible injury (Hill and Littlefield, 1969). In the current study, injury due to ozone appeared on sugarbeet within 1-3 d of the end of exposure. This was in agreement with another investigation, which determined that in subterranean (*T. subterraneum*) and white (*T. repens*) clover, visible injury required 7 h mean ozone concentrations to be greater than 35 ppb over the growing season for induction but may also need lower ozone concentrations for 1 d before expression (Benton *et al*, 1995).

The exposure regime used in the present study was equal to an AOT40 of approximately 840 ppb.h and was in excess of the 200-500 ppb.h short term critical level for visible injury. These critical levels were based on work carried out on subterranean clover, a species which is considerably more sensitive to ozone than sugarbeet.

Small decreases in total chlorophyll, and total xanthrophyll and carotenoid, contents of the first two leaves were observed in sugarbeet, although this effect was transitory and did not affect the photosynthetic rate. A prolonged loss of pigments would be associated with an increase in membrane leakage, since the secondary products of ozone are likely to have damaged the plasma membrane prior to damaging

Table 7.1 Summary of experimental results for ozone presented as percentage increase above control values over a time course of 10 days. Statistical analysis was performed on the raw data (see appendices). NS means differences were not significant; percentage changes followed by * indicate a significant difference at $p<0.05$; and blanks indicate measurements were not made on that day.

	PERCENTAGE INCREASE ABOVE CONTROL VALUE										
Day before/after herbicide treatment	**-3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Shoot Dry Weight											NS
Leaf Area											**-11.3 ***
Photosynthesis	NS	NS	NS	NS	NS	NS	NS	NS	NS		
Total Chlorophyll		NS								NS	
Total Xanthrophyll and Carotenoids		NS								NS	
Stomatal Conductance	NS	NS	NS	NS	NS	NS	NS	NS	NS		
Membrane Leakage			NS	NS	NS	NS	NS	NS	NS	NS	
Sodium				NS	NS	NS	NS	NS		NS	
Potassium				NS	NS	NS	NS	NS		NS	
Magnesium				NS	NS	NS	NS	NS		NS	
Ammonium											
Nitrate				NS	NS	NS	NS	NS		NS	
Phosphate				NS	NS	NS	NS	NS		NS	
Starch Grains per Chloroplast							NS				NS
Thylakoid Appression							NS				**-16.6 ***
Protein		NS	NS	NS	NS	**38.0 ***	NS	NS		NS	
Superoxide Dismutase		NS	NS	**-22.1 ***	NS	NS	NS	NS		NS	
Ascorbate Peroxidase		NS	NS	NS	NS	**59.6 ***	NS	NS		**82.4 ***	
Monodehydroascorbate Reductase		NS	NS	**54.3 ***	NS	**67.4 ***	NS	NS		NS	
Glutathione Reductase		NS	NS	NS	NS	**48.1 ***	**54.6 ***	**27.1 ***		**35.2 ***	
Catalase		NS	NS	NS	NS	**229.6 ***	**158.1 ***	NS			
Guaiacol Peroxidase		NS	NS	**98.3 ***	NS	**72.2 ***	**245.5 ***	**84.3 ***		**295.6 ***	
Glutathione S-Transferase		NS	NS	NS	NS	**22.0 ***	**29.9 ***	NS		NS	
Total Glutathione				**-48.3 ***	NS	**-44.9 ***	NS	NS		NS	

the chloroplast. No alterations in membrane leakage in response to exposure to ozone were observed at any time after exposure. Previous studies have indicated that the primary site of action of ozone is the plasma membrane, which would lead to an effect on membrane leakage. This could occur *via* a breakdown in membrane structure with the onset of lipid peroxidation, an increase of the membrane fluidity or through an alteration in the activities of the solute transport pumps and ports within the membrane (Dominy & Heath, 1985, Chimiklis & Heath, 1975).

Results would suggest that secondary products of ozone (active oxygen species) did not reach the chloroplast, since no reductions in photosynthetic rate was observed. In a study investigating the relative sensitivity of *P. vulgaris* cultivars, the tolerance of the cultivar Goldcrop to ozone was not due to stomatal closure but possibly due to mechanisms of preventing injury to membranes i.e. antioxidants (McKersie *et al*, 1982). Damage was prevented after initial injury had occurred, which also seemed to be occurring in sugarbeet in this study. If this is occurring then the activities of antioxidant enzymes located in the extra-cellular spaces would be expected to be elevated. The only apparent consequences of exposure to ozone noted in this study were those on the system of antioxidant enzymes (Figure 7.1). Activities of MDHAR and GPOD were increased 3 d after the end of exposure whilst the remaining enzymes in the ascorbate-glutathione cycle were unaffected. GPOD is a apoplastic enzyme whilst MDHAR occurs in the chloroplast and cytosol Since activities of individual isozymes were not determined and considering no physiological effects were observed in the chloroplast, the responses were likely to have occurred either in the cytosol or in the apoplast.

APX has been noted to be very sensitive to ozone exposure even when no visible injury occurs (Bender *et al*, 1994; Conklin & Last, 1995). In sugarbeet, APX activities were not significantly elevated until 5 d after the end of exposure and only after injury became visible. Increases in APX activity have been correlated with ozone-induced increases in SOD activity, particularly in sensitive species where reductions in photosynthesis are also observed (Mehlhorn, 1990). Interference of ozone with normal photosynthesis produces superoxide in some sensitive species (Sheng *et al*, 1993). However, neither photosynthesis nor SOD activity responded to exposure to ozone in sugarbeet, again suggesting the secondary products of ozone did not reach the chloroplast.

The observed increase in GR activity would suggest an increase in the amount of

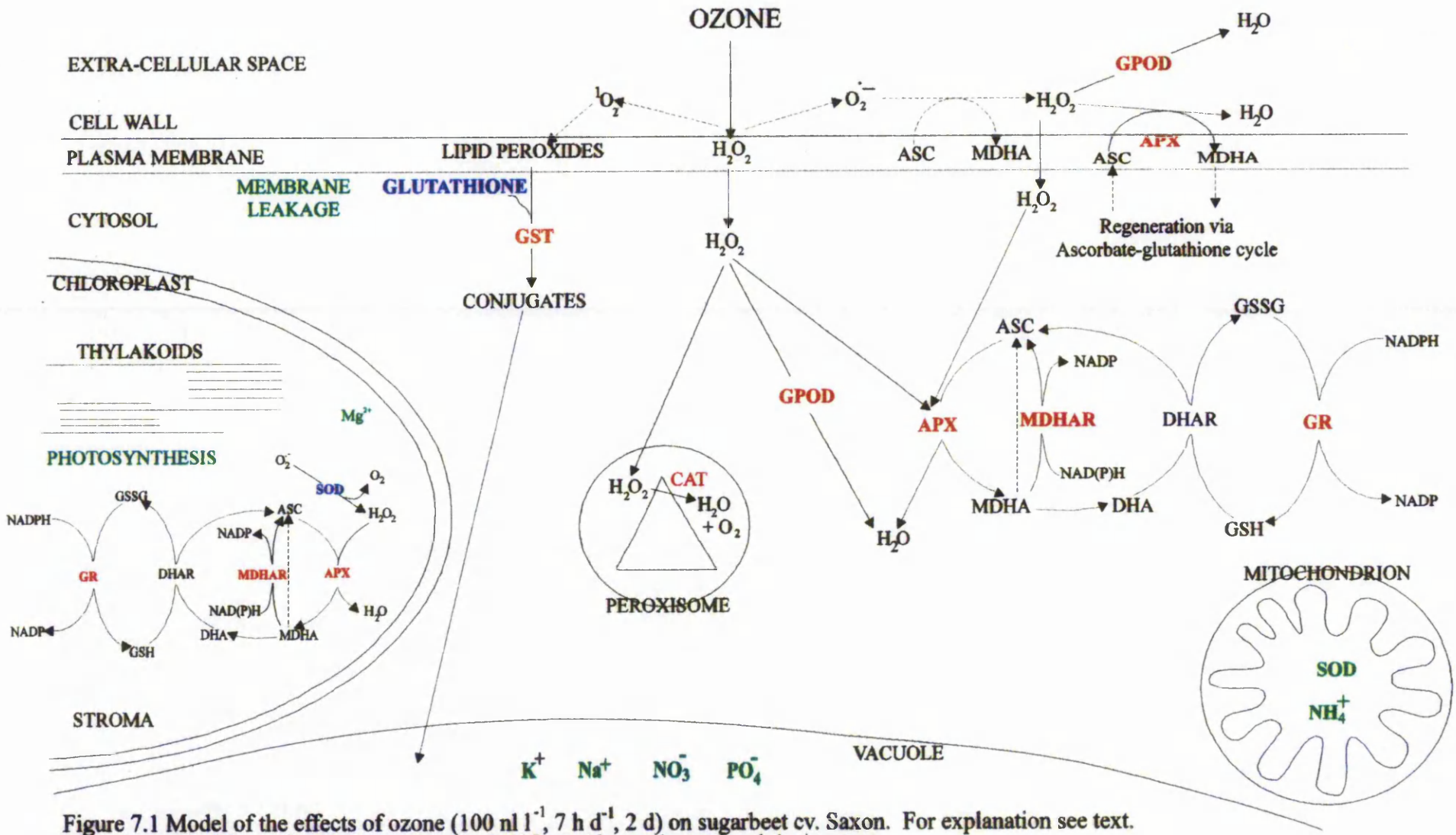


Figure 7.1 Model of the effects of ozone (100 nl l^{-1}, 7 h d^{-1}, 2 d) on sugarbeet cv. Saxon. For explanation see text.
(red = increase; blue = decrease; green = no significant change in total activity/content;
— enzymatic reaction; --- non-enzymatic reaction)

oxidised glutathione (GSSG) available for reduction to GSH, i.e. a faster cycling capacity of GSSG. This could arise from either an increase in direct superoxide scavenging by GSH or the regeneration of ascorbate from DHAR by GSH. The loss of GSH through conjugation by GST would not result in an increase in GR activity. Since both GR and GST activities increase and total glutathione (GSH and GSSG) content declines a combination of these events is likely to occur in sugarbeet. GST conjugates toxins to glutathione and has additional activity as a selenium-independent glutathione peroxidase (Lamoureux *et al*, 1991; Kreuz *et al*, 1996). Several studies have noted the importance of GSTs after exposure to ozone (Price *et al*, 1990; Sharma & Davis, 1994). It has also been noted that a doubling of activity measured with the artificial substrate, 1-chloro-2,4-di-nitrobenzene (CDNB), can actually represent a 30-fold increase in activity to an endogenous substrate, such as herbicides (Grunwald *et al*, 1987). If this is occurring in sugarbeet it would indicate a large involvement of GSTs in the tolerance of the plants to ozone pollution. Sugarbeet may therefore utilise GSTs to limit any membrane damage by detoxifying fatty acid hydroperoxides or conjugating hydrophobic electrophiles with glutathione.

General peroxidase activity increased as injury in the form of a chlorotic stipple became visible on the leaf. These enzymes are thought to be activated by calcium (Castillo *et al*, 1984). However, in sugarbeet no alteration in calcium content of the tissue was observed, although the activity of GPOD was greatly increased by exposure to ozone. GPOD is involved in the polymerisation of lignin precursors, suberisation and cross-linking proteins or other molecules with wall material (Castillo *et al*, 1984).

Sugarbeet is tolerant to ozone pollution within the bounds of the concentration and exposure regime utilised in this study. No effects were observed on photosynthesis, membrane leakage or ion leakage, indicating an elevation in the activities of selected enzymes of the antioxidant system was sufficient to prevent persistent damage other than visible injury. It is hypothesised that exposure to ozone increases the activities of MDHAR and GPOD rapidly enough and to a great enough extent to restrict damage to small areas of cells, with very little effect on the plant as a whole. Small amounts of injury can therefore be tolerated with no lasting damage to the plant.

7.3 EFFECTS OF PHENMEDIPHAM ON SUGARBEET

Sugarbeet is susceptible to injury from phenmedipham, one of the most utilised herbicides in the crop, although this damage is known to be transient in the field. Phenmedipham decreased the shoot dry weight of seedlings grown in the glasshouse. A proposed model for the action of phenmedipham on sugarbeet cv. Saxon is shown in Figure 7.2. Phenmedipham acts at the D1 protein, inhibiting photosynthetic electron transport in isolated chloroplasts of both tolerant and susceptible plants at similar rates and producing a 50 % reduction in whole plant photosynthesis within 3-4 h of application (Chapter 5; Arndt & Kotter, 1968; Voss *et al*, 1984; Cobb, 1992; C. Unsworth, unpublished data). Transport of phenmedipham from the leaf surface to the thylakoid membrane takes approximately 2 h in sugarbeet (Voss *et al*, 1984). In the present study, photosynthesis was reduced for at least 7 d after phenmedipham application and probably accounted for the decline in shoot dry weight of seedlings. Tolerant species, such as sugarbeet, normally recover within 10 d of treatment due to detoxification of the herbicide (Voss *et al*, 1984; Prodoehl *et al*, 1992) by hydroxylation and monoglycosylation (Davies *et al*, 1990).

When photosynthetic electron transport was blocked by phenmedipham, reductions in stomatal conductance and a steady increase in membrane leakage and ion leakage from the cells of sugarbeet were observed (Table 7.2). The observed decline in photosynthetic rate would have increased the sub-stomatal carbon dioxide concentration resulting in the observed reduction in stomatal conductance. Herbicides which inhibit photosynthetic electron transport trigger the production of superoxide, singlet oxygen and other active oxygen species within the chloroplast due to excess excitation energy. Effects on the plant became apparent as chlorotic lesions merged to cover large areas of the leaf tissue. Necrotic patches also arose, associated with contact injury of the formulation. The nature of the injury indicated that the chlorophyll content would be decreased by treatment with phenmedipham. Reductions in total chlorophyll, and total xanthrophyll and carotenoid contents in the first two leaves of herbicide treated plants were observed 7 d after application of phenmedipham. Where a reduction in photosynthetic rate occurred, a decline in the starch grain content was also be observed. This decline may have also been due to an increase in the utilisation of starch in repair processes within the cell. A reduction in photosynthesis would have reduced the amount of NADPH available for scavenging and repair processes, further increasing the

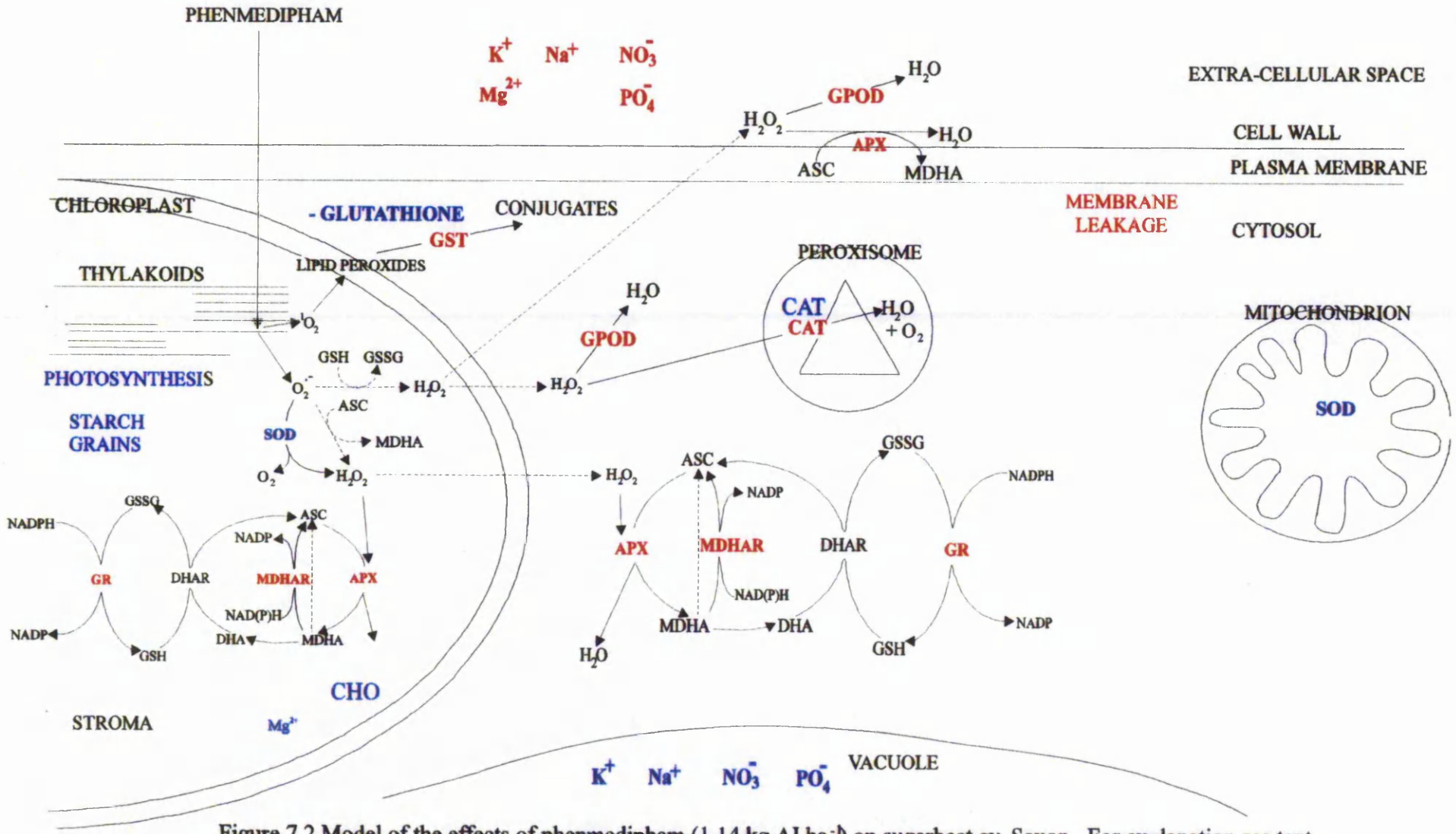


Figure 7.2 Model of the effects of phenmedipham (1.14 kg AI ha^{-1}) on sugarbeet cv. Saxon. For explanation see text.
(red = increase; blue = decrease; green = no significant change in activity/content;
– enzymatic reaction; --- non-enzymatic reaction)

Table 7.2 Summary of experimental results for phenmedipham presented as percentage increase above control values over a time course of 7 days. Statistical analysis was performed on the raw data (see appendices). NS means differences were not significant; percentage changes followed by * indicate a significant difference at $p<0.05$; and blanks indicate measurements were not made on that day.

	PERCENTAGE INCREASE ABOVE CONTROL VALUE						
Day before/after herbicide treatment	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Shoot Dry Weight								**-35.8 ***
Leaf Area								**-38.2 ***
Photosynthesis		**-55.7 ***	**-45.1 ***	**-38.1 ***	**-39.6 ***		**-36 ***	
Total Chlorophyll								**-31.8 ***
Total Xanthrophyll and Carotenoids								**-23.8 ***
Stomatal Conductance		**-50.5 ***	NS	NS	NS		NS	
Membrane Leakage		**162.2 ***	**187.3 ***	**216.7 ***	**182.4 ***	**194.9 ***	**132 ***	
Sodium		NS	NS	**399.7 ***	**283.5 ***		NS	
Potassium		NS	NS	**432.3 ***	**202.6 ***		NS	
Magnesium		NS	NS	**321.6 ***	**159.5 ***		NS	
Ammonium		NS	NS	NS	NS		NS	
Nitrate		NS	NS	**451.5 ***	**3552.6 ***		NS	
Phosphate		NS	NS	NS	NS		**629.4 ***	
Starch Grains per Chloroplast				**-36.1 ***				**-71.2 ***
Thylakoid Appression				NS				NS
Protein		**-12.2 ***	**21.2 ***	NS	NS		NS	
Superoxide Dismutase		NS	**-41.3 ***	NS	NS		**38.6 ***	
Ascorbate Peroxidase		NS	NS	**37.4 ***	NS		NS	
Monodehydroascorbate Reductase		NS	**46.2 ***	NS	NS		NS	
Glutathione Reductase		NS	**71.3 ***	**50.4 ***	**33.8 ***		**52.6 ***	
Catalase		**-31.6 ***	NS	**253.5 ***	**36.3 ***			
Guaiacol Peroxidase		NS	**142.0 ***	**513.3 ***	**128.4 ***		**459.7 ***	
Glutathione S-Transferase		NS	**30.8 ***	**69.6 ***	NS		NS	
Total Glutathione		**-26.9 ***	**-51.5 ***	NS	NS		**-42.6 ***	

stress on the plant.

Antioxidant enzymes were utilised by the plant to scavenge active oxygen species reducing the amount of damage. No work had previously been carried out on the effects of phenmedipham on the activities of antioxidant enzymes. Since the herbicide acts on the chloroplast, those enzymes found there would be expected to increase first, followed by those in the cytosol or apoplast. However, SOD, a major chloroplastic enzyme, decreased after treatment with phenmedipham, suggesting either very little superoxide was produced or the radicals were detoxified via ascorbate or glutathione. An indication that the latter may be occurring was the increase in activity of GR regenerating GSSG to GSH, extending over the duration of the experiment. GR activity may have been expected to be inhibited due to the reduction in the production of NADPH by photosynthesis. However, it would seem likely that either enough NADPH was available or normal cell metabolism utilising this substrate was lowered. An increase in the regeneration of ascorbate would have been expected to be observed as an elevation in the activity of MDHAR.

GST activity was elevated for days 2 and 3, whilst total glutathione was reduced by 50 % on day 3. Increases in the activity of GR indicated a shift in the ratio of GSSG to GSH, which normally induces the synthesis of GSH, which did not occur in the present study (Rennenburg, 1982). GST is known to play a role in the detoxification of certain herbicides (Cole, 1994). However, phenmedipham is detoxified in sugarbeet by hydroxylation and monoglycosylation (Davies *et al*, 1990) and GST is not thought to play a part in this, although another thiocarbamate herbicide, EPTC, is conjugated to glutathione by GSTs (Carringer *et al*, 1978). GSTs are also known to detoxify lipid peroxides, formed from the action of hydroxyl radicals on methylene groups and the subsequent reaction of the resulting diene with oxygen. This secondary action may account for the increase in GST activity and decline in glutathione as a result of treatment with phenmedipham.

Activities of all the enzymes, except SOD, were elevated 2 d after treatment with phenmedipham, during the period when membrane leakage was at a maximum. Increases in the amounts of hydrogen peroxide probably induced the activities of the scavengers, although APX can be inhibited by high concentrations of this oxygen species during periods of darkness (Hossain & Asada, 1984). Increases in the activity of GPOD have previously been shown to be important in the tolerance of maize (*Zea*

mays) to atrazine (Alla, 1995). However, the observed alterations in membrane leakage of the cell 1-3 d after herbicide treatment may suggest lipid peroxide and/or hydroxyl radical production at various sites within the cell. Effects on membrane leakage have been observed for other photosystem II herbicides, such as linuron, prometryne and bromacil (Crowley & Prendeville, 1980). Alterations in the fluidity of the membranes through affects on the double bonds of the fatty acids or damage to the transport systems for various solutes into and out of the cytosol seem the likely causes of the increases in the conductivity of the leachate. Repair systems in the leaf tissue limited the damage occurring and eventually led to a reduction in membrane leakage 6 d after herbicide treatment, although photosynthesis had not recovered to control values by this time.

Analysis of the ion content of the leachate hints at damage to the plasma membrane, tonoplast, chloroplast and mitochondrial membranes. The chloroplast envelope would be expected to be damaged due to the close proximity to the thylakoids and the source of active oxygen species. However, analysis of magnesium and sulphate content of the leachate indicated that although there was some damage the concentrations were small compared to those of potassium and sodium leaking from the vacuole. Observed increases in the amounts of sodium, potassium, nitrate and phosphate indicated effects on the tonoplast membrane. These may have arisen from alterations in the activities of the pumps and ports into and out of the vacuole. Increases in ammonium ion concentration in the leachate could be due to disruption of the mitochondrial membrane, where it is formed during photorespiration, or reductions in the activity of glutamine synthetase, which incorporates the ion into glutamine (Givan, 1979; Sarojini & Oliver, 1983). Reduction in the activities of this enzyme and glutamate dehydrogenase have been shown in sugarbeet suspension cultures treated with phenmedipham (Zelmer & Günther, 1988). Membrane leakage reached a peak 3-4 d after application and then started to decline as antioxidants prevented further damage and repair processes began. Growth effects observed at 7 d were likely to be due to a 50 % reduction in photosynthesis decreasing the amount of carbohydrates available for normal metabolism and growth. Sugarbeet would be expected to recover from treatment with phenmedipham as has been observed in the field (Hendrick, 1973; Schweizer, 1974; Prodoehl *et al*, 1992).

7.4 EFFECTS OF EXPOSURE TO OZONE FOLLOWED 3 DAYS LATER BY APPLICATION OF PHENMEDIPHAM

Growth studies indicated an antagonistic interaction between ozone pollution and phenmedipham in sugarbeet (Table 7.3). Previous studies looking at interactions between ozone and herbicides have concentrated on the effects on growth or on the metabolism of the herbicide (Hodgson & Hoffer, 1977; Hatzios & Yang, 1983). The present investigation aimed to look in detail at the physiological and biochemical basis of the interaction between ozone and phenmedipham. However, it should be noted that interactions which occur depend on several factors, as illustrated in previous studies, and discussed in more detail in Chapter 1, section 1.5.

A proposed model of the interaction between ozone and phenmedipham is shown in Figure 7.3. Stomatal conductance and photosynthetic rate in plants exposed to ozone for 2 d, followed 3 d later by treatment with phenmedipham did not differ from plants treated with phenmedipham alone at any time over the experimental period. Activities of MDHAR and GPOD were elevated due to exposure to ozone at the time when the plants were treated with phenmedipham. SOD activity and total glutathione content was reduced over the same period. These antioxidants did not prevent the observed 50 % reduction in photosynthetic rate caused by phenmedipham.

The enzymes would be available to prevent subsequent damage to pigments and membranes. In the present study this was noted as the contents of total chlorophyll and total carotenoids were intermediate between control values and plants sprayed with phenmedipham alone at the end of the experimental period. Significant interactions were observed between ozone and phenmedipham on d 1 for MDHAR, GR, CAT and GPOD. MDHAR, GR and GPOD showed antagonistic interactions whilst that of CAT was less than expected. The prior treatment with ozone may have sensitised the plant to the effects of oxidative stress, allowing a faster response to the herbicide. In peas, cytosolic SOD has been shown to be more responsive to ozone, whilst chloroplastic SOD activity increased with the development of injury (Pitcher & Zilinskas, 1996). Increased activities of chloroplastic isozymes of the antioxidants would have reduced the damage occurring within the chloroplast.

Since phenmedipham affects photosynthetic electron transport, generating active oxygen species, an increase in the titre of scavenging enzymes would decrease the effects at the cellular level. Antioxidant enzymes were all elevated during the period

Table 7.3 Summary of experimental results for ozone followed by phenmedipham presented as percentage increase above control values over a time course of 7 days. Statistical analysis was performed on the raw data (see appendices). NS means differences were not significant; percentage changes followed by * indicate a significant difference at $p<0.05$; and blanks indicate measurements were not made on that day.

	PERCENTAGE INCREASE ABOVE CONTROL VALUE							
Day before/after herbicide treatment	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Shoot Dry Weight								**-34.2 ***
Leaf Area								**35.3 ***
Photosynthesis	NS	**-58.2 ***	**-48.2 ***	**-44.0 ***	**-42.8 ***		**-35.2 ***	
Total Chlorophyll								**-18.6 ***
Total Xanthrophyll and Carotenoids								**-14.9 ***
Stomatal Conductance	NS	**-50.7 ***	**-48.9 ***	NS	NS		NS	
Membrane Leakage	NS	**140.7 ***	**129.9 ***	**211.6 ***	**157.3 ***	**84.7**	NS	
Sodium	NS	NS	NS	**4710.0 ***	NS		NS	
Potassium	NS	NS	NS	**405.1 ***	**170.8 ***		NS	
Magnesium	NS	NS	NS	**410.9 ***	**115.4 ***		NS	
Ammonium								
Nitrate	NS	NS	NS	NS	NS		**16698 ***	
Phosphate	NS	NS	NS	NS	NS		NS	
Starch Grains per Chloroplast				**-67.9 ***				**-41.8 ***
Thylakoid Appression				**46.1 ***				**42.9 ***
Protein	NS	**-10.2 ***	NS	NS	**-36.6 ***		NS	
Superoxide Dismutase	**-22.1 ***	NS	NS	**18.0 ***	NS		NS	
Ascorbate Peroxidase	NS	NS	NS	NS	NS		NS	
Monodehydroascorbate Reductase	**54.3 ***	**45.8 ***	**47.7 ***	NS	NS		**-17.2 ***	
Glutathione Reductase	NS	**80.8 ***	**76.0 ***	**80.3 ***	NS		**36.2 ***	
Catalase	NS	NS	**102.6 ***	**133.0 ***	**-43.7 ***			
Guaiacol Peroxidase	**98.3 ***	**81.9 ***	**137.4 ***	**382.6 ***	**88.9 ***		**347.1 ***	
Glutathione S-Transferase	NS	**30.9 ***	**44.0 ***	**42.3 ***	NS		NS	
Total Glutathione	**-48.3 ***	**-62.5 ***	**-48.8 ***	NS	NS		NS	

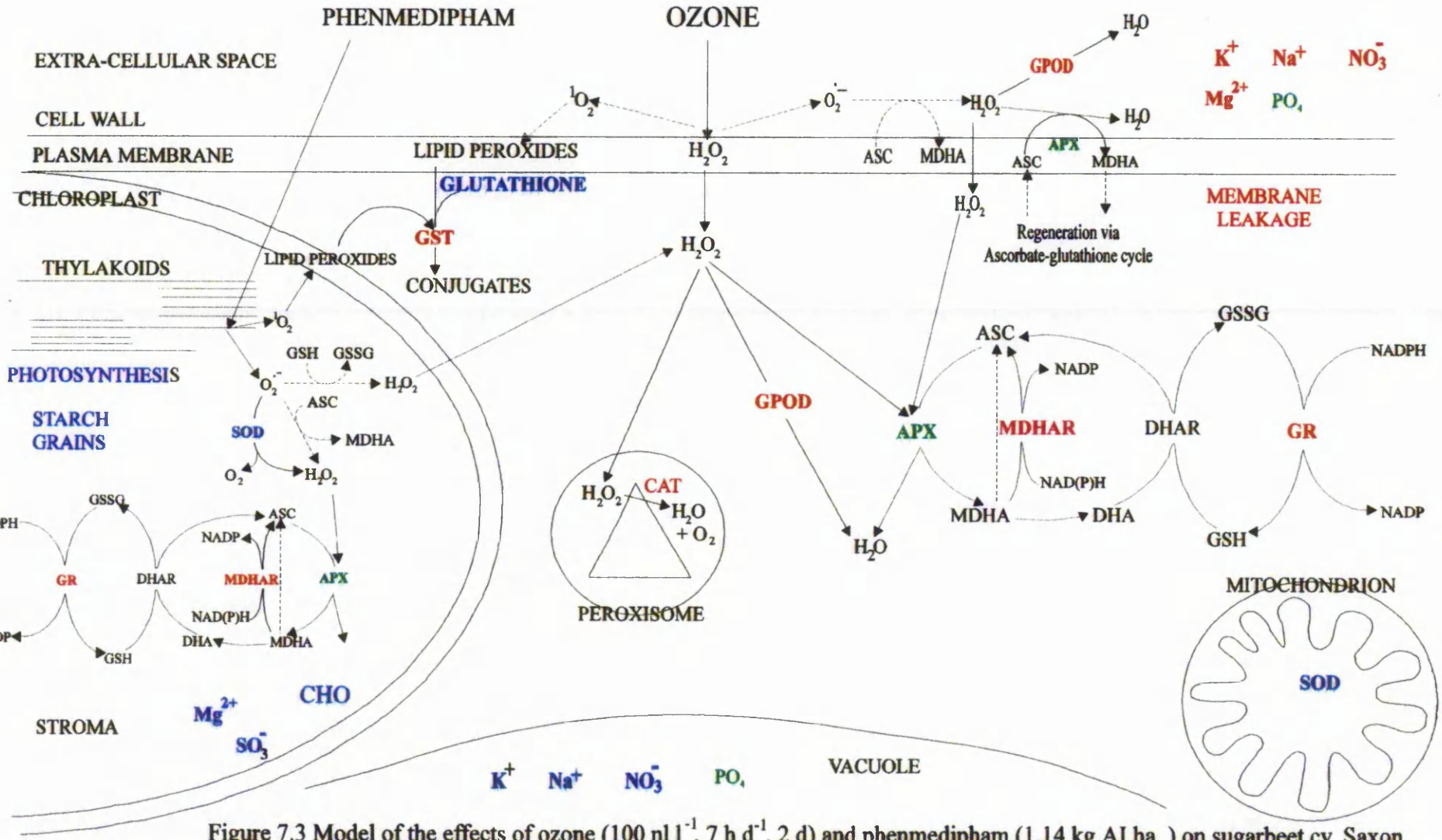


Figure 7.3 Model of the effects of ozone (100 nl l^{-1}, 7 h d^{-1}, 2 d) and phenmedipham (1.14 kg AI ha) on sugarbeet cv. Saxon. For explanation see text. (red = increase; blue = decrease; green = no significant change in total activity/content; — enzymatic reaction; --- non-enzymatic reaction)

after the two treatments. However, at different stages during the experimental period, the activities of each of the enzymes measured were greater than those expected in an additive interaction. One day after treatment with phenmedipham, the activity of GPOD was elevated to a greater extent than expected, indicating an increased response at the membranes to hydrogen peroxide. Membrane leakage was lower 2 d after herbicide treatment in plants exposed to both treatments than in those treated with phenmedipham alone. An elevation in GPOD is normally associated with the appearance of visible injury following treatment with ozone (Nouchi, 1993). However, the effect of ozone was to increase the levels of the enzyme prior to phenmedipham application which may have increased the cell wall hardening processes at the membrane. This would reduce the amount of membrane leakage if this was occurring due to physical damage. Injury was first noted 1-3 d after treatment with phenmedipham. Symptoms were in the form of chlorotic areas surrounding initial contact injury (due to the herbicide) and appearance corresponded with the time period when membrane leakage increased.

The primary sites of damage of ozone and phenmedipham are different (Luwe *et al*, 1993). Ozone damages the plasma membrane and consequently activities of enzymes in this vicinity are stimulated, for example, GPOD, GST and isozymes of GR. Induction of largely chloroplastic enzymes including APX and SOD is a secondary response in sugarbeet to ozone. Conversely, the primary mode of action of phenmedipham is in the chloroplast where it blocks electron transport and produces active oxygen species at the thylakoid membrane and consequently, enzymes largely associated with the chloroplast are induced. When the herbicide is sprayed after ozone exposure then both the plasma membrane and the chloroplast are potential damage sites. From the findings presented in this study, it would appear that ozone stimulates the antioxidant system so that if an additional oxidative stress, such as a PSII herbicide, is imposed on the plant, it is more able to deal with the generation of active oxygen species. The net effect is the antagonistic response seen in the reduction in the effect on leaf area since the herbicide does not exert the full predicted effect. This study supports the hypothesis that improving the endogenous antioxidant capacity of plants can lead to increased stress tolerance (Foyer *et al*, 1994).

The antagonistic interaction noted in this study may reduce the effect of phenmedipham on sugarbeet in field sown crops as episodes of the type simulated in these experiments do occur when sugarbeet is at the young seedling stage. For example,

in 1995 sugarbeet plants, sown in March/April, were at around the 2-3 leaf stage when a 4-5 day ozone episode occurred during the first few days of May. Since this is the stage when phenmedipham is applied to sugarbeet, it is possible that both herbicide treatment and ozone exposure occurred within a few days of each other.

7.5 SUGGESTIONS FOR FURTHER STUDY

Further work which would have increased the usefulness of the findings of the present study include field work during and after ozone episodes such as that described above in early May, 1995. Field-based methods for measuring photosynthesis and observation of herbicide symptoms could have been employed. Similarly, field studies looking at the relative timing of the pollutant and the herbicide may have also revealed important implications for crop husbandry. Multiple episodes of ozone and the other major pollutants, sulphur dioxide and oxides of nitrogen, occurring throughout the growing season of the crop may also affect the efficacy of the pesticides and could be studies further.

Analysis of the relative activities of the isozymes of the antioxidant enzymes already studied would give clearer indications of exactly where effects of the treatments were occurring. Similarly the activity of dehydroascorbate reductase, and the contents of ascorbate, oxidised and reduced glutathione, and alpha-tocopherol would have provided further information on the effects of ozone and phenmedipham on sugarbeet. The short-term response of the plants to ozone and phenmedipham, looking at the first 24 or 48 hours after treatment, would have shown whether sugarbeet was tolerant to the pollutant or if effects were not observed due to the time lapsed between the end of exposure and the start of analysis. The response of glutamine synthetase and glutamate dehydrogenase activities could be studied to determine whether the increase in ammonium ion concentration in the leachate was due to alterations in the mitochondrial membrane or reductions in detoxification of the ion.

Looking at other interactions, such as the antagonism occurring in oilseed rape between ozone and clopyralid, may also further the understanding of the effects of both herbicide and ozone on the crop. Clopyralid is an auxin-type herbicide and may influence the response to ozone by inducing the production of ethylene. Other interactions may occur between the herbicides and different pollutants, for example, sulphur dioxide and nitrogen dioxide.

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Appendix 1.1 Effects of clopyralid rate (0, 0.035, 0.07, 0.14 kg AI ha^{-1}) on the shoot dry weights of 4 spring barley cultivars. Results of one-way ANOVA tests conducted on data.

Dependent Variable: **TYNE SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
clopyralid rate	0.011	3	0.004	0.456	0.718
Error	0.099	12	0.008		
Total	0.110	15	0.007		

Dependent Variable: **NUGGET SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
clopyralid rate	0.004	3	0.001	0.529	0.671
Error	0.032	12	0.003		
Total	0.036	15	0.002		

Dependent Variable: **SHERPA SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
clopyralid rate	0.012	3	0.004	2.011	0.166
Error	0.024	12	0.002		
Total	0.036	15	0.002		

Dependent Variable: **CORGI SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
clopyralid rate	0.007	3	0.002	0.715	0.562
Error	0.040	12	0.003		
Total	0.047	15	0.003		

Appendix 1.2 Effects of diclofop-methyl rate (0, 0.475, 0.95, 1.9 kg AI ha^{-1}) on the shoot dry weights of 4 spring barley cultivars. Results of one-way ANOVA and Duncan's Multiple Range Tests for shoot dry weight, classified by rate of herbicide. In the comparisons table, accept indicates that the two rates are not significantly different at $p \leq 0.05$ and * denotes significantly different pairs.

Dependent Variable: **TYNE SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
diclofop-methyl rate	0.003	3	0.001	0.236	0.870
Error	0.044	12	0.004		
Total	0.047	15	0.003		

Dependent Variable: **NUGGET SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
diclofop-methyl rate	0.009	3	0.003	2.360	0.123
Error	0.016	12	0.001		
Total	0.025	15	0.002		

Dependent Variable: **SHERPA SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
diclofop-methyl rate	0.092	3	0.031	7.068	0.005
Error	0.052	12	0.004		
Total	0.144	15	0.010		

For **SHERPA SHOOT DRY WEIGHT**, classified by diclofop-methyl rate

Group	Cases	Mean	1.9	0.95	0	0.475
1.9	4	0.1638		*	*	*
0.95	4	0.3106	*			
0	4	0.3461	*			
0.475	4	0.3496	*			

Comparison	Difference	Std Error	q Stat	Table q	Result
1.9 - 0.475	0.1859	0.0330	5.6385	3.3193	Reject
1.9 - 0	0.1824	0.0330	5.5323	3.2216	Reject
1.9 - 0.95	0.1469	0.0330	4.4555	3.0734	Reject
0.95 - 0.475	0.0390	0.0330	1.1831	3.2216	Accept
0.95 - 0	0.0355	0.0330	1.0769	3.0734	Accept
0 - 0.475	0.0035	0.0330	0.1062	3.0734	Accept

Homogeneous Subsets:

Group 1:	1.9		Group 2:	0, 0.475, 0.95,	
Pooled mean =	0.16375		Pooled mean =	0.3355	
95% Confidence Interval =	0.0919	0.2356	95% Confidence Interval =	0.2940	0.3769

Dependent Variable: **CORGI SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
diclofop-methyl rate	0.091	3	0.030	31.851	<0.0001
Error	0.011	12	0.001		
Total	0.102	15	0.007		

For **CORGI SHOOT DRY WEIGHT**, classified by diclofop-methyl rate

Group	Cases	Mean	1.9	0.95	0.475	0
1.9	4	0.1701		*	*	*
0.95	4	0.2846	*			*
0.475	4	0.3303	*			
0	4	0.3718	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
1.9 - 0	0.2016	0.0154	13.0815	3.3193	Reject
1.9 - 0.475	0.1601	0.0154	10.3890	3.2216	Reject
1.9 - 0.95	0.1145	0.0154	7.4288	3.0734	Reject
0.95 - 0	0.0871	0.0154	5.6527	3.2216	Reject
0.95 - 0.475	0.0456	0.0154	2.9602	3.0734	Accept
0.475 - 0	0.0415	0.0154	2.6925	3.0734	Accept

Homogeneous Subsets:

Group 1:	1.9		Group 2:	0.95, 0.475	
Pooled mean =	0.1701		Pooled mean =	0.3074	
95% Confidence Interval =	0.1365	0.2037	95% Confidence Interval =	0.2837	0.3312
Group 3:	0.475, 0				
Pooled mean =	0.3510				
95% Confidence Interval =	0.3273	0.3747			

Appendix 1.2.1 The effects of various rates (0, 0.475, 0.95, 1.9 kg AI ha^{-1}) of diclofop-methyl with and without fenpropimorph (0.75 kg AI ha^{-1}), on the visible injury of 4 spring barley cultivars, where n=4 or 8. Results of two-way ANOVA and Duncan's Multiple Range Tests for visible injury, classified by diclofop-methyl rate and ± fungicide. In the comparisons table, accept indicates that the two rates are not significantly different at $p \leq 0.05$ and * denotes significantly different pairs.

Dependent Variable: **SHERPA VISIBLE INJURY**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
fungicide	0.021	1	0.021	0.272	0.6055
d-m rate	6.228	2	3.114	39.780	<0.0001
fungicide × d-m rate	0.232	2	0.116	1.480	0.2437
Error	2.348	30	0.078		
Total	8.829	35	0.252		

For **SHERPA VISIBLE INJURY**, classified by diclofop-methyl rate ± fungicide

Group	Cases	Mean	0.475-F	0.475+F	0.95+F	0.95-F	1.9-F	1.9+F
0.475-F	8	0.1331			*	*	*	*
0.475+F	4	0.1418				*	*	*
0.95+F	4	0.5127	*				*	*
0.95-F	8	0.7963	*	*			*	*
1.9-F	8	1.1126	*	*	*	*		
1.9+F	4	1.2326	*	*	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
0.475-F - 1.9+F	1.0996	0.1212	9.0760	3.2507	Reject
0.475-F - 1.9-F	0.9796	0.0989	9.9027	3.1985	Reject
0.475-F - 0.95-F	0.6632	0.0989	6.7047	3.1344	Reject
0.475-F - 0.95+F	0.3796	0.1212	3.1334	3.0338	Reject
0.475-F - 0.475+F	0.0087	0.1212	0.0720	2.8863	Accept
0.475+F - 1.9+F	1.0908	0.1399	7.7976	3.1985	Reject
0.475+F - 1.9-F	0.9708	0.1212	8.0135	3.1344	Reject
0.475+F - 0.95-F	0.6545	0.1212	5.4024	3.0338	Reject
0.475+F - 0.95+F	0.3709	0.1399	2.6512	2.8863	Accept
0.95+F - 1.9+F	0.7199	0.1399	5.1464	3.1344	Reject
0.95+F - 1.9-F	0.6000	0.1212	4.9522	3.0338	Reject
0.95+F - 0.95-F	0.2836	0.1212	2.3410	2.8863	Accept
0.95-F - 1.9+F	0.4363	0.1212	3.6016	3.0338	Reject
0.95-F - 1.9-F	0.3163	0.0989	3.1980	2.8863	Reject
1.9-F - 1.9+F	0.1200	0.1212	0.9904	2.8863	Accept

Homogeneous Subsets:

Group 1:	0.475-F	0.475+F	Group 2:	0.475+F	0.95+F
Pooled mean =	0.136		Pooled mean =	0.327	
95% Confidence Interval =	-0.029	0.301	95% Confidence Interval =	0.125	0.529
Group 3:	0.95+F	0.95-F	Group 4:	1.9-F	1.9+F
Pooled mean =	0.702		Pooled mean =	1.153	
95% Confidence Interval =	0.537	0.867	95% Confidence Interval =	0.988	1.318

Dependent Variable: **CORGI VISIBLE INJURY**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
fungicide	0.022	1	0.022	0.616	0.4387
d-m rate	4.944	2	2.472	69.429	<0.0001
fungicide × d-m rate	0.195	2	0.098	2.740	0.0807
Error	1.068	30	0.036		

Total	6.229	35	0.178	

For **CORGI VISIBLE INJURY**, classified by diclofop-methyl rate ± fungicide

Group	Cases	Mean	0.475+F	0.475-F	0.95+F	0.95-F	1.9-F	1.9+F
0.475+F	4	0.0153			*	*	*	*
0.475-F	8	0.0600			*	*	*	*
0.95+F	4	0.4363	*	*			*	*
0.95-F	8	0.5072	*	*			*	*
1.9-F	8	0.8618	*	*	*	*		*
1.9+F	4	1.1345	*	*	*	*	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
0.475+F - 1.9+F	1.1192	0.0943	11.8626	3.2507	Reject
0.475+F - 1.9-F	0.8465	0.0817	10.3601	3.1985	Reject
0.475+F - 0.95-F	0.4920	0.0817	6.0212	3.1344	Reject
0.475+F - 0.95+F	0.4211	0.0943	4.4629	3.0338	Reject
0.475+F - 0.475-F	0.0447	0.0817	0.5474	2.8863	Accept
0.475-F - 1.9+F	1.0745	0.0817	13.1504	3.1985	Reject
0.475-F - 1.9-F	0.8018	0.0667	12.0181	3.1344	Reject
0.475-F - 0.95-F	0.4472	0.0667	6.7040	3.0338	Reject
0.475-F - 0.95+F	0.3763	0.0817	4.6060	2.8863	Reject
0.95+F - 1.9+F	0.6981	0.0943	7.3997	3.1344	Reject
0.95+F - 1.9-F	0.4254	0.0817	5.2068	3.0338	Reject
0.95+F - 0.95-F	0.0709	0.0817	0.8678	2.8863	Accept
0.95-F - 1.9+F	0.6272	0.0817	7.6766	3.0338	Reject
0.95-F - 1.9-F	0.3545	0.0667	5.3141	2.8863	Reject
1.9-F - 1.9+F	0.2727	0.0817	3.3377	2.8863	Reject

Homogeneous Subsets:

Group 1:	0.475+F	0.475-F	Group 2:	0.95+F	0.95-F
Pooled mean =	0.045		Pooled mean =	0.484	
95% Confidence Interval =	-0.066	0.156	95% Confidence Interval =	0.372	0.595
Group 3:	1.9-F		Group 4:	1.9+F	
Pooled mean =	0.862		Pooled mean =	1.134	
95% Confidence Interval =	0.726	0.998	95% Confidence Interval =	0.942	1.327

Dependent Variable: **TYNE VISIBLE INJURY** (no fungicide applied)

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
d-m rate	0.003	2	0.001	0.057	0.9453
Error	0.219	9	0.024		
Total	0.222	11	0.020		

Dependent Variable: **NUGGET VISIBLE INJURY** (no fungicide applied)

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
d-m rate	0.141	2	0.070	4.348	0.0477
Error	0.146	9	0.016		
Total	0.286	11	0.026		

For **NUGGET VISIBLE INJURY**, classified by diclofop-methyl rate

Group	Cases	Mean	0.475-F	1.9-F	0.95-F
0.475-F	4	0.0611		*	*
1.9-F	4	0.2727	*		
0.95-F	4	0.3054	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
0.475-F - 0.95-F	0.2443	0.0636	3.8413	3.3361	Reject

0.475-F - 1.9-F	0.2116	0.0636	3.3269	3.1903	Reject
1.9-F - 0.95-F	0.0327	0.0636	0.5145	3.1903	Accept

Homogeneous Subsets:

Group 1:	0.475-F		Group 2:	1.9-F	0.95-F
Pooled mean =	0.061		Pooled mean =	0.289	
95% Confidence Interval =	-0.083	0.205	95% Confidence Interval =	0.187	0.391

Appendix 1.2.2 The effects of various rates (0, 0.475, 0.95, 1.9 kg AI ha^{-1}) of diclofop-methyl with and without fenpropimorph (0.75 kg AI ha^{-1}), on the shoot dry weight of 2 spring barley cultivars, Sherpa and Corgi. Results of two-way ANOVA and Duncan's Multiple Range Tests for shoot dry weight, classified by rate of herbicide with and without fungicide. In the comparisons table, accept indicates that the two rates are not significantly different at $p \leq 0.05$ and * denotes significantly different pairs.

Dependent Variable: **SHERPA SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
fungicide	0.010	1	0.010	5.168	0.032
diclofop-methyl rate	0.081	3	0.027	13.962	<0.001
fungicide × diclofop-methyl rate	0.013	3	0.004	2.244	0.109
Error	0.046	24	0.002		
Total	0.150	31	0.005		

for **SHERPA SHOOT DRY WEIGHT WITHOUT FUNGICIDE**

Group	Cases	Mean	1.9	0.95	0.475	0
1.9	4	0.153			*	*
0.95	4	0.219				*
0.475	4	0.239	*			*
0	4	0.346	*	*	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
1.9 - 0	0.194	0.023	8.468	3.319	Reject
1.9 - 0.475	0.086	0.023	3.770	3.222	Reject
1.9 - 0.95	0.066	0.023	2.896	3.073	Accept
0.95 - 0	0.128	0.023	5.572	3.222	Reject
0.95 - 0.475	0.020	0.023	0.874	3.073	Accept
0.475 - 0	0.108	0.023	4.698	3.073	Reject

Homogeneous Subsets:

Group 1:	1.9 0.95		Group 2:	0.95 0.475	
Pooled mean =	0.186		Pooled mean =	0.229	
95% Confidence Interval =	0.150	0.221	95% Confidence Interval =	0.194	0.264
Group 3:	0				
Pooled mean =	0.346				
95% Confidence Interval =	0.296	0.396			

for **SHERPA SHOOT DRY WEIGHT**, classified by diclofop-methyl with fungicide

Group	Cases	Mean	1.9	0.95	0.475	0
1.9	4	0.160				*
0.95	4	0.190				
0.475	4	0.220				
0	4	0.245	*			

Comparison	Difference	Std Error	q Stat	Table q	Result
1.9 - 0	0.085	0.021	4.045	3.319	Reject
1.9 - 0.475	0.060	0.021	2.855	3.222	Accept
1.9 - 0.95	0.030	0.021	1.428	3.073	Accept
0.95 - 0	0.055	0.021	2.617	3.222	Accept
0.95 - 0.475	0.030	0.021	1.428	3.073	Accept
0.475 - 0	0.025	0.021	1.190	3.073	Accept

Homogeneous Subsets:

Group 1:	1.9, 0.95, 0.475		Group 2:	0.95, 0.475, 0	
Pooled mean =	0.19		Pooled mean =	0.218	
95% Confidence Interval =	0.164	0.216	95% Confidence Interval =	0.192	0.245

Dependent Variable: **CORGI SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
fungicide	0.001	1	0.001	0.665	0.423
diclofop-methyl rate	0.053	3	0.018	9.365	<0.001
fungicide × diclofop-methyl rate	0.009	3	0.003	1.527	0.233
Error	0.045	24	0.002		
Total	0.108	31	0.003		

for **CORGI SHOOT DRY WEIGHT**, classified by diclofop-methyl without fungicide

Group	Cases	Mean	1.9	0.95	0.475	0
1.9	4	0.158		*	*	*
0.95	4	0.229	*			
0.475	4	0.256	*			
0	4	0.299	*			

Comparison	Difference	Std Error	q Stat	Table q	Result
1.9 - 0	0.141	0.022	6.400	3.319	Reject
1.9 - 0.475	0.099	0.022	4.474	3.222	Reject
1.9 - 0.95	0.071	0.022	3.228	3.073	Reject
0.95 - 0	0.070	0.022	3.172	3.222	Accept
0.95 - 0.475	0.028	0.022	1.246	3.073	Accept
0.475 - 0	0.043	0.022	1.926	3.073	Accept

Homogeneous Subsets:

Group 1:	1.9		Group 2:	0.95, 0.475, 0	
Pooled mean =	0.158		Pooled mean =	0.261	
95% Confidence Interval =	0.109	0.206	95% Confidence Interval =	0.233	0.289

for **CORGI SHOOT DRY WEIGHT**, classified by diclofop-methyl with fungicide

Group	Cases	Mean	1.9	0.475	0	0.95
1.9	4	0.1963			*	*
0.475	4	0.2363				
0	4	0.2788	*			
0.95	4	0.2800	*			

Comparison	Difference	Std Error	q Stat	Table q	Result
1.9 - 0.95	0.084	0.021	3.938	3.319	Reject
1.9 - 0	0.083	0.021	3.880	3.222	Reject
1.9 - 0.475	0.040	0.021	1.881	3.073	Accept
0.475 - 0.95	0.044	0.021	2.057	3.222	Accept
0.475 - 0	0.043	0.021	1.999	3.073	Accept
0 - 0.95	0.001	0.021	0.059	3.073	Accept

Homogeneous Subsets:

Group 1:	1.9, 0.475		Group 2:	0.475, 0, 0.95	
Pooled mean =	0.216		Pooled mean =	0.265	
95% Confidence Interval =	0.183	0.249	95% Confidence Interval =	0.238	0.292

Appendix 1.3 Effects of mecoprop-p (0, 0.69, 1.38, 2.76 kg AI ha^{-1}) on the shoot dry weights of 4 spring barley cultivars. Results of one-way ANOVA and Duncan's Multiple Range Tests for shoot dry weight, classified by rate of herbicide. In the comparisons table, accept indicates that the two rates are not significantly different at $p < 0.05$ and * denotes significantly different pairs.

Dependent Variable: **TYNE SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
mecoprop-p rate	0.016	3	0.005	2.769	0.088
Error	0.023	12	0.002		
Total	0.039	15	0.003		

Dependent Variable: **NUGGET SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
mecoprop-p rate	0.018	3	0.006	3.721	0.042
Error	0.020	12	0.002		
Total	0.038	15	0.003		

For **NUGGET SHOOT DRY WEIGHT**, classified by mecoprop-p rate

Group	Cases	Mean	1.38	2.76	0.69	0
1.38	4	0.2590				*
2.76	4	0.2709				*
0.69	4	0.2781				*
0	4	0.3457	*	*	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
1.38 - 0	0.0867	0.0202	4.2893	3.3193	Reject
1.38 - 0.69	0.0191	0.0202	0.9433	3.2216	Accept
1.38 - 2.76	0.0119	0.0202	0.5885	3.0734	Accept
2.76 - 0	0.0748	0.0202	3.7009	3.2216	Reject
2.76 - 0.69	0.0072	0.0202	0.3548	3.0734	Accept
0.69 - 0	0.0677	0.0202	3.3460	3.0734	Reject

Homogeneous Subsets:

Group 1:	1.38 2.76 0.69		Group 2:	0	
Pooled mean =	0.2693		Pooled mean =	0.3457	
95% Confidence Interval =	0.2439	0.2948	95% Confidence Interval =	0.3017	0.3898

Dependent Variable: **SHERPA SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
mecoprop-p rate	0.014	3	0.005	2.348	0.124
Error	0.024	12	0.002		
Total	0.039	15	0.003		

Dependent Variable: **CORGI SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
mecoprop-p rate	0.023	3	0.008	2.781	0.087
Error	0.033	12	0.003		
Total	0.056	15	0.004		

Appendix 1.3.1 The effects of the fungicide fenpropimorph (0.75 kg AI ha^{-1}), on the shoot dry weights of 4 spring barley cultivars treated with mecoprop-p at various rates (0, 0.69, 1.38, 2.76 kg AI ha^{-1}). Results of two-way ANOVA and Duncan's Multiple Range Tests for shoot dry weight, classified by rate of herbicide with and without fungicide. In the comparisons table, accept indicates that the two rates are not significantly different at $p < 0.05$ and * denotes significantly different pairs.

Dependent Variable: **TYNE SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
fungicide	0.001	1	0.001	0.741	0.340
mecoprop-p rate	0.034	3	0.011	5.713	0.004
fungicide × mecoprop-p rate	0.001	3	0.000	0.136	0.938
Error	0.047	24	0.002		
Total	0.083	31	0.003		

For **TYNE SHOOT DRY WEIGHT**, classified by mecoprop-p rate without fungicide

Group	Cases	Mean	0.5	2	1	0
0.5	4	0.2090				*
2	4	0.2091				*
1	4	0.2420				
0	4	0.2862	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
.5 - 0	0.0773	0.0220	3.5158	3.3193	Reject
.5 - 1	0.0331	0.0220	1.5048	3.2216	Accept
.5 - 2	0.0001	0.0220	0.0068	3.0734	Accept
2 - 0	0.0771	0.0220	3.5089	3.2216	Reject
2 - 1	0.0329	0.0220	1.4980	3.0734	Accept
1 - 0	0.0442	0.0220	2.0110	3.0734	Accept

Homogeneous Subsets:

Group 1:	.5 2 1		Group 2:	1 0	
Pooled mean =	0.2200		Pooled mean =	0.2641	
95% Confidence Interval =	0.1924	0.2477	95% Confidence Interval =	0.2303	0.2980

For **TYNE SHOOT DRY WEIGHT**, classified by mecoprop-p rate with fungicide

Group	Cases	Mean	2	0.5	1	0
2	4	0.1801				*
0.5	4	0.2044				
1	4	0.2372				
0	4	0.2705	*			

Comparison	Difference	Std Error	q Stat	Table q	Result
2 - 0	0.0904	0.0224	4.0287	3.3193	Reject
2 - 1	0.0571	0.0224	2.5451	3.2216	Accept
2 - .5	0.0244	0.0224	1.0849	3.0734	Accept
.5 - 0	0.0661	0.0224	2.9438	3.2216	Accept
.5 - 1	0.0328	0.0224	1.4602	3.0734	Accept
1 - 0	0.0333	0.0224	1.4836	3.0734	Accept

Homogeneous Subsets:

Group 1:	2 .5 1		Group 2:	.5 1 0	
Pooled mean =	0.2072		Pooled mean =	0.2374	
95% Confidence Interval =	0.1790	0.2355	95% Confidence Interval =	0.2092	0.2656

Dependent Variable: **NUGGET SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
fungicide	0.006	1	0.006	3.084	0.092
mecoprop-p rate	0.047	3	0.016	8.228	0.001
fungicide × mecoprop-p rate	0.001	3	0.000	0.215	0.885
Error	0.046	24	0.002		
Total	0.100	31	0.003		

For **NUGGET SHOOT DRY WEIGHT**, classified by mecoprop-p rate -

Group	Cases	Mean	1	2	0.5	0
1	4	0.2593				*
2	4	0.2709				*
0.5	4	0.2781				*
0	4	0.3469	*	*	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
1 - 0	0.0876	0.0201	4.3626	3.3193	Reject
1 - .5	0.0188	0.0201	0.9358	3.2216	Accept
1 - 2	0.0116	0.0201	0.5784	3.0734	Accept
2 - 0	0.0760	0.0201	3.7842	3.2216	Reject
2 - .5	0.0072	0.0201	0.3574	3.0734	Accept
.5 - 0	0.0688	0.0201	3.4268	3.0734	Reject

Homogeneous Subsets:

Group 1:	1 2 .5		Group 2:	0	
Pooled mean =	0.2694		Pooled mean =	0.3469	
95% Confidence Interval =	0.2442	0.2947	95% Confidence Interval =	0.3031	0.3906

For **NUGGET SHOOT DRY WEIGHT**, classified by mecoprop-p rate with fungicide

Group	Cases	Mean	1	2	0.5	0
1	4	0.2735				*
2	4	0.2863				*
0.5	4	0.3191				
0	4	0.3850	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
1 - 0	0.1115	0.0236	4.7318	3.3193	Reject
1 - .5	0.0456	0.0236	1.9346	3.2216	Accept
1 - 2	0.0128	0.0236	0.5443	3.0734	Accept
2 - 0	0.0987	0.0236	4.1875	3.2216	Reject
2 - .5	0.0328	0.0236	1.3904	3.0734	Accept
.5 - 0	0.0659	0.0236	2.7971	3.0734	Accept

Homogeneous Subsets:

Group 1:	1 2 .5		Group 2:	.5 0	
Pooled mean =	0.2929		Pooled mean =	0.3520	
95% Confidence Interval =	0.2633	0.3226	95% Confidence Interval =	0.3157	0.3883

Dependent Variable: **SHERPA SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
fungicide	0.000	1	0.000	0.000	0.986
mecoprop-p rate	0.090	3	0.030	9.525	<0.001
fungicide × mecoprop-p rate	0.048	3	0.016	5.074	0.007
Error	0.076	24	0.003		
Total	0.214	31	0.007		

For **SHERPA SHOOT DRY WEIGHT**, classified by mecoprop-p rate without fungicide

Group	Cases	Mean	2	0.5	0	1
2	4	0.2486				
0.5	4	0.3113				
0	4	0.3127				
1	4	0.3198				

Comparison	Difference	Std Error	q Stat	Table q	Result
2 - 1	0.0712	0.0229	3.1076	3.3193	Accept
2 - 0	0.0641	0.0229	2.7967	3.2216	Accept
2 - .5	0.0627	0.0229	2.7367	3.0734	Accept
.5 - 1	0.0085	0.0229	0.3709	3.2216	Accept
.5 - 0	0.0014	0.0229	0.0600	3.0734	Accept
0 - 1	0.0071	0.0229	0.3109	3.0734	Accept

Homogeneous Subsets:

Group 1:	2 .5 0 1	
Pooled mean =	0.2981	
95% Confidence Interval =	0.2732	0.3231

For **SHERPA SHOOT DRY WEIGHT**, classified by mecoprop-p rate with fungicide

Group	Cases	Mean	2	1	0.5	0
2	4	0.2102				*
1	4	0.2478				*
0.5	4	0.2904				*
0	4	0.4428	*	*	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
2 - 0	0.2326	0.0324	7.1678	3.3193	Reject
2 - .5	0.0802	0.0324	2.4702	3.2216	Accept
2 - 1	0.0376	0.0324	1.1592	3.0734	Accept
1 - 0	0.1950	0.0324	6.0086	3.2216	Reject
1 - .5	0.0425	0.0324	1.3110	3.0734	Accept
.5 - 0	0.1524	0.0324	4.6977	3.0734	Reject

Homogeneous Subsets:

Group 1:	2 1 .5		Group 2:	0	
Pooled mean =	0.2495		Pooled mean =	0.4428	
95% Confidence Interval =	0.2086	0.2903	95% Confidence Interval =	0.3721	0.5135

Dependent Variable: **CORGI SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
fungicide	0.012	1	0.012	4.119	0.054
mecoprop-p rate	0.029	3	0.010	3.293	0.038
fungicide × mecoprop-p rate	0.002	3	0.001	0.271	0.846
Error	0.069	24	0.003		
Total	0.112	31	0.004		

Appendix 1.4 Effects of clopyralid (0, 0.05, 0.1, 0.2 kg AI ha^{-1}) on the shoot dry weights of 3 sugarbeet cultivars. Results of one-way ANOVA tests conducted on data.

Dependent Variable: **AMETHYST SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
clopyralid rate	0.001	3	0.000	0.044	0.987
Error	0.057	12	0.005		
Total	0.058	15	0.004		

Dependent Variable: **CELT SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
clopyralid rate	0.005	3	0.002	1.067	0.400
Error	0.018	12	0.001		
Total	0.023	15	0.002		

Dependent Variable: **SAXON SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
clopyralid rate	0.015	3	0.005	3.274	0.059
Error	0.018	12	0.002		
Total	0.033	15	0.002		

Appendix 1.5 Effects of diclofop-methyl (0, 0.57, 1.14, 2.28 kg AI ha^{-1}) on the shoot dry weights of 3 sugarbeet cultivars. Results of one-way ANOVA tests conducted on data.

Dependent Variable: **AMETHYST SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
diclofop-methyl rate	0.011	3	0.004	1.912	0.182
Error	0.024	12	0.002		
Total	0.035	15	0.002		

Dependent Variable: **CELT SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
diclofop-methyl rate	0.012	3	0.004	2.277	0.132
Error	0.020	12	0.002		
Total	0.032	15	0.002		

Dependent Variable: **SAXON SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
diclofop-methyl rate	0.038	3	0.013	2.896	0.079
Error	0.053	12	0.004		
Total	0.091	15	0.006		

Appendix 1.6 Effects of phenmedipham (0, 0.57, 1.14, 2.28 kg AI ha^{-1}) on the shoot dry weights of 3 sugarbeet cultivars. Results of one-way ANOVA and Duncan's Multiple Range Tests for shoot dry weight, classified by rate of herbicide. In the comparisons table, accept indicates that the two rates are not significantly different at $p \leq 0.05$ and * denotes significantly different pairs.

Dependent Variable: **AMETHYST SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
phenmedipham rate	0.162	3	0.054	22.349	<0.001
Error	0.029	12	0.002		
Total	0.191	15	0.013		

For **AMETHYST SHOOT DRY WEIGHT**, classified by phenmedipham rate

Group	Cases	Mean	0.57	2.28	1.14	0
0.57	4	0.098				*
2.28	4	0.100				*
1.14	4	0.153				*
0	4	0.344	*	*	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
0.57 - 0	0.246	0.025	10.007	3.319	Reject
0.57 - 1.14	0.055	0.025	2.235	3.222	Accept
0.57 - 2.28	0.003	0.025	0.102	3.073	Accept
2.28 - 0	0.244	0.025	9.905	3.222	Reject
2.28- 1.14	0.053	0.025	2.133	3.073	Accept
1.14 - 0	0.191	0.025	7.772	3.073	Reject

Homogeneous Subsets:

Group 1:	0.57, 2.28, 1.14		Group 2:	0	
Pooled mean =	0.117		Pooled mean =	0.344	
95% Confidence Interval =	0.009	0.148	95% Confidence Interval =	0.290	0.397

Dependent Variable: **CELT SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
phenmedipham rate	0.034	3	0.011	5.931	0.010
Error	0.023	12	0.002		
Total	0.057	15	0.004		

For **CELT SHOOT DRY WEIGHT**, classified by phenmedipham rate

Group	Cases	Mean	2.28	1.14	0.57	0
2.28	4	0.065			*	*
1.14	4	0.124				
0.57	4	0.166	*			
0	4	0.185	*			

Comparison	Difference	Std Error	q Stat	Table q	Result
2.28 - 0	0.120	0.022	5.490	3.319	Reject
2.28 - 0.57	0.101	0.022	4.632	3.222	Reject
2.28 - 1.14	0.059	0.022	2.688	3.073	Accept
1.14 - 0	0.061	0.022	2.802	3.222	Accept
1.14 - 0.57	0.043	0.022	1.944	3.073	Accept
0.57 - 0	0.019	0.022	0.858	3.073	Accept

Homogeneous Subsets:

Group 1:	2.28, 1.14	Group 2:	1.14, 0.57, 0

Pooled mean =	0.094		Pooled mean =	0.158	
95% Confidence Interval =	0.061	0.128	95% Confidence Interval =	0.131	0.186

Dependent Variable: **SAXON SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
phenmedipham rate	0.074	3	0.025	8.578	0.003
Error	0.035	12	0.003		
Total	0.109	15	0.007		

For **SAXON SHOOT DRY WEIGHT**, classified by phenmedipham rate

Group	Cases	Mean	2.28	1.14	0.57	0
2.28	4	0.086			*	*
1.14	4	0.163				*
0.57	4	0.195	*			
0	4	0.276	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
2.28 - 0	0.190	0.027	7.070	3.319	Reject
2.28 - 0.57	0.109	0.027	4.047	3.222	Reject
2.28 - 1.14	0.076	0.027	2.838	3.073	Accept
1.14 - 0	0.114	0.027	4.233	3.222	Reject
1.14 - 0.57	0.033	0.027	1.209	3.073	Accept
0.57 - 0	0.081	0.027	3.024	3.073	Accept

Homogeneous Subsets:

Group 1:	2.28, 1.14		Group 2:	1.14, 0.57	
Pooled mean =	0.124		Pooled mean =	0.179	
95% Confidence Interval =	0.008	0.166	95% Confidence Interval =	0.137	0.220
Group 3:	0.57, 0				
Pooled mean =	0.236				
95% Confidence Interval =	0.194	0.277			

Appendix 2.1 Effects of diclofop-methyl (0.95kg AI ha^{-1}) and ozone (100 nl l^{-1}, 7 h d^{-1}, 2 d) on the shoot dry weights of 4 spring barley cultivars, where n=4. Results of two-way ANOVA tests conducted on data. (See Figure 4.1)

Dependent Variable: **SHERPA SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.003	1	0.003	3.578	0.083
diclofop-methyl	0.000	1	0.000	0.071	0.795
ozone × diclofop-methyl	0.002	1	0.002	2.132	0.170
Error	0.009	12	0.001		
Total	0.013	15	0.001		

Dependent Variable: **SHERPA LEAF AREA**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	22.4	1	22.4	0.192	0.669
diclofop-methyl	29.8	1	29.8	0.255	0.622
ozone × diclofop-methyl	123.8	1	123.8	1.061	0.323
Error	1400.0	12	116.7		
Total	1576.0	15	105.1		

Dependent Variable: **CORGI SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.015	1	0.015	5.542	0.036
diclofop-methyl	0.001	1	0.001	0.415	0.532
ozone × diclofop-methyl	0.000	1	0.000	0.000	0.999
Error	0.032	12	0.003		
Total	0.048	15	0.003		

Dependent Variable: **CORGI LEAF AREA**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	443.866	1	443.9	1.978	0.185
diclofop-methyl	673.759	1	673.8	3.002	0.109
ozone × diclofop-methyl	217.618	1	217.6	0.970	0.344
Error	2693.325	12	224.4		
Total	4028.569	15	268.6		

Appendix 2.2 Effects of diclofop-methyl (1.14 kg AI ha^{-1}) and ozone (100 nl l^{-1}, 7 h d^{-1}, 2 d) on the shoot dry weights of 2 spring oilseed rape cultivars, where n=12. Results of two-way ANOVA tests conducted on data. (See Figure 4.2)

Dependent Variable: **GALAXY SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.004	1	0.004	0.757	0.389
diclofop-methyl	0.023	1	0.023	3.843	0.056
ozone × diclofop-methyl	0.009	1	0.009	1.471	0.232
Error	0.258	44	0.006		
Total	0.294	47	0.006		

Dependent Variable: **STARLIGHT SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.070	1	0.070	2.944	0.093
diclofop-methyl	0.007	1	0.007	0.301	0.586
ozone × diclofop-methyl	0.000	1	0.000	0.006	0.937
Error	1.041	44	0.024		
Total	1.117	47	0.024		

Appendix 2.2.1 Effects of diclofop-methyl (1.14 kg AI ha^{-1}) and ozone (100 nl l^{-1}, 7 h d^{-1}, 2 d) on visible injury on 2 spring oilseed rape cultivars, where n=12. Results of two-way ANOVA tests conducted on data and Duncan's Multiple Range Test. (See Table 4.1). Analysis conducted on data which has been ARC-SIN transformed.

Dependent Variable: **GALAXY VISIBLE INJURY**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.543	1	0.543	11.534	0.0043
dm	0.021	1	0.021	0.440	0.5179
ozone × dm	0.000	1	0.000	0.000	1.0000
Error	0.659	14	0.047		
Total	1.269	17	0.075		

Higher interactions cannot be estimated due to multi-collinearity

For **GALAXY VISIBLE INJURY**, classified by treatment

Group	Cases	Mean	dm	ozone	dm.o3
dm	6	0.2842		*	*
ozone	6	0.6265	*		
dm.o3	6	0.7096	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
dm - dm.o3	0.4254	0.0856	4.9715	3.1564	Reject
dm - ozone	0.3423	0.0856	4.0006	3.0077	Reject
ozone - dm.o3	0.0831	0.0856	0.9709	3.0077	Accept

Homogeneous Subsets:

Group 1:	dm		Group 2:	ozone	dm.o3
Pooled mean =	0.2842		Pooled mean =	0.6680	
95% Confidence Interval =	0.1018	0.4666	95% Confidence Interval =	0.5391	0.7970

Dependent Variable: **STARLIGHT VISIBLE INJURY**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	1.215	1	1.215	32.052	0.0001
dm	0.060	1	0.060	1.579	0.2295
ozone × dm	0.000	1	0.000	0.000	1.0000
Error	0.531	14	0.038		
Total	1.872	17	0.110		

Higher interactions cannot be estimated due to multi-collinearity

For **STARLIGHT VISIBLE INJURY**, classified by treatment

Group	Cases	Mean	dm	ozone	dm.o3
dm	6	0.1905		*	*
ozone	6	0.6858	*		
dm.o3	6	0.8270	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
dm - dm.o3	0.6365	0.0768	8.2875	3.1564	Reject
dm - ozone	0.4952	0.0768	6.4482	3.0077	Reject

ozone - dm.o3	0.1413	0.0768	1.8393	3.0077	Accept

Homogeneous Subsets:

Group 1:	dm		Group 2:	ozone dm.o3	
Pooled mean =	0.1905		Pooled mean =	0.7564	
95% Confidence Interval =	0.0268	0.3542	95% Confidence Interval =	0.6406	0.8722

Appendix 2.3 Effects of metazachlor (0.75 kg AI ha^{-1}) and ozone (100 nl l^{-1}, 7 h d^{-1}, 2 d) on the shoot dry weights of 2 spring oilseed rape cultivars, where n=4. Results of two-way ANOVA tests conducted on data. (See Figure 4.3)

Dependent Variable: **GALAXY SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.044	1	0.044	3.200	0.099
metazachlor	0.005	1	0.005	0.334	0.574
ozone × metazachlor	0.006	1	0.006	0.443	0.518
Error	0.167	12	0.014		
Total	0.222	15	0.015		

Dependent Variable: **STARLIGHT SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.059	1	0.059	2.626	0.131
metazachlor	0.002	1	0.002	0.101	0.757
ozone × metazachlor	0.050	1	0.050	2.220	0.162
Error	0.272	12	0.023		
Total	0.384	15	0.026		

Appendix 2.4 Effects of clopyralid (0.10 kg AI ha^{-1}) and ozone (100 nl l^{-1}, 7 h d^{-1}, 2 d) on the shoot dry weights of 2 spring oilseed rape cultivars, where n=4. Results of two-way ANOVA tests conducted on data. (See Figure 4.4)

Dependent Variable: **GALAXY SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.017	1	0.017	1.257	0.284
clopyralid	0.008	1	0.008	0.564	0.467
ozone × clopyralid	0.070	1	0.070	5.063	0.044
Error	0.165	12	0.014		
Total	0.260	15	0.017		

Dependent Variable: **STARLIGHT SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.082	1	0.082	3.347	0.092
clopyralid	0.000	1	0.000	0.000	0.984
ozone × clopyralid	0.033	1	0.033	1.365	0.265
Error	0.292	12	0.024		
Total	0.407	15	0.027		

Appendix 2.4.1 Effects of clopyralid (0.10 kg AI ha^{-1}) and ozone (100 nl l^{-1}, 7 h d^{-1}, 2 d) on the shoot dry weights of 2 spring oilseed rape cultivars, where n=4. Results of Duncan's Multiple Range Tests for shoot dry weight, classified by clopyralid. In the comparisons table, accept indicates that the two treatments are not significantly different at $p < 0.05$ and * denotes significantly different pairs. (See Figure 4.4)

For **GALAXY SHOOT DRY WEIGHT**, classified by treatment

Group	Cases	Mean	clopyralid/ ozone	control	ozone	clopyralid
clopyralid/ozone	4	0.415				*
control	4	0.437				
ozone	4	0.503				
clopyralid	4	0.613	*			

Comparison	Difference	Std Error	q Stat	Table q	Result
clopyralid/ozone - clopyralid	0.198	0.059	3.371	3.319	Reject
clopyralid/ozone - ozone	0.088	0.059	1.499	3.222	Accept
clopyralid/ozone - control	0.022	0.059	0.370	3.073	Accept
control - clopyralid	0.176	0.059	3.001	3.222	Accept
control - ozone	0.066	0.059	1.129	3.073	Accept
ozone - clopyralid	0.110	0.059	1.872	3.073	Accept

Homogeneous Subsets:

Group 1:	clopyralid/ozone, control, ozone		Group 2:	control, ozone, clopyralid	
Pooled mean =	0.451		Pooled mean =	0.517	
95% Confidence Interval =	0.378	0.525	95% Confidence Interval =	0.444	0.591

Appendix 2.5 Effects of phenmedipham (1.14 kg AI ha^{-1}) and ozone (100 nl l^{-1}, 7 h d^{-1}, 2 d) on Saxon leaf area 7 and 14 days after the end of exposure to ozone, where n=4 Results of two-way ANOVA tests conducted on data. (See Figure 4.5)

Dependent Variable: **7 DAY LEAF AREA**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
phenmedipham	16.621	1	16.621	5.418	0.0382
ozone	47.584	1	47.584	15.511	0.0020
phenmedipham x ozone	26.658	1	26.658	8.690	0.0122
Error	36.813	12	3.068		
Total	127.676	15	8.512		

Dependent Variable: **14 DAY LEAF AREA**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
phenmedipham	54.234	1	54.234	5.400	0.0385
ozone	10.550	1	10.550	1.050	0.3256
phenmedipham x ozone	34.538	1	34.538	3.439	0.0884
Error	120.523	12	10.044		
Total	219.845	15	14.656		

Appendix 2.5.1 Effects of phenmedipham (1.14 kg AI ha^{-1}) and ozone (100 nl l^{-1}, 7 h d^{-1}, 2 d) on sugarbeet cultivar, Saxon, leaf area, harvested 7 and 14 days after the end of exposure to ozone, where n=4 Results of Duncan's Multiple Range Tests for leaf area, classified by treatment. In the comparisons table, accept indicates that the two treatments are not significantly different at $p < 0.05$ and * denotes significantly different pairs. (See Figure 4.5)

For 7 **DAY LEAF AREA**, classified by treatment

Group	Cases	Mean	phenmedipham/ozone	ozone	control	phenmedipham
phenmedipham/ozone	4	21.97		*	*	*
ozone	4	26.59	*			
control	4	27.45	*			
phenmedipham	4	28.00	*			

Comparison	Difference	Std Error	q Stat	Table q	Result
phenmedipham/ozone - phenmedipham	6.03	0.8758	6.8862	3.3193	Reject
phenmedipham/ozone - control	5.49	0.8758	6.2660	3.2216	Reject
phenmedipham/ozone - ozone	4.62	0.8758	5.2755	3.0734	Reject
ozone - phenmedipham	1.41	0.8758	1.6108	3.2216	Accept
ozone - control	0.87	0.8758	0.9906	3.0734	Accept
control - phenmedipham	0.54	0.8758	0.6202	3.0734	Accept

Homogeneous Subsets:

Group 1:	phenmedipham/ozone		Group 2:	ozone control phenmedipham	
Pooled mean =	21.97		Pooled mean =	27.35	
95% Confidence Interval =	20.06	23.87	95% Confidence Interval =	26.24	28.45

Appendix 2.6 Effects of phenmedipham (1.14 kg AI ha^{-1}) and ozone (100 nl l^{-1}, 7 h d^{-1}, 2 d) on sugarbeet cultivar, Saxon, shoot dry weight 7 and 14 days after the end of exposure to ozone, where n=4 Results of two-way ANOVA tests conducted on data. (See Figure 4.5)

Dependent Variable: **7 DAY SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
phenmedipham	0.031	1	0.031	55.732	0.0000
ozone	0.007	1	0.007	11.661	0.0051
phenmedipham × ozone	0.003	1	0.003	5.490	0.0372
Error	0.007	12	0.001		
Total	0.048	15	0.003		

Dependent Variable: **14 DAY SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
phenmedipham	0.076	1	0.076	27.625	0.0002
ozone	0.032	1	0.032	11.645	0.0051
phenmedipham × ozone	0.018	1	0.018	6.423	0.0262
Error	0.033	12	0.003		
Total	0.158	15	0.011		

Appendix 2.6.1 Effects of phenmedipham (1.14 kg AI ha^{-1}) and ozone (100 nl l^{-1}, 7 h d^{-1}, 2 d) on sugarbeet cultivar, Saxon, shoot dry weight 7 and 14 days after the end of exposure to ozone, where n=4 Results of Duncan's Multiple Range Tests for shoot dry weight, classified by treatment. In the comparisons table, accept indicates that the two treatments are not significantly different at $p < 0.05$ and * denotes significantly different pairs. (See Figure 4.5)

For **7 DAY SHOOT DRY WEIGHT**, classified by treatment

Group	Cases	Mean	phen /ozone	phen	ozone	control
phenmedipham/ozone	4	0.3009		*	*	*
phenmedipham	4	0.3693	*		*	*
ozone	4	0.4174	*	*		
control	4	0.4301	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
phenmedipham/ozone - control	0.1293	0.0119	10.8802	3.3193	Reject
phenmedipham/ozone - ozone	0.1165	0.0119	9.8085	3.2216	Reject
phenmedipham/ozone - phenmedipham	0.0684	0.0119	5.7579	3.0734	Reject
phenmedipham - control	0.0609	0.0119	5.1223	3.2216	Reject
phenmedipham - ozone	0.0481	0.0119	4.0506	3.0734	Reject
ozone - control	0.0127	0.0119	1.0717	3.0734	Accept

Homogeneous Subsets:

Group 1:	phenmedipham /ozone		Group 2:	phenmedipham	
Pooled mean =	0.3009		Pooled mean =	0.3693	
95% Confidence Interval =	0.2750	0.3268	95% Confidence Interval =	0.3434	0.3952
Group 3:	ozone control				
Pooled mean =	0.4238				
95% Confidence Interval =	0.4055	0.4421			

For **14 DAY SHOOT DRY WEIGHT**, classified by treatment

Group	Cases	Mean	phen /ozone	phen	ozone	control
phenmedipham/ozone	4	0.4917				*
phenmedipham	4	0.5147				*
ozone	4	0.5629				*
control	4	0.7186	*	*	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
phenmedipham/ozone - control	0.2269	0.0262	8.6684	3.3193	Reject
phenmedipham/ozone - ozone	0.0712	0.0262	2.7216	3.2216	Accept
phenmedipham/ozone - phenmedipham	0.0230	0.0262	0.8781	3.0734	Accept
phenmedipham - control	0.2039	0.0262	7.7903	3.2216	Reject
phenmedipham - ozone	0.0482	0.0262	1.8435	3.0734	Accept
ozone - control	0.1556	0.0262	5.9468	3.0734	Reject

Homogeneous Subsets:

Group 1:	phenmedipham /ozone phenmedipham ozone		Group 2:	control	
Pooled mean =	0.5231		Pooled mean =	0.7186	
95% Confidence Interval =	0.4902	0.5560	95% Confidence Interval =	0.6616	0.7756

Appendix 2.7 Effects of phenmedipham (1.14 kg AI ha^{-1}) and ozone (100 nl l^{-1}, 7 h d^{-1}, 2 d) on Saxon visible injury 7 and 14 days after the end of exposure to ozone, where n=4 Results of two-way ANOVA tests conducted on data. (See Table 4.2)

Dependent Variable: **7 DAY VISIBLE INJURY**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.166	1	0.166	10.859	0.0109
phen	0.093	1	0.093	6.108	0.0386
ozone × phen	0.000	1	0.000	0.000	1.0000
Error	0.122	8	0.015		
Total	0.302	11	0.027		

Higher interactions cannot be estimated due to multi-collinearity

For **7 DAY VISIBLE INJURY**, classified by treatment

Group	Cases	Mean	phen	ozone	phen.ozo
phen	4	0.2705			*
ozone	4	0.3425			*
phen.ozo	4	0.5585	*	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
phen - phen.ozo	0.2880	0.0583	4.9431	3.3361	Reject
phen - ozone	0.0720	0.0583	1.2358	3.1903	Accept
ozone - phen.ozo	0.2160	0.0583	3.7073	3.1903	Reject

Homogeneous Subsets:

Group 1:	phen ozone		Group 2:	phen.ozo	
Pooled mean =	0.307		Pooled mean =	0.559	
95% Confidence Interval =	0.213	0.400	95% Confidence Interval =	0.427	0.690

Dependent Variable: **14 DAY VISIBLE INJURY**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.381	1	0.381	11.951	0.0086
phen	0.711	1	0.711	22.308	0.0015
ozone × phen	0.000	1	0.000	0.000	1.0000
Error	0.255	8	0.032		
Total	1.017	11	0.092		

Higher interactions cannot be estimated due to multi-collinearity

For **14 DAY VISIBLE INJURY**, classified by treatment

Group	Cases	Mean	ozone	phen	phen.ozo
ozone	4	0.2258			*
phen	4	0.3856			*
phen.ozo	4	0.8219	*	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
ozone - phen.ozo	0.5961	0.0841	7.0847	3.3361	Reject
ozone - phen	0.1598	0.0841	1.8992	3.1903	Accept
phen - phen.ozo	0.4363	0.0841	5.1855	3.1903	Reject

Homogeneous Subsets:

Group 1:	ozone phen		Group 2:	phen.ozo	
Pooled mean =	0.3057		Pooled mean =	0.8219	
95% Confidence Interval =	0.1711	0.4403	95% Confidence Interval =	0.6316	1.0123

Appendix 2.8 Effects of ozone (100 nl l^{-1}, 7 h d^{-1}, 2 d) and phenmedipham (1.14 kg AI ha^{-1}) on leaf area of sugarbeet cultivar, Saxon, 7 days after treatment with phenmedipham, where n=8. Results of two-way ANOVA conducted on data. (see Figure 4.6a)

Dependent Variable: **SAXON LEAF AREA**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	22.37	1	22.37	1.515	0.2286
phenmedipham	1225.06	1	1225.06	82.982	0.0000
ozone × phenmedipham	64.37	1	64.37	4.360	0.0460
Error	413.36	28	14.76		
Total	1725.17	31	55.65		

Appendix 2.8.1 Effects of ozone (100 nl l^{-1}, 7 h d^{-1}, 2 d) and phenmedipham (1.14 kg AI ha^{-1}) on leaf area of sugarbeet cultivar, Saxon, 7 days after treatment with phenmedipham, where n=8. Results of Duncan's Multiple Range Tests for leaf area, classified by treatment. In the comparisons table, accept indicates that the two treatments are not significantly different at $p < 0.05$ and *denotes significantly different pairs. (See Figure 4.6)

For **SAXON LEAF AREA**, classified by treatment

Group	Cases	Mean	phen	ozone /phen	ozone	control
phenmedipham	8	24.61			*	*
ozone/phenmedipham	8	25.77			*	*
ozone	8	35.31	*	*		*
control	8	39.82	*	*	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
phenmedipham - control	15.21	1.3584	11.1975	3.1432	Reject
phenmedipham - ozone	10.70	1.3584	7.8785	3.0423	Reject
phenmedipham - ozone/phenmedipham	1.16	1.3584	0.8571	2.8945	Accept
ozone/phenmedipham - control	14.05	1.3584	10.3404	3.0423	Reject
ozone/phenmedipham - ozone	9.54	1.3584	7.0214	2.8945	Reject
ozone - control	4.51	1.3584	3.3191	2.8945	Reject

Homogeneous Subsets:

Group 1:	phenmedipham ozone /phenmedipham		Group 2:	ozone	
Pooled mean =	25.19		Pooled mean =	35.31	
95% Confidence Interval =	23.22	27.15	95% Confidence Interval =	32.52	38.09
Group 3:	control				
Pooled mean =	39.82				
95% Confidence Interval =	37.03	42.60			

Appendix 2.9 Effects of ozone (100 nl l^{-1}, 7 h d^{-1}, 2 d) and phenmedipham (1.14 kg AI ha^{-1}) on shoot dry weight of sugarbeet cultivar Saxon 7 days after treatment with phenmedipham, where n=8. Results of two-way ANOVA conducted on data. (see Figure 4.6b)

Dependent Variable: **SAXON SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.000	1	0.000	1.109	0.3014
phenmedipham	0.045	1	0.045	220.557	0.0000
ozone × phenmedipham	0.001	1	0.001	3.274	0.0811
Error	0.006	28	0.000		
Total	0.052	31	0.002		

Appendix 2.9.1 Effects of ozone (100 nl l^{-1}, 7 h d^{-1}, 2 d) and phenmedipham (1.14 kg AI ha^{-1}) on shoot dry weight of sugarbeet cultivar, Saxon, 7 days after treatment with phenmedipham, where n=8. Results of Duncan's Multiple Range Tests for shoot dry weight, classified by treatment. In the comparisons table, accept indicates that the two treatments are not significantly different at $p \leq 0.05$ and *denotes significantly different pairs.

For **SAXON SHOOT DRY WEIGHT**, classified by treatment

Group	Cases	Mean	phen	ozone /phen	ozone	control
phenmedipham	8	0.1516			*	*
ozone/phenmedipham	8	0.1554			*	*
ozone	8	0.2216	*	*		
control	8	0.2362	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
phenmedipham - control	0.0846	0.0051	16.6606	3.1432	Reject
phenmedipham - ozone	0.0701	0.0051	13.7983	3.0423	Reject
phenmedipham - ozone/phenmedipham	0.0038	0.0051	0.7565	2.8945	Accept
ozone/phenmedipham - control	0.0807	0.0051	15.9041	3.0423	Reject
ozone/phenmedipham - ozone	0.0662	0.0051	13.0418	2.8945	Reject
ozone - control	0.0145	0.0051	2.8623	2.8945	Accept

Homogeneous Subsets:

Group 1:	phenmedipham ozone/phenmedipham		Group 2:	ozone control	
Pooled mean =	0.1535		Pooled mean =	0.2289	
95% Confidence Interval =	0.1461	0.1608	95% Confidence Interval =	0.2215	0.2362

Appendix 2.10 Effects of ozone (100 nl l^{-1}, 7 h d^{-1}, 2 d) and phenmedipham (1.14 kg AI ha^{-1}) on leaf area of sugarbeet cultivar, Celt, 7 days after treatment with phenmedipham, where n=8 Results of two-way ANOVA conducted on data. (see Figure 4.6c)

Dependent Variable: **CELT LEAF AREA**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	64.241	1	64.241	2.354	0.1362
phenmedipham	559.451	1	559.451	20.501	0.0001
ozone × phenmedipham	263.007	1	263.007	9.638	0.0043
Error	764.095	28	27.289		
Total	1650.795	31	53.251		

Appendix 2.10.1 Effects of ozone (100 nl l^{-1}, 7 h d^{-1}, 2 d) and phenmedipham (1.14 kg AI ha^{-1}) on leaf area of sugarbeet cultivar, Celt, 7 days after treatment with phenmedipham, where n=8. Results of Duncan's Multiple Range Tests for leaf area, classified by treatment. In the comparisons table, accept indicates that the two treatments are not significantly different at $p < 0.05$ and *denotes significantly different pairs.

For **CELT LEAF AREA**, classified by treatment

Group	Cases	Mean	phen	ozone /phen	ozone	control
phenmedipham	8	20.34				*
ozone/phenmedipham	8	23.24				*
ozone	8	25.87				*
control	8	34.43	*	*	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
phenmedipham - control	14.10	1.8469	7.6323	3.1432	Reject
phenmedipham - ozone	5.53	1.8469	2.9935	3.0423	Accept
phenmedipham - ozone/phenmedipham	2.90	1.8469	1.5702	2.8945	Accept
ozone/phenmedipham - control	11.20	1.8469	6.0621	3.0423	Reject
ozone/phenmedipham - ozone	2.63	1.8469	1.4233	2.8945	Accept
ozone - control	8.57	1.8469	4.6388	2.8945	Reject

Homogeneous Subsets:

Group 1: phenmedipham ozone/phenmedipham ozone			Group 2: control		
Pooled mean =	23.15		Pooled mean =	34.43	
95% Conf Int =	20.96	25.33	95% Conf Int =	30.65	38.22

Appendix 2.11 Effects of ozone (100 nl l^{-1}, 7 h d^{-1}, 2 d) and phenmedipham (1.14 kg AI ha^{-1}) on shoot dry weight of sugarbeet cultivar Celt 7 days after treatment with phenmedipham, n=8. Results of ANOVA and Duncan's Multiple Range Tests for shoot dry weight, classified by treatment. In the comparisons table, accept indicates that the two treatments are not significantly different at $p < 0.05$ and *denotes significantly different pairs. (see Figure 4.6d)

Dependent Variable: **CELT SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.000	1	0.000	0.309	0.5827
phenmedipham	0.014	1	0.014	36.644	0.0000
ozone × phenmedipham	0.002	1	0.002	6.307	0.0181
Error	0.011	28	0.000		
Total	0.028	31	0.001		

For **CELT SHOOT DRY WEIGHT**, classified by treatment

Group	Cases	Mean	phen	ozone /phen	ozone	control
phenmedipham	8	0.0642			*	*
ozone/phenmedipham	8	0.0778			*	*
ozone	8	0.1024	*	*		*
control	8	0.1237	*	*	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
phenmedipham - control	0.0595	0.0069	8.5648	3.1432	Reject
phenmedipham - ozone	0.0382	0.0069	5.4976	3.0423	Reject
phenmedipham - ozone/phenmedipham	0.0136	0.0069	1.9555	2.8945	Accept
ozone/phenmedipham - control	0.0459	0.0069	6.6093	3.0423	Reject
ozone/phenmedipham - ozone	0.0246	0.0069	3.5421	2.8945	Reject
ozone - control	0.0213	0.0069	3.0672	2.8945	Reject

Homogeneous Subsets:

Group 1:	phenmedipham ozone/phenmedipham		Group 2:	ozone	
Pooled mean =	0.0710		Pooled mean =	0.1024	
95% Confidence Interval =	0.0609	0.0811	95% Confidence Interval =	0.0882	0.1166
Group 3:	control				
Pooled mean =	0.1237				
95% Confidence Interval =	0.1095	0.1379			

Appendix 2.12 Effects of ozone (100 nl l^{-1}, 7 h d^{-1}, 2 d) and diclofop-methyl (1.14 kg AI ha^{-1}) on the leaf area and shoot dry weight of 2 sugarbeet cultivars, 7 days after treatment with diclofop-methyl, where n=4. Results of two-way ANOVA tests conducted on data. (See Figure 4.7)

Dependent Variable: **SAXON LEAF AREA**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	25.3	1	25.3	0.839	0.378
diclofop-methyl	92.2	1	92.2	3.064	0.106
ozone × diclofop-methyl	17.9	1	17.9	0.593	0.456
Error	361.3	12	30.1		
Total	496.7	15	33.1		

Dependent Variable: **SAXON SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.000	1	0.000	0.747	0.404
diclofop-methyl	0.000	1	0.000	0.019	0.893
ozone × diclofop-methyl	0.000	1	0.000	0.017	0.898
Error	0.006	12	0.001		
Total	0.007	15	0.000		

Dependent Variable: **CELT LEAF AREA**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	21.1	1	21.1	1.050	0.326
diclofop-methyl	154.4	1	154.4	7.664	0.017
ozone × diclofop-methyl	28.1	1	28.1	1.392	0.261
Error	241.7	12	20.1		
Total	445.3	15	29.7		

Dependent Variable: **CELT SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.000	1	0.000	0.206	0.658
diclofop-methyl	0.003	1	0.003	12.415	0.004
ozone × diclofop-methyl	0.000	1	0.000	0.064	0.805
Error	0.003	12	0.000		
Total	0.006	15	0.000		

Appendix 2.12.1 Effects of ozone (100 nl l^{-1}, 7 h d^{-1}, 2 d) and diclofop-methyl (1.14 kg AI ha^{-1}) on leaf area of sugarbeet cultivar, Celt, 7 days after treatment with diclofop-methyl, where n=4. Results of Duncan's Multiple Range Tests for shoot dry weight, classified by treatment. In the comparisons table, accept indicates that the two treatments are not significantly different at $p < 0.05$ and * denotes significantly different pairs.

For **CELT LEAF AREA**, classified by treatment

Group	Cases	Mean	diclofop-methyl /ozone	diclofop-methyl	control	ozone
diclofop-methyl/ozone	4	22.31			*	*
diclofop-methyl	4	27.26				
control	4	30.82	*			
ozone	4	31.17	*			

Comparison	Difference	Std Error	q Stat	Table q	Result
diclofop-methyl/ozone - ozone	8.86	2.244	3.948	3.319	Reject
diclofop-methyl/ozone - control	8.51	2.244	3.793	3.222	Reject
diclofop-methyl/ozone - diclofop-methyl	4.95	2.244	2.205	3.073	Accept
diclofop-methyl - ozone	3.91	2.244	1.744	3.222	Accept
diclofop-methyl - control	3.56	2.244	1.588	3.073	Accept
control - ozone	0.35	2.244	0.155	3.073	Accept

Homogeneous Subsets:

Group 1:	diclofop-methyl/ ozone, diclofop-methyl		Group 2:	diclofop-methyl, control, ozone	
Pooled mean =	24.79		Pooled mean =	29.75	
95% Confidence Interval =	21.33	28.24	95% Confidence Interval =	26.93	32.57

For **CELT SHOOT DRY WEIGHT**, classified by treatment

Group	Cases	Mean	diclofop-methyl /ozone	diclofop-methyl	ozone	control
diclofop-methyl/ozone	4	0.130			*	*
diclofop-methyl	4	0.136				*
ozone	4	0.159	*			
control	4	0.161	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
diclofop-methyl/ozone - control	0.031	0.008	3.978	3.319	Reject
diclofop-methyl/ozone - ozone	0.029	0.008	3.777	3.222	Reject
diclofop-methyl/ozone - diclofop-methyl	0.006	0.008	0.707	3.073	Accept
diclofop-methyl - control	0.026	0.008	3.271	3.222	Reject
diclofop-methyl - ozone	0.024	0.008	3.069	3.073	Accept
ozone - control	0.002	0.008	0.201	3.073	Accept

Homogeneous Subsets:

Group 1:	diclofop-methyl/ ozone, diclofop-methyl		Group 2:	diclofop-methyl, ozone	
Pooled mean =	0.133		Pooled mean =	0.147	
95% Confidence Interval =	0.121	0.145	95% Confidence Interval =	0.135	0.159
Group 3:	ozone, control				
Pooled mean =	0.160				
95% Confidence Interval =	0.148	0.172			

Appendix 2.13 Effects of ozone (100 nl l^{-1}, 7 h d^{-1}, 2 d) and clopyralid (0.10 kg AI ha^{-1}) on the leaf area and shoot dry weight of 2 sugarbeet cultivars, 7 days after exposure to ozone, where n=4. Results of two-way ANOVA tests conducted on data. (See Figure 4.8)

Dependent Variable: **SAXON LEAF AREA**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	180.2	1	180.2	0.647	0.4369
clopyralid	4.6	1	4.6	0.017	0.8995
ozone × clopyralid	135.4	1	135.4	0.486	0.4991
Error	3343.7	12	278.6		
Total	3664.0	15	244.3		

Dependent Variable: **SAXON SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.001	1	0.001	0.243	0.6307
clopyralid	0.001	1	0.001	0.233	0.6377
ozone × clopyralid	0.000	1	0.000	0.112	0.7432
Error	0.045	12	0.004		
Total	0.047	15	0.003		

Dependent Variable: **CELT LEAF AREA**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	5.2	1	5.2	0.101	0.7557
clopyralid	473.4	1	473.4	9.187	0.0104
ozone × clopyralid	12.1	1	12.1	0.235	0.6364
Error	618.3	12	51.5		
Total	1109.1	15	73.9		

Dependent Variable: **CELT SHOOT DRY WEIGHT**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.001	1	0.001	0.781	0.3942
clopyralid	0.001	1	0.001	1.167	0.3013
ozone × clopyralid	0.001	1	0.001	0.742	0.4060
Error	0.008	12	0.001		
Total	0.010	15	0.001		

Appendix 2.13.1 Effects of ozone (100 nl l^{-1}, 7 h d^{-1}, 2 d) and clopyralid (0.10 kg AI ha^{-1}) on leaf area of sugarbeet cultivar, Celt, 7 days after treatment with clopyralid, where n=4. Results of Duncan's Multiple Range Tests for leaf area, classified by treatment. In the comparisons table, accept indicates that the two treatments are not significantly different at $p < 0.05$ and * denotes significantly different pairs.

For **CELT LEAF AREA**, classified by treatment

Group	Cases	Mean	3	4	2	1
3	4	57.61			*	*
4	4	60.49				
2	4	69.63	*			
1	4	70.23	*			

Comparison	Difference	Std Error	q Stat	Table q	Result
3 - 1	12.619	3.589	3.5160	3.3193	Reject
3 - 2	12.021	3.589	3.3494	3.2216	Reject
3 - 4	2.883	3.589	0.8033	3.0734	Accept
4 - 1	9.736	3.589	2.7127	3.2216	Accept
4 - 2	9.138	3.589	2.5461	3.0734	Accept
2 - 1	0.598	3.589	0.1666	3.0734	Accept

Homogeneous Subsets:

Group 1:	3 4		Group 2:	4 2 1	
Pooled mean =	59.05		Pooled mean =	66.78	
95% Confidence Interval =	53.52	64.58	95% Confidence Interval =	62.27	71.30

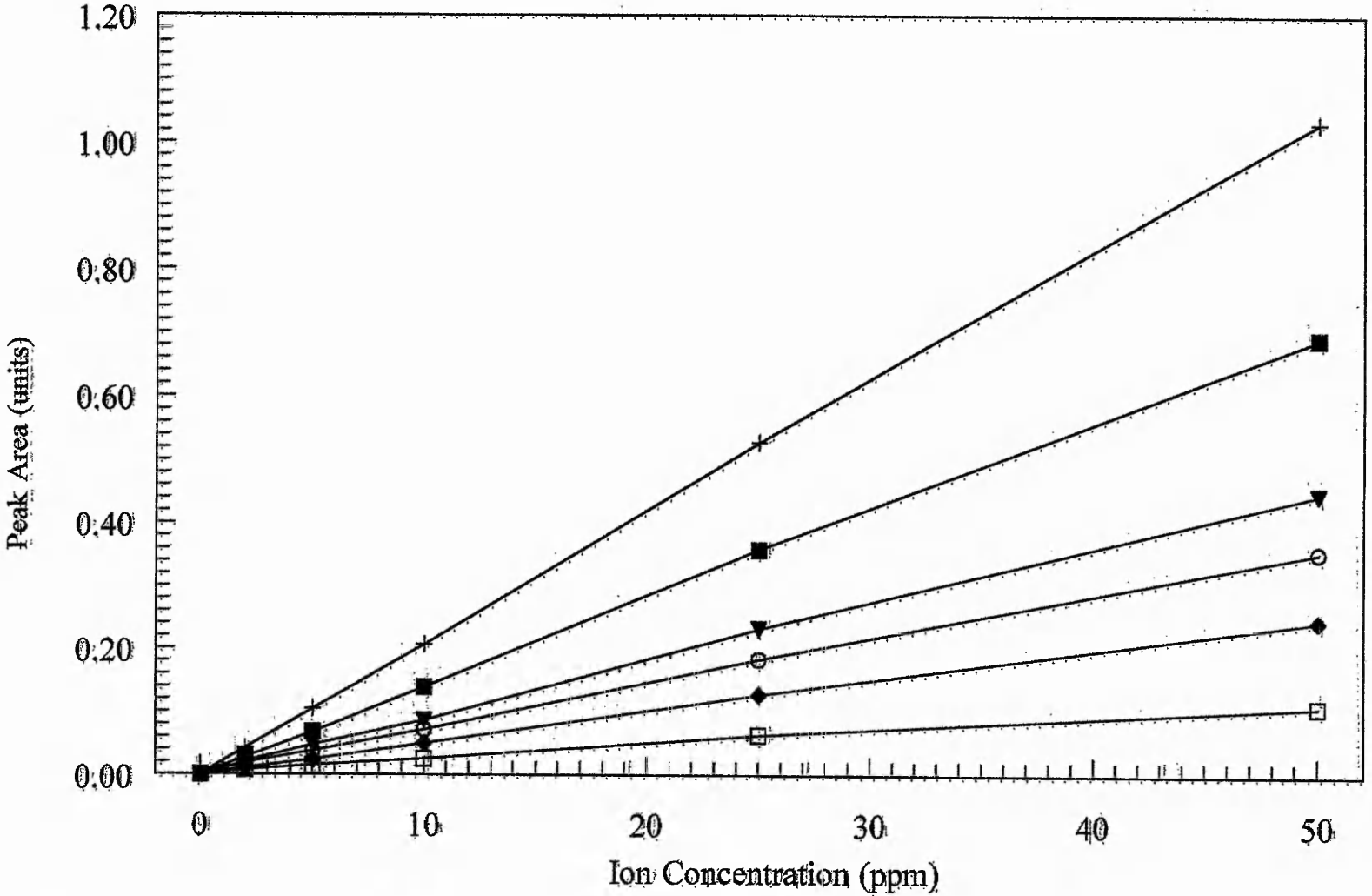

Figure A3.1 Cation standard curve constructed on an ion chromatograph (DX-100, Dionex).
Key: Lithium (+); Sodium (O); Ammonium (□); Potassium (◆); Magnesium (■); Calcium (□).

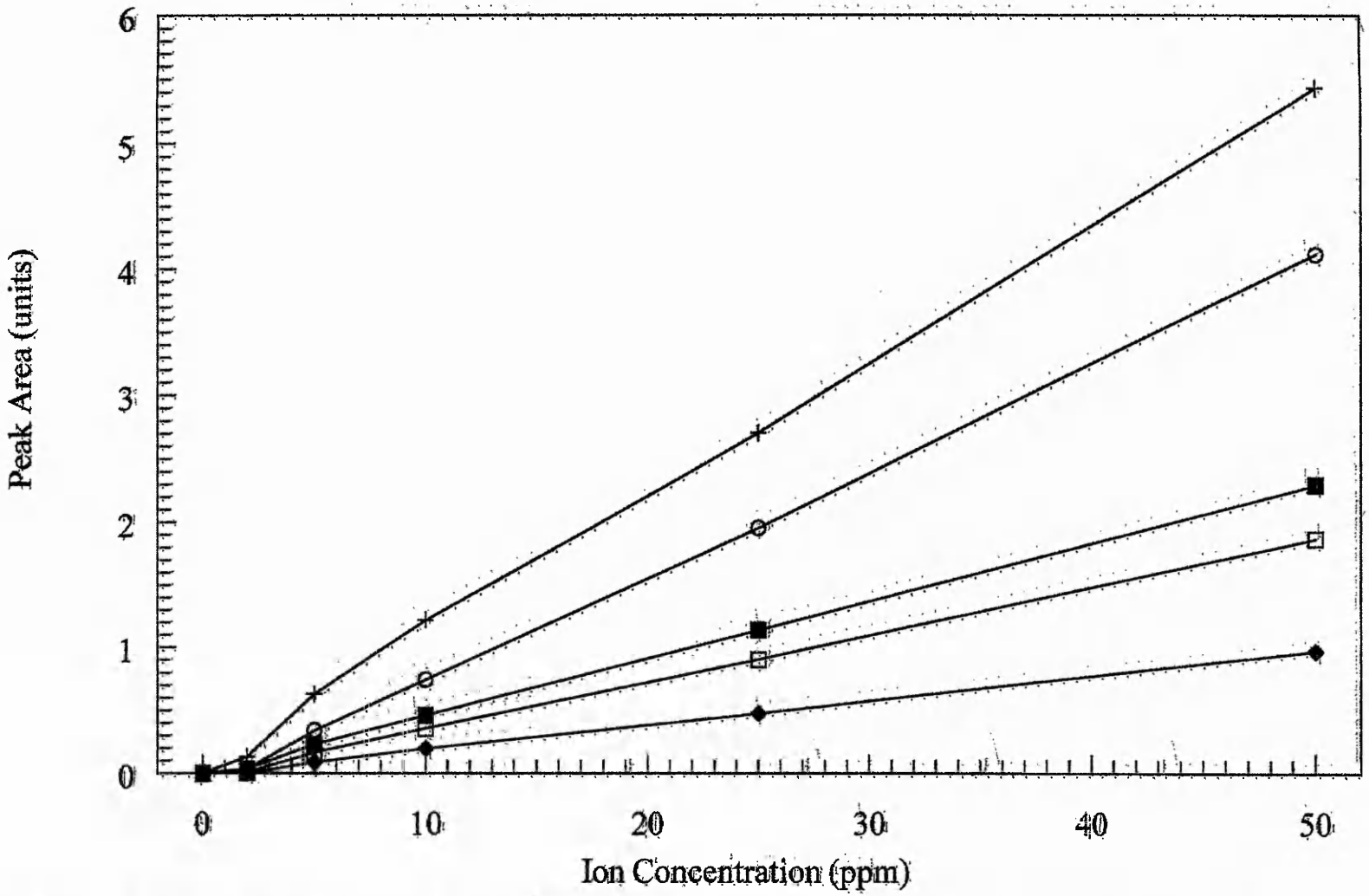

Figure A3.2 Anion standard curve constructed on an ion chromatograph (DX-100, Dionex).
Key: Fluoride (+); Chloride (O); Nitrate (□); Phosphate (◆); Sulphate (■).

Appendix 3.1 (Figure 5.2) Effects of ozone on photosynthetic rate of sugarbeet cv Saxon, where n=2-4 on days -4, -3, -2, -1 and 0. Results of two-way ANOVA, classified by ozone and time.

Dependent Variable: **PHOTOSYNTHETIC RATE**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
time	3.736	4	0.934	1.286	0.2977
ozone	0.054	1	0.054	0.074	0.7873
time × ozone	0.160	4	0.040	0.055	0.9940
Error	21.798	30	0.727		
Total	25.748	39	0.660		

Appendix 3.1.1 (Figure 5.2) Effects of ozone on photosynthetic rate of sugarbeet cv Saxon, where n=2-4 on days -3, -2, -1 and 0. Results of one-way ANOVA, classified by ozone.

Dependent Variable: **PHOTOSYNTHETIC RATE -3**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.034	1	0.034	0.012	0.9175
Error	17.575	6	2.929		
Total	17.609	7	2.516		

Dependent Variable: **PHOTOSYNTHETIC RATE -2**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.001	1	0.001	0.008	0.9372
Error	0.356	2	0.178		
Total	0.357	3	0.119		

Dependent Variable: **PHOTOSYNTHETIC RATE -1**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.021	1	0.021	0.107	0.7504
Error	1.987	10	0.199		
Total	2.008	11	0.183		

Dependent Variable: **PHOTOSYNTHETIC RATE 0**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.157	1	0.157	0.866	0.3739
Error	1.817	10	0.182		
Total	1.975	11	0.180		

Appendix 3.2 (Figure 5.2) Effects of ozone and phenmedipham on photosynthetic rate of sugarbeet cv Saxon, where n=6-8 on days 0, 1, 2, 3, 4 and 6. Results of three-way ANOVA, classified by ozone, phenmedipham and time.

Dependent Variable: **PHOTOSYNTHETIC RATE**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
time	20.190	5	4.038	16.131	0.0000
ozone	0.478	1	0.478	1.911	0.1693
phen	113.000	1	113.000	451.403	0.0000
time × ozone	0.429	5	0.086	0.343	0.8860
time × phen	26.263	5	5.253	20.983	0.0000
ozone × phen	0.069	1	0.069	0.275	0.6009
time × ozone × phen	0.046	5	0.009	0.037	0.9993
Error	32.042	128	0.250		
Total	192.519	151	1.275		

Appendix 3.2.1 Effects of ozone and phenmedipham on photosynthetic rate of sugarbeet cv Saxon, where n=5-8 on days 0, 1, 2, 3, 4 and 6. Results of two-way ANOVA and Duncan's Multiple Range Tests for photosynthetic rate, classified by ozone and/or phenmedipham. In the comparisons table, accept indicates that the two treatments are not significantly different at $p \ll 0.05$ and * denotes significantly different pairs.

Dependent Variable: **PHOTOSYNTHETIC RATE 1**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.127	1	0.127	0.317	0.5781
phen	53.186	1	53.186	132.883	0.0000
ozone × phen	0.001	1	0.001	0.002	0.9664
Error	11.207	28	0.400		
Total	64.520	31	2.081		

For **PHOTOSYNTHETIC RATE 1**, classified by treatment

Group	Cases	Mean	o3phen 1	phen 1	ozone 1	con 1
o3phen 1	8	1.9280			*	*
phen 1	8	2.0634			*	*
ozone 1	8	4.5160	*	*		
con 1	8	4.6323	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
o3phen 1 - con 1	2.7043	0.2237	12.0902	3.1432	Reject
o3phen 1 - ozone 1	2.5879	0.2237	11.5700	3.0423	Reject
o3phen 1 - phen 1	0.1354	0.2237	0.6052	2.8945	Accept
phen 1 - con 1	2.5689	0.2237	11.4850	3.0423	Reject
phen 1 - ozone 1	2.4526	0.2237	10.9648	2.8945	Reject
ozone 1 - con 1	0.1163	0.2237	0.5201	2.8945	Accept

Homogeneous Subsets:

Group 1:	o3phen 1	phen 1	Group 2:	ozone 1	con 1
Pooled mean =	1.996		Pooled mean =	4.574	
95% Confidence Interval =	1.672	2.320	95% Confidence Interval =	4.250	4.898

Dependent Variable: **PHOTOSYNTHETIC RATE 2**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.062	1	0.062	0.232	0.6338

phen	38.280	1	38.280	142.616	0.0000
ozone × phen	0.077	1	0.077	0.287	0.5966
Error	7.515	28	0.268		
Total	45.934	31	1.482		

For **PHOTOSYNTHETIC RATE 2**, classified by treatment

Group	Cases	Mean	o3phen 2	phen 2	con 2	ozone 2
o3phen 2	8	2.3796			*	*
phen 2	8	2.5659			*	*
con 2	8	4.6553	*	*		
ozone 2	8	4.6652	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
o3phen 2 - ozone 2	2.2855	0.1832	12.4776	3.1432	Reject
o3phen 2 - con 2	2.2757	0.1832	12.4239	3.0423	Reject
o3phen 2 - phen 2	0.1863	0.1832	1.0172	2.8945	Accept
phen 2 - ozone 2	2.0992	0.1832	11.4605	3.0423	Reject
phen 2 - con 2	2.0894	0.1832	11.4067	2.8945	Reject
con 2 - ozone 2	0.0098	0.1832	0.0537	2.8945	Accept

Homogeneous Subsets:

Group 1:	o3phen 2 phen 2		Group 2:	con 2 ozone 2	
Pooled mean =	2.473		Pooled mean =	4.660	
95% Confidence Interval =	2.207	2.738	95% Confidence Interval =	4.395	4.926

Dependent Variable: **PHOTOSYNTHETIC RATE 3**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.279	1	0.279	1.441	0.2475
phen	16.460	1	16.460	84.893	0.0000
ozone × phen	0.007	1	0.007	0.036	0.8528
Error	3.102	16	0.194		
Total	19.848	19	1.045		

For **PHOTOSYNTHETIC RATE 3**, classified by treatment

Group	Cases	Mean	o3phen 3	phen 3	ozone 3	con 3
o3phen 3	5	2.6130			*	*
phen 3	5	2.8865			*	*
ozone 3	5	4.4645	*	*		
con 3	5	4.6638	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
o3phen 3 - con 3	2.0508	0.1969	10.4141	3.2421	Reject
o3phen 3 - ozone 3	1.8515	0.1969	9.4023	3.1405	Reject
o3phen 3 - phen 3	0.2735	0.1969	1.3889	2.9918	Accept
phen 3 - con 3	1.7773	0.1969	9.0252	3.1405	Reject
phen 3 - ozone 3	1.5780	0.1969	8.0134	2.9918	Reject
ozone 3 - con 3	0.1993	0.1969	1.0118	2.9918	Accept

Homogeneous Subsets:

Group 1:	o3phen 3 phen 3		Group 2:	ozone 3 con 3	
Pooled mean =	2.750		Pooled mean =	4.564	
95% Confidence Interval =	2.455	3.045	95% Confidence Interval =	4.269	4.859

Dependent Variable: **PHOTOSYNTHETIC RATE 4**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.067	1	0.067	0.275	0.6073

phen	19.222	1	19.222	78.605	0.0000
ozone × phen	0.009	1	0.009	0.039	0.8466
Error	3.913	16	0.245		
Total	23.212	19	1.222		

For **PHOTOSYNTHETIC RATE 4**, classified by treatment

Group	Cases	Mean	o3phen 4	phen 4	ozone 4	con 4
o3phen 4	5	2.7729			*	*
phen 4	5	2.9323			*	*
ozone 4	5	4.7771	*	*		
con 4	5	4.8495	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
o3phen 4 - con 4	2.0767	0.2212	9.3901	3.2421	Reject
o3phen 4 - ozone 4	2.0042	0.2212	9.0625	3.1405	Reject
o3phen 4 - phen 4	0.1594	0.2212	0.7208	2.9918	Accept
phen 4 - con 4	1.9173	0.2212	8.6693	3.1405	Reject
phen 4 - ozone 4	1.8448	0.2212	8.3417	2.9918	Reject
ozone 4 - con 4	0.0724	0.2212	0.3276	2.9918	Accept

Homogeneous Subsets:

Group 1:	o3phen 4 phen 4		Group 2:	ozone 4 con 4	
Pooled mean =	2.853		Pooled mean =	4.813	
95% Confidence Interval =	2.521	3.184	95% Confidence Interval =	4.482	5.145

Dependent Variable: **PHOTOSYNTHETIC RATE 6**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.057	1	0.057	0.428	0.5205
phen	12.116	1	12.116	90.727	0.0000
ozone × phen	0.021	1	0.021	0.156	0.6969
Error	2.671	20	0.134		
Total	14.865	23	0.646		

For photosynthetic rate 6, classified by treatment

Group	Cases	Mean	phen 6	o3phen 6	con 6	ozone 6
phen 6	6	2.4160			*	*
o3phen 6	6	2.4546			*	*
con 6	6	3.7780	*	*		
ozone 6	6	3.9346	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
phen 6 - ozone 6	1.5186	0.1492	10.1792	3.1960	Reject
phen 6 - con 6	1.3621	0.1492	9.1299	3.0938	Reject
phen 6 - o3phen 6	0.0386	0.1492	0.2589	2.9453	Accept
o3phen 6 - ozone 6	1.4800	0.1492	9.9203	3.0938	Reject
o3phen 6 - con 6	1.3235	0.1492	8.8710	2.9453	Reject
con 6 - ozone 6	0.1565	0.1492	1.0493	2.9453	Accept

Homogeneous Subsets:

Group 1:	phen 6 o3phen 6		Group 2:	con 6 ozone 6	
Pooled mean =	2.435		Pooled mean =	3.856	
95% Confidence Interval =	2.215	2.655	95% Confidence Interval =	3.636	4.076

Appendix 3.3 (Figure 5.3) Effects of ozone on stomatal conductance of sugarbeet cv Saxon, where n=2-4 on days -4, -3, -2, -1 and 0. Results of two-way ANOVA, classified by ozone and time.

Dependent Variable: **STOMATAL CONDUCTANCE**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
time	0.305	4	0.076	4.716	0.0045
ozone	0.001	1	0.001	0.088	0.7686
time × ozone	0.003	4	0.001	0.041	0.9966
Error	0.485	30	0.016		
Total	0.795	39	0.020		

Appendix 3.3.1 (Figure 5.3) Effects of ozone on stomatal conductance of sugarbeet cv Saxon, where n=2-4 on days -3, -2, -1 and 0. Results of one-way ANOVA, classified by ozone.

Dependent Variable: **STOMATAL CONDUCTANCE -3**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.000	1	0.000	0.030	0.8679
Error	0.004	6	0.001		
Total	0.004	7	0.001		

Dependent Variable: **STOMATAL CONDUCTANCE -2**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.002	1	0.002	0.941	0.4343
Error	0.003	2	0.002		
Total	0.005	3	0.002		

Dependent Variable: **STOMATAL CONDUCTANCE -1**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.002	1	0.002	0.056	0.8182
Error	0.432	10	0.043		
Total	0.435	11	0.040		

Dependent Variable: **STOMATAL CONDUCTANCE 0**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.000	1	0.000	0.020	0.8895
Error	0.025	10	0.002		
Total	0.025	11	0.002		

Appendix 3.4 (Figure 5.3) Effects of ozone and phenmedipham on stomatal conductance of sugarbeet cv Saxon, where n=6-8 on days 0, 1, 2, 3, 4 and 6. Results of three-way ANOVA, classified by ozone, phenmedipham and time.

Dependent Variable: **STOMATAL CONDUCTANCE**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
time	0.252	5	0.050	4.003	0.0021
ozone	0.006	1	0.006	0.503	0.4797
phen	0.308	1	0.308	24.412	0.0000
time × ozone	0.031	5	0.006	0.495	0.7794
time × phen	0.090	5	0.018	1.436	0.2158
ozone × phen	0.000	1	0.000	0.017	0.8975
time × ozone × phen	0.030	5	0.006	0.469	0.7985
Error	1.614	128	0.013		
Total	2.331	151	0.015		

Appendix 3.4.1 Effects of ozone and phenmedipham on stomatal conductance of sugarbeet cv Saxon, where n=5-8 on days 0, 1, 2, 3, 4 and 6. Results of two-way ANOVA and Duncan's Multiple Range Tests for transpiration rate, classified by ozone and/or phenmedipham. In the comparisons table, accept indicates that the two treatments are not significantly different at $p < 0.05$ and * denotes significantly different pairs.

Dependent Variable: **STOMATAL CONDUCTANCE 1**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.000	1	0.000	0.003	0.9585
phen	0.149	1	0.149	13.608	0.0010
ozone × phen	0.000	1	0.000	0.004	0.9479
Error	0.306	28	0.011		
Total	0.454	31	0.015		

For **STOMATAL CONDUCTANCE 1**, classified by treatment

Group	Cases	Mean	o3phen 1	phen 1	con 1	ozone 1
o3phen 1	8	0.1307			*	*
phen 1	8	0.1312			*	*
con 1	8	0.2651	*	*		
ozone 1	8	0.2694	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
o3phen 1 - ozone 1	0.1387	0.0369	3.7549	3.1432	Reject
o3phen 1 - con 1	0.1343	0.0369	3.6365	3.0423	Reject
o3phen 1 - phen 1	0.0005	0.0369	0.0135	2.8945	Accept
phen 1 - ozone 1	0.1382	0.0369	3.7414	3.0423	Reject
phen 1 - con 1	0.1338	0.0369	3.6229	2.8945	Reject
con 1 - ozone 1	0.0044	0.0369	0.1184	2.8945	Accept

Homogeneous Subsets:

Group 1:	o3phen 1	phen 1	Group 2:	con 1	ozone 1
Pooled mean =	0.131		Pooled mean =	0.267	
95% Confidence Interval =	0.077	0.184	95% Confidence Interval =	0.214	0.321

Dependent Variable: **STOMATAL CONDUCTANCE 2**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif

ozone	0.001	1	0.001	0.052	0.8206
phen	0.133	1	0.133	8.714	0.0063
ozone × phen	0.000	1	0.000	0.032	0.8583
Error	0.426	28	0.015		
Total	0.560	31	0.018		

For **STOMATAL CONDUCTANCE 2**, classified by treatment

Group	Cases	Mean	o3phen 2	phen 2	ozone 2	con 2
o3phen 2	8	0.1449			*	*
phen 2	8	0.1627				
ozone 2	8	0.2815	*			
con 2	8	0.2836	*			

Comparison	Difference	Std Error	q Stat	Table q	Result
o3phen 2 - con 2	0.1388	0.0436	3.1809	3.1432	Reject
o3phen 2 - ozone 2	0.1366	0.0436	3.1322	3.0423	Reject
o3phen 2 - phen 2	0.0178	0.0436	0.4091	2.8945	Accept
phen 2 - con 2	0.1209	0.0436	2.7718	3.0423	Accept
phen 2 - ozone 2	0.1188	0.0436	2.7231	2.8945	Accept
ozone 2 - con 2	0.0021	0.0436	0.0487	2.8945	Accept

Homogeneous Subsets:

Group 1:	o3phen 2	phen 2	Group 2:	phen 2	ozone 2 con 2
Pooled mean =	0.154		Pooled mean =	0.243	
95% Confidence Interval =	0.091	0.217	95% Confidence Interval =	0.191	0.294

Dependent Variable: **STOMATAL CONDUCTANCE 3**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.017	1	0.017	1.618	0.2215
phen	0.015	1	0.015	1.469	0.2431
ozone × phen	0.000	1	0.000	0.000	0.9936
Error	0.167	16	0.010		
Total	0.199	19	0.010		

Dependent Variable: **STOMATAL CONDUCTANCE 4**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.004	1	0.004	0.186	0.6722
phen	0.072	1	0.072	3.173	0.0939
ozone × phen	0.016	1	0.016	0.716	0.4098
Error	0.362	16	0.023		
Total	0.454	19	0.024		

Dependent Variable: **STOMATAL CONDUCTANCE 6**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.016	1	0.016	1.024	0.3237
phen	0.030	1	0.030	1.969	0.1759
ozone × phen	0.013	1	0.013	0.860	0.3649
Error	0.303	20	0.015		
Total	0.362	23	0.016		

Appendix 3.5 (Table 5.1) Effects of ozone on total chlorophyll concentration of sugarbeet cv Saxon, where n=4 on day -2. Results of one-way ANOVA, classified by ozone.

Dependent Variable: µg/g **TOTAL CHLOROPHYLL**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	61309.4	1	61309.4	2.380	0.1738
Error	154548.0	6	25758.0		
Total	215857.3	7	30836.8		

Appendix 3.5.1 Effects of ozone and phenmedipham on total chlorophyll concentration of sugarbeet cv Saxon, where n=4 on day 7. Results of two-way ANOVA and Duncan's Multiple Range Tests for total chlorophyll concentration, classified by ozone and/or phenmedipham. In the comparisons table, accept indicates that the two treatments are not significantly different at $p < 0.05$ and * denotes significantly different pairs.

Dependent Variable: **µg/g TOTAL CHLOROPHYLL**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	19295.9	1	19295.9	5.661	0.0348
phenmedipham	181783.8	1	181783.8	53.333	0.0000
ozone × phenmedipham	4174.3	1	4174.3	1.225	0.2901
Error	40901.6	12	3408.5		
Total	246155.6	15	16410.4		

For **µg/g TOTAL CHLOROPHYLL**, classified by treatment

Group	Cases	Mean	p	op	c	o
p	4	526.4		*	*	*
op	4	628.1	*		*	*
c	4	771.9	*	*		
o	4	809.0	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
p - o	282.6	29.2	9.68	3.32	Reject
p - c	245.5	29.2	8.41	3.22	Reject
p - op	101.8	29.2	3.49	3.07	Reject
op - o	180.9	29.2	6.20	3.22	Reject
op - c	143.7	29.2	4.92	3.07	Reject
c - o	37.2	29.2	1.27	3.07	Accept

Homogeneous Subsets:

Group 1:	p		Group 2:	op	
Pooled mean =	526.4		Pooled mean =	628.1	
95% Confidence Interval =	462.8	590.0	95% Confidence Interval =	564.5	691.7
Group 3:	c o				
Pooled mean =	790.4				
95% Confidence Interval =	745.5	835.4			

Appendix 3.6 (Table 5.1) Effects of ozone on total xanthrophyll and carotenoid concentrations of sugarbeet cv Saxon, where n=4 on day -2. Results of one-way ANOVA, classified by ozone.

Dependent Variable: µg/g **TOTAL XANTHROPHYLL + CAROTENOIDS**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	7439.2	1	7439.2	2.832	0.1434
Error	15760.0	6	2626.7		
Total	23199.3	7	3314.2		

Appendix 3.6.1 Effects of ozone and phenmedipham on total xanthrophyll and carotenoid concentrations of sugarbeet cv Saxon, where n=4 on day 7. Results of two-way ANOVA and Duncan's Multiple Range Tests for total xanthrophyll and carotenoid concentration, classified by ozone and/or phenmedipham. In the comparisons table, accept indicates that the two treatments are not significantly different at $p < 0.05$ and * denotes significantly different pairs.

Dependent Variable: **µg/g TOTAL XANTHROPHYLL + CAROTENOIDS**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	343.3	1	343.3	3.218	0.0981
phenmedipham	2735.1	1	2735.1	25.637	0.0003
ozone × phenmedipham	2.7	1	2.7	0.025	0.8761
Error	1280.3	12	106.7		
Total	4361.4	15	290.8		

For **µg/g TOTAL XANTHROPHYLL + CAROTENOIDS**, classified by treatment

Group	Cases	Mean	p	op	c	o
p	4	86.21			*	*
op	4	96.29			*	*
c	4	113.18	*	*		
o	4	121.62	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
p - o	35.41	5.16	6.86	3.32	Reject
p - c	26.97	5.16	5.22	3.22	Reject
p - op	10.09	5.16	1.95	3.07	Accept
op - o	25.33	5.16	4.90	3.22	Reject
op - c	16.89	5.16	3.27	3.07	Reject
c - o	8.44	5.16	1.63	3.07	Accept

Homogeneous Subsets:

Group 1:	p op		Group 2:	c o	
Pooled mean =	91.25		Pooled mean =	117.40	
95% Confidence Interval =	83.29	99.21	95% Confidence Interval =	109.44	125.36

Appendix 3.7 (Figure 5.4) Effects of ozone and phenmedipham on membrane permeability of sugarbeet cv Saxon, where n=4-12 on days 0, 1, 2, 3, 4, 5 and 6. Results of three-way ANOVA, classified by ozone, phenmedipham and time.

Dependent Variable: **MEMBRANE PERMEABILITY**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
time	141896.325	6	23649.388	13.079	0.0000
ozone	6049.028	1	6049.028	3.345	0.0691
phen	426496.902	1	426496.902	235.866	0.0000
time × ozone	7215.942	6	1202.657	0.665	0.6779
time × phen	74915.137	6	12485.856	6.905	0.0000
ozone × phen	7984.887	1	7984.887	4.416	0.0371
time × ozone × phen	6891.946	6	1148.658	0.635	0.7019
Error	311013.732	172	1808.219		
Total	982463.899	199	4937.005		

Appendix 3.7.1 Effects of ozone and phenmedipham on membrane permeability of sugarbeet cv Saxon, where n=4-12 on days -1, 0, 1, 2, 3, 4, 5 and 6. Results of two-way ANOVA and Duncan's Multiple Range Tests for membrane permeability, classified by ozone and/or phenmedipham. In the comparisons table, accept indicates that the two treatments are not significantly different at $p < 0.05$ and * denotes significantly different pairs.

Dependent Variable: **MEMBRANE PERMEABILITY -1**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	37.761	1	37.761	1.946	0.2978
Error	38.812	2	19.406		
Total	76.573	3	25.524		

Dependent Variable: **MEMBRANE PERMEABILITY 0**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	48.545	1	48.545	0.084	0.7773
Error	5749.108	10	574.911		
Total	5797.653	11	527.059		

Dependent Variable: **MEMBRANE PERMEABILITY 1**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	201.221	1	201.221	0.084	0.7744
phen	48638.288	1	48638.288	20.254	0.0001
ozone × phen	299.030	1	299.030	0.125	0.7268
Error	67238.348	28	2401.370		
Total	116376.887	31	3754.093		

For **MEMBRANE PERMEABILITY 1**, classified by treatment

Group	Cases	Mean	con 1	ozone 1	o3phen 1	phen 1
con 1	8	51.8425			*	*
ozone 1	8	52.9411			*	*
o3phen 1	8	124.8002	*	*		
phen 1	8	135.9293	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result

con 1 - phen 1	84.0868	17.3254	4.8534	3.1432	Reject
con 1 - o3phen 1	72.9577	17.3254	4.2110	3.0423	Reject
con 1 - ozone 1	1.0986	17.3254	0.0634	2.8945	Accept
ozone 1 - phen 1	82.9882	17.3254	4.7900	3.0423	Reject
ozone 1 - o3phen 1	71.8592	17.3254	4.1476	2.8945	Reject
o3phen 1 - phen 1	11.1291	17.3254	0.6424	2.8945	Accept

Homogeneous Subsets:

Group 1:	con 1 ozone 1		Group 2:	o3phen 1 phen 1	
Pooled mean =	52.4		Pooled mean =	130.4	
95% Confidence Interval =	27.3	77.5	95% Confidence Interval =	105.3	155.5

Dependent Variable: **MEMBRANE PERMEABILITY 2**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	3314.022	1	3314.022	1.883	0.1769
phen	147098.342	1	147098.342	83.602	0.0000
ozone × phen	7455.639	1	7455.639	4.237	0.0455
Error	77418.510	44	1759.512		
Total	235286.512	47	5006.096		

For **MEMBRANE PERMEABILITY 2**, classified by treatment

Group	Cases	Mean	con 2	ozone 2	o3phen 2	phen 2
con 2	12	72.4301			*	*
ozone 2	12	80.7377			*	*
o3phen 2	12	166.5285	*	*		*
phen 2	12	208.0728	*	*	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
con 2 - phen 2	135.6427	12.1089	11.2019	3.0951	Reject
con 2 - o3phen 2	94.0984	12.1089	7.7710	2.9970	Reject
con 2 - ozone 2	8.3076	12.1089	0.6861	2.8505	Accept
ozone 2 - phen 2	127.3351	12.1089	10.5158	2.9970	Reject
ozone 2 - o3phen 2	85.7908	12.1089	7.0849	2.8505	Reject
o3phen 2 - phen 2	41.5443	12.1089	3.4309	2.8505	Reject

Homogeneous Subsets:

Group 1:	con 2 ozone 2		Group 2:	o3phen 2	
Pooled mean =	76.6		Pooled mean =	166.5	
95% Confidence Interval =	59.3	93.8	95% Confidence Interval =	142.1	190.9
Group 3:	phen 2				
Pooled mean =	208.1				
95% Confidence Interval =	183.7	232.5			

Dependent Variable: **MEMBRANE PERMEABILITY 3**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	2.250	1	2.250	0.001	0.9777
phen	133765.472	1	133765.472	47.090	0.0000
ozone × phen	53.507	1	53.507	0.019	0.8918
Error	79538.501	28	2840.661		
Total	213359.730	31	6882.572		

For **MEMBRANE PERMEABILITY 3**, classified by treatment

Group	Cases	Mean	con 3	ozone 3	o3phen 3	phen 3
con 3	8	60.8677			*	*
ozone 3	8	62.9236			*	*
o3phen 3	8	189.6459	*	*		

phen 3	8	192.7624	*	*

Comparison	Difference	Std Error	q Stat	Table q	Result
con 3 - phen 3	131.8947	18.8436	6.9994	3.1432	Reject
con 3 - o3phen 3	128.7782	18.8436	6.8340	3.0423	Reject
con 3 - ozone 3	2.0559	18.8436	0.1091	2.8945	Accept
ozone 3 - phen 3	129.8388	18.8436	6.8903	3.0423	Reject
ozone 3 - o3phen 3	126.7223	18.8436	6.7249	2.8945	Reject
o3phen 3 - phen 3	3.1165	18.8436	0.1654	2.8945	Accept

Homogeneous Subsets:

Group 1:	con 3 ozone 3		Group 2:	o3phen 3 phen 3	
Pooled mean =	61.9		Pooled mean =	191.2	
95% Confidence Interval =	34.6	89.2	95% Confidence Interval =	163.9	218.5

Dependent Variable: membrane permeability 4

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	591.796	1	591.796	0.321	0.5754
phen	109998.863	1	109998.863	59.710	0.0000
ozone × phen	613.527	1	613.527	0.333	0.5685
Error	51582.067	28	1842.217		
Total	162786.252	31	5251.169		

For **MEMBRANE PERMEABILITY 4**, classified by treatment

Group	Cases	Mean	con 4	ozone 4	o3phen 4	phen 4
con 4	8	69.0815			*	*
ozone 4	8	69.2380			*	*
o3phen 4	8	177.7405	*	*		
phen 4	8	195.0987	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
con 4 - phen 4	126.0171	15.1749	8.3043	3.1432	Reject
con 4 - o3phen 4	108.6589	15.1749	7.1604	3.0423	Reject
con 4 - ozone 4	0.1565	15.1749	0.0103	2.8945	Accept
ozone 4 - phen 4	125.8606	15.1749	8.2940	3.0423	Reject
ozone 4 - o3phen 4	108.5025	15.1749	7.1501	2.8945	Reject
o3phen 4 - phen 4	17.3582	15.1749	1.1439	2.8945	Accept

Homogeneous Subsets:

Group 1:	con 4 ozone 4		Group 2:	o3phen 4 phen 4	
Pooled mean =	69.2		Pooled mean =	186.4	
95% Confidence Interval =	47.2	91.1	95% Confidence Interval =	164.4	208.4

Dependent Variable: **MEMBRANE PERMEABILITY 5**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	6618.768	1	6618.768	6.246	0.0280
phen	41601.452	1	41601.452	39.256	0.0000
ozone × phen	6119.673	1	6119.673	5.775	0.0333
Error	12716.860	12	1059.738		
Total	67056.753	15	4470.450		

For **MEMBRANE PERMEABILITY 5**, classified by treatment

Group	Cases	Mean	ozone 5	con 5	o3phen 5	phen 5
ozone 5	4	70.8408			*	*
con 5	4	72.4045			*	*
o3phen 5	4	133.7088	*	*		*

phen 5	4	213.5008	*	*	*

Comparison	Difference	Std Error	q Stat	Table q	Result
ozone 5 - phen 5	142.6601	16.2768	8.7646	3.3193	Reject
ozone 5 - o3phen 5	62.8680	16.2768	3.8624	3.2216	Reject
ozone 5 - con 5	1.5637	16.2768	0.0961	3.0734	Accept
con 5 - phen 5	141.0963	16.2768	8.6685	3.2216	Reject
con 5 - o3phen 5	61.3043	16.2768	3.7664	3.0734	Reject
o3phen 5 - phen 5	79.7921	16.2768	4.9022	3.0734	Reject

Homogeneous Subsets:

Group 1:	ozone 5 con 5		Group 2:	o3phen 5	
Pooled mean =	71.6		Pooled mean =	133.7	
95% Confidence Interval =	46.5	96.7	95% Confidence Interval =	98.2	169.2
Group 3:	phen 5				
Pooled mean =	213.5				
95% Confidence Interval =	178.0	249.0			

Dependent Variable: **MEMBRANE PERMEABILITY 6**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	2439.823	1	2439.823	2.656	0.1291
phen	20309.622	1	20309.622	22.113	0.0005
ozone × phen	335.459	1	335.459	0.365	0.5569
Error	11021.229	12	918.436		
Total	34106.134	15	2273.742		

For **MEMBRANE PERMEABILITY 6**, classified by treatment

Group	Cases	Mean	ozone 6	con 6	o3phen 6	phen 6
ozone 6	4	45.3621			*	*
con 6	4	60.9016				*
o3phen 6	4	107.4602	*			
phen 6	4	141.3153	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
ozone 6 - phen 6	95.9532	15.1529	6.3324	3.3193	Reject
ozone 6 - o3phen 6	62.0981	15.1529	4.0981	3.2216	Reject
ozone 6 - con 6	15.5395	15.1529	1.0255	3.0734	Accept
con 6 - phen 6	80.4137	15.1529	5.3068	3.2216	Reject
con 6 - o3phen 6	46.5586	15.1529	3.0726	3.0734	Accept
o3phen 6 - phen 6	33.8551	15.1529	2.2342	3.0734	Accept

Homogeneous Subsets:

Group 1:	ozone 6 con 6		Group 2:	con 6 o3phen 6	
Pooled mean =	53.1		Pooled mean =	84.2	
95% Confidence Interval =	29.8	76.5	95% Confidence Interval =	60.8	107.5
Group 3:	o3phen 6 phen 6				
Pooled mean =	124.4				
95% Confidence Interval =	101.0	147.7			

Appendix 3.8 (Figure 5.5) Effects of ozone and phenmedipham on sodium leakage of sugarbeet cv Saxon, where n=2-4 on days 0, 1, 2, 3, 4 and 6. Results of three-way ANOVA, classified by ozone, phenmedipham and time.

Dependent Variable: **log [SODIUM]*10**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
time	8.345	5	1.669	14.282	0.0000
ozone	0.018	1	0.018	0.153	0.6971
phen	2.714	1	2.714	23.224	0.0000
time × ozone	0.200	5	0.040	0.342	0.8853
time × phen	1.681	5	0.336	2.876	0.0210
ozone × phen	0.000	1	0.000	0.002	0.9658
time × ozone × phen	0.160	5	0.032	0.274	0.9257
Error	7.479	64	0.117		
Total	20.597	87	0.237		

Appendix 3.8.1 Effects of ozone and phenmedipham on sodium leakage of sugarbeet cv Saxon, where n=2-4 on days 0, 1, 2, 3, 4 and 6. Results of two-way ANOVA and Duncan's Multiple Range Tests for sodium leakage, classified by ozone and/or phenmedipham. In the comparisons table, accept indicates that the two treatments are not significantly different at $p < 0.05$ and * denotes significantly different pairs.

Dependent Variable: **log [Na]*10 d0**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.026	1	0.026	0.360	0.5707
Error	0.433	6	0.072		
Total	0.459	7	0.066		

Dependent Variable: **log [Na]*10 d1**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.016	1	0.016	0.272	0.6114
phen	0.105	1	0.105	1.802	0.2043
ozone × phen	0.083	1	0.083	1.422	0.2561
Error	0.699	12	0.058		
Total	0.903	15	0.060		

Dependent Variable: **log [Na]*10 d2**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.042	1	0.042	0.144	0.7105
phen	0.239	1	0.239	0.815	0.3845
ozone × phen	0.011	1	0.011	0.037	0.8499
Error	3.518	12	0.293		
Total	3.810	15	0.254		

Dependent Variable: **log [Na]*10 d3**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.006	1	0.006	0.096	0.7623
phen	2.393	1	2.393	38.495	0.0000
ozone × phen	0.031	1	0.031	0.505	0.4910
Error	0.746	12	0.062		
Total	3.176	15	0.212		

For **log [Na]*10 d3**, classified by treatment

Group	Cases	Mean	ozone 3	con 3	phen 3	o3phen 3
ozone 3	4	1.4894			*	*
con 3	4	1.6165			*	*
phen 3	4	2.3014	*	*		
o3phen 3	4	2.3514	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
ozone 3 - o3phen 3	0.8620	0.1247	6.9148	3.3193	Reject
ozone 3 - phen 3	0.8121	0.1247	6.5139	3.2216	Reject
ozone 3 - con 3	0.1271	0.1247	1.0199	3.0734	Accept
con 3 - o3phen 3	0.7349	0.1247	5.8949	3.2216	Reject
con 3 - phen 3	0.6849	0.1247	5.4940	3.0734	Reject
phen 3 - o3phen 3	0.0500	0.1247	0.4009	3.0734	Accept

Homogeneous Subsets:

Group 1:	ozone 3	con 3	Group 2:	phen 3	o3phen 3
Pooled mean =	1.553		Pooled mean =	2.326	
95% Confidence Interval =	1.361	1.745	95% Confidence Interval =	2.134	2.518

Dependent Variable: **log [Na]*10 d4**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.049	1	0.049	0.384	0.5471
phen	1.503	1	1.503	11.658	0.0051
ozone × phen	0.016	1	0.016	0.124	0.7310
Error	1.547	12	0.129		
Total	3.116	15	0.208		

For **log [Na]*10 d4**, classified by treatment

Group	Cases	Mean	ozone 4	con 4	o3phen 4	phen 4
ozone 4	4	1.4013				*
con 4	4	1.4493				*
o3phen 4	4	1.9511				
phen 4	4	2.1256	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
ozone 4 - phen 4	0.7243	0.1796	4.0340	3.3193	Reject
ozone 4 - o3phen 4	0.5499	0.1796	3.0625	3.2216	Accept
ozone 4 - con 4	0.0481	0.1796	0.2677	3.0734	Accept
con 4 - phen 4	0.6762	0.1796	3.7663	3.2216	Reject
con 4 - o3phen 4	0.5018	0.1796	2.7949	3.0734	Accept
o3phen 4 - phen 4	0.1744	0.1796	0.9714	3.0734	Accept

Homogeneous Subsets:

Group 1:	ozone 4	con 4 o3phen 4	Group 2:	o3phen 4	phen 4
Pooled mean =	1.601		Pooled mean =	2.038	
95% Confidence Interval =	1.375	1.826	95% Confidence Interval =	1.762	2.315

Dependent Variable: **log [Na]*10 d6**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.052	1	0.052	2.051	0.2253
phen	0.154	1	0.154	6.042	0.0698
ozone × phen	0.019	1	0.019	0.751	0.4349
Error	0.102	4	0.026		
Total	0.328	7	0.047		

Appendix 3.9 (Figure 5.6) Effects of ozone and phenmedipham on potassium leakage of sugarbeet cv Saxon, where n=2-4 on days 0, 1, 2, 3, 4 and 6. Results of three-way ANOVA, classified by ozone, phenmedipham and time.

Dependent Variable: **log [POTASSIUM]*10**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
time	12.262	5	2.452	19.093	0.0000
ozone	0.006	1	0.006	0.048	0.8279
phen	2.816	1	2.816	21.919	0.0000
time × ozone	0.119	5	0.024	0.185	0.9673
time × phen	1.312	5	0.262	2.042	0.0845
ozone × phen	0.006	1	0.006	0.044	0.8353
time × ozone × phen	0.088	5	0.018	0.137	0.9831
Error	8.221	64	0.128		
Total	24.829	87	0.285		

Appendix 3.9.1 (Figure 5.6) Effects of ozone and phenmedipham on potassium leakage of sugarbeet cv Saxon, where n=2-4 on days 0, 1, 2, 3, 4 and 6. Results of two-way ANOVA and Duncan's Multiple Range Tests for potassium leakage, classified by ozone and/or phenmedipham. In the comparisons table, accept indicates that the two treatments are not significantly different at $p < 0.05$ and * denotes significantly different pairs.

Dependent Variable: **log [K]*10 d0**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.028	1	0.028	1.296	0.2983
Error	0.128	6	0.021		
Total	0.155	7	0.022		

Dependent Variable: **log [K]*10 d1**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.002	1	0.002	0.017	0.8998
phen	0.145	1	0.145	1.589	0.2314
ozone × phen	0.010	1	0.010	0.104	0.7523
Error	1.095	12	0.091		
Total	1.251	15	0.083		

Dependent Variable: **log [K]*10 d2**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.015	1	0.015	0.040	0.8451
phen	0.466	1	0.466	1.248	0.2858
ozone × phen	0.000	1	0.000	0.001	0.9766
Error	4.482	12	0.374		
Total	4.964	15	0.331		

Dependent Variable: **log [K]*10 d3**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.001	1	0.001	0.010	0.9229
phen	2.197	1	2.197	42.710	0.0000
ozone × phen	0.006	1	0.006	0.110	0.7456
Error	0.617	12	0.051		
Total	2.821	15	0.188		

For **log [K]*10 d3**, classified by treatment

Group	Cases	Mean	con 3	ozone 3	o3phen 3	phen 3
con 3	4	2.0407			*	*
ozone 3	4	2.0895			*	*
o3phen 3	4	2.7931	*	*		
phen 3	4	2.8195	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
con 3 - phen 3	0.7788	0.1134	6.8672	3.3193	Reject
con 3 - o3phen 3	0.7524	0.1134	6.6341	3.2216	Reject
con 3 - ozone 3	0.0489	0.1134	0.4308	3.0734	Accept
ozone 3 - phen 3	0.7299	0.1134	6.4364	3.2216	Reject
ozone 3 - o3phen 3	0.7035	0.1134	6.2033	3.0734	Reject
o3phen 3 - phen 3	0.0264	0.1134	0.2331	3.0734	Accept

Homogeneous Subsets:

Group 1:	con 3	ozone 3	Group 2:	o3phen 3	phen 3
Pooled mean =	2.065		Pooled mean =	2.806	
95% Confidence Interval =	1.890	2.240	95% Confidence Interval =	2.632	2.981

Dependent Variable: **log [K]*10 d4**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.041	1	0.041	0.282	0.6051
phen	1.060	1	1.060	7.370	0.0188
ozone × phen	0.031	1	0.031	0.213	0.6526
Error	1.726	12	0.144		
Total	2.857	15	0.190		

Dependent Variable: **log [K]*10 d6**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.012	1	0.012	1.079	0.3576
phen	0.259	1	0.259	22.665	0.0089
ozone × phen	0.048	1	0.048	4.158	0.1111
Error	0.046	4	0.011		
Total	0.364	7	0.052		

For **log [K]*10 d6**, classified by treatment

Group	Cases	Mean	ozone 6	con 6	phen 6	o3phen 6
ozone 6	2	1.8636			*	*
con 6	2	2.0962				
phen 6	2	2.3019	*			
o3phen 6	2	2.3775	*			

Comparison	Difference	Std Error	q Stat	Table q	Result
ozone 6 - o3phen 6	0.5139	0.0756	6.7999	4.0317	Reject
ozone 6 - phen 6	0.4383	0.0756	5.7996	4.0169	Reject
ozone 6 - con 6	0.2326	0.0756	3.0780	3.9151	Accept
con 6 - o3phen 6	0.2813	0.0756	3.7219	4.0169	Accept
con 6 - phen 6	0.2057	0.0756	2.7216	3.9151	Accept
phen 6 - o3phen 6	0.0756	0.0756	1.0003	3.9151	Accept

Homogeneous Subsets:

Group 1:	ozone 6	con 6	Group 2:	con 6	phen 6 o3phen 6
Pooled mean =	1.980		Pooled mean =	2.259	
95% Confidence Interval =	1.831	2.128	95% Confidence Interval =	2.137	2.380

Appendix 3.10 (Figure 5.7) Effects of ozone and phenmedipham on magnesium leakage of sugarbeet cv Saxon, where n=2-4 on days 0, 1, 2, 3, 4 and 6. Results of three-way ANOVA, classified by ozone, phenmedipham and time.

Dependent Variable: **log [MAGNESIUM]*10**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
time	4.759	5	0.952	13.749	0.0000
ozone	0.040	1	0.040	0.572	0.4524
phen	1.040	1	1.040	15.022	0.0003
time × ozone	0.109	5	0.022	0.315	0.9022
time × phen	1.044	5	0.209	3.016	0.0166
ozone × phen	0.000	1	0.000	0.003	0.9549
time × ozone × phen	0.048	5	0.010	0.140	0.9824
Error	4.431	64	0.069		
Total	11.471	87	0.132		

Appendix 3.10.1 (Figure 5.7) Effects of ozone and phenmedipham on magnesium leakage of sugarbeet cv Saxon, where n=2-4 on days 0, 1, 2, 3, 4 and 6. Results of two-way ANOVA and Duncan's Multiple Range Tests for magnesium leakage, classified by ozone and/or phenmedipham. In the comparisons table, accept indicates that the two treatments are not significantly different at $p < 0.05$ and * denotes significantly different pairs.

Dependent Variable: **log [Mg]*10 d0**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.003	1	0.003	0.054	0.8242
Error	0.336	6	0.056		
Total	0.339	7	0.048		

Dependent Variable: **log [Mg]*10 d1**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.006	1	0.006	0.174	0.6840
phen	0.039	1	0.039	1.075	0.3202
ozone × phen	0.023	1	0.023	0.627	0.4438
Error	0.438	12	0.036		
Total	0.506	15	0.034		

Dependent Variable: **log [Mg]*10 d2**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.069	1	0.069	0.356	0.5620
phen	0.006	1	0.006	0.029	0.8670
ozone × phen	0.024	1	0.024	0.122	0.7332
Error	2.329	12	0.194		
Total	2.427	15	0.162		

Dependent Variable: **log [Mg]*10 d3**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.008	1	0.008	0.171	0.6869
phen	1.347	1	1.347	27.547	0.0002
ozone × phen	0.001	1	0.001	0.021	0.8860
Error	0.587	12	0.049		
Total	1.943	15	0.130		

For **log [Mg]*10 d3**, classified by treatment

Group	Cases	Mean	con 3	ozone 3	phen 3	o3phen 3
con 3	4	1.4656			*	*
ozone 3	4	1.5274			*	*
phen 3	4	2.0621	*	*		
o3phen 3	4	2.0916	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
con 3 - o3phen 3	0.6260	0.1106	5.6615	3.3193	Reject
con 3 - phen 3	0.5965	0.1106	5.3949	3.2216	Reject
con 3 - ozone 3	0.0619	0.1106	0.5594	3.0734	Accept
ozone 3 - o3phen 3	0.5641	0.1106	5.1021	3.2216	Reject
ozone 3 - phen 3	0.5346	0.1106	4.8354	3.0734	Reject
phen 3 - o3phen 3	0.0295	0.1106	0.2667	3.0734	Accept

Homogeneous Subsets:

Group 1:	con 3	ozone 3	Group 2:	phen 3	o3phen 3
Pooled mean =	1.497		Pooled mean =	2.077	
95% Confidence Interval =	1.326	1.667	95% Confidence Interval =	1.906	2.247

Dependent Variable: **log [Mg]*10 d4**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.039	1	0.039	1.298	0.2767
phen	0.644	1	0.644	21.160	0.0006
ozone × phen	0.000	1	0.000	0.011	0.9189
Error	0.365	12	0.030		
Total	1.048	15	0.070		

For **log [Mg]*10 d4**, classified by treatment

Group	Cases	Mean	ozone 4	con 4	o3phen 4	phen 4
ozone 4	4	1.3835			*	*
con 4	4	1.4738			*	*
o3phen 4	4	1.7756	*	*		
phen 4	4	1.8840	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
ozone 4 - phen 4	0.5005	0.0872	5.7395	3.3193	Reject
ozone 4 - o3phen 4	0.3921	0.0872	4.4961	3.2216	Reject
ozone 4 - con 4	0.0903	0.0872	1.0356	3.0734	Accept
con 4 - phen 4	0.4102	0.0872	4.7039	3.2216	Reject
con 4 - o3phen 4	0.3018	0.0872	3.4605	3.0734	Reject
o3phen 4 - phen 4	0.1084	0.0872	1.2434	3.0734	Accept

Homogeneous Subsets:

Group 1:	ozone 4	con 4	Group 2:	o3phen 4	phen 4
Pooled mean =	1.429		Pooled mean =	1.830	
95% Confidence Interval =	1.294	1.563	95% Confidence Interval =	1.695	1.964

Dependent Variable: **log [Mg]*10 d6**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.019	1	0.019	1.996	0.2306
phen	0.048	1	0.048	4.968	0.0897
ozone × phen	0.001	1	0.001	0.066	0.8097
Error	0.039	4	0.010		
Total	0.107	7	0.015		

Appendix 3.11 (Figure 5.8) Effects of ozone and phenmedipham on nitrate leakage of sugarbeet cv Saxon, where n=4-6 on days 0, 1, 2, 3, 4 and 6. Results of three-way ANOVA, classified by ozone, phenmedipham and time

Dependent Variable: **NITRATE**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
time	1244.644	5	248.929	5.568	0.0002
ozone	0.000	1	0.000	0.000	0.9976
phen	1509.042	1	1509.042	33.756	0.0000
time × ozone	89.479	5	17.896	0.400	0.8474
time × phen	943.775	5	188.755	4.222	0.0017
ozone × phen	5.851	1	5.851	0.131	0.7184
time × ozone × phen	74.423	5	14.885	0.333	0.8918
Error	3933.990	88	44.704		
Total	7801.203	111	70.281		

Appendix 3.11.1 Effects of ozone and phenmedipham on nitrate leakage of sugarbeet cv Saxon, where n=4-6 on days 0, 1, 2, 3, 4 and 6. Results of two-way ANOVA and Duncan's Multiple Range Tests for nitrate leakage, classified by ozone and/or phenmedipham. In the comparisons table, accept indicates that the two treatments are not significantly different at $p < 0.05$ and * denotes significantly different pairs.

Dependent Variable: **NITRATE 0**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.764	1	0.764	0.491	0.4997
Error	15.584	10	1.558		
Total	16.348	11	1.486		

Dependent Variable: **NITRATE 1**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	5.848	1	5.848	0.190	0.6678
phen	124.113	1	124.113	4.026	0.0585
ozone × phen	2.173	1	2.173	0.070	0.7933
Error	616.525	20	30.826		
Total	748.659	23	32.550		

Dependent Variable: **NITRATE 2**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	19.352	1	19.352	0.166	0.6913
phen	616.401	1	616.401	5.273	0.0405
ozone × phen	21.818	1	21.818	0.187	0.6734
Error	1402.652	12	116.888		
Total	2060.223	15	137.348		

Dependent Variable: **NITRATE 3**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	3.143	1	3.143	0.039	0.8471
phen	844.219	1	844.219	10.429	0.0072
ozone × phen	1.305	1	1.305	0.016	0.9011
Error	971.356	12	80.946		
Total	1820.023	15	121.335		

For **NITRATE 3**, classified by treatment

Group	Cases	Mean	ozone 3	con 3	o3phen 3	phen 3
ozone 3	4	3.0290				*
con 3	4	3.3443				*
o3phen 3	4	16.9856				
phen 3	4	18.4432	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
ozone 3 - phen 3	15.4142	4.4985	3.4265	3.3193	Reject
ozone 3 - o3phen 3	13.9566	4.4985	3.1025	3.2216	Accept
ozone 3 - con 3	0.3153	4.4985	0.0701	3.0734	Accept
con 3 - phen 3	15.0989	4.4985	3.3564	3.2216	Reject
con 3 - o3phen 3	13.6413	4.4985	3.0324	3.0734	Accept
o3phen 3 - phen 3	1.4576	4.4985	0.3240	3.0734	Accept

Homogeneous Subsets:

Group 1:	ozone 3	con 3	o3phen 3	Group 2:	o3phen 3	phen 3
Pooled mean =	7.786			Pooled mean =	17.714	
95% Confidence Interval =	2.127		13.445	95% Confidence Interval =	10.784	24.645

Dependent Variable: **NITRATE 4**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	20.936	1	20.936	0.276	0.6090
phen	825.611	1	825.611	10.879	0.0064
ozone × phen	21.141	1	21.141	0.279	0.6073
Error	910.644	12	75.887		
Total	1778.331	15	118.555		

For **NITRATE 4**, classified by treatment

Group	Cases	Mean	con 4	ozone 4	o3phen 4	phen 4
con 4	4	0.4691				*
ozone 4	4	0.4803				*
o3phen 4	4	12.5480				
phen 4	4	17.1348	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
con 4 - phen 4	16.6657	4.3557	3.8262	3.3193	Reject
con 4 - o3phen 4	12.0789	4.3557	2.7732	3.2216	Accept
con 4 - ozone 4	0.0112	4.3557	0.0026	3.0734	Accept
ozone 4 - phen 4	16.6545	4.3557	3.8236	3.2216	Reject
ozone 4 - o3phen 4	12.0678	4.3557	2.7706	3.0734	Accept
o3phen 4 - phen 4	4.5867	4.3557	1.0530	3.0734	Accept

Homogeneous Subsets:

Group 1:	con 4	ozone 4	o3phen 4	Group 2:	o3phen 4	phen 4
Pooled mean =	4.499			Pooled mean =	14.841	
95% Confidence Interval =	-0.980		9.978	95% Confidence Interval =	8.131	21.552

Dependent Variable: **NITRATE 6**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	38.672	1	38.672	281.959	0.0000
phen	42.474	1	42.474	309.681	0.0000
ozone × phen	33.837	1	33.837	246.713	0.0000
Error	1.646	12	0.137		
Total	116.629	15	7.775		

For **NITRATE 6**, classified by treatment

Group	Cases	Mean	con 6	ozone 6	phen 6	o3phen 6
con 6	4	0.0381				*
ozone 6	4	0.2390				*
phen 6	4	0.3882				*
o3phen 6	4	6.4061	*	*	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
con 6 - o3phen 6	6.3679	0.1852	34.3894	3.3193	Reject
con 6 - phen 6	0.3501	0.1852	1.8907	3.2216	Accept
con 6 - ozone 6	0.2008	0.1852	1.0845	3.0734	Accept
ozone 6 - o3phen 6	6.1671	0.1852	33.3049	3.2216	Reject
ozone 6 - phen 6	0.1493	0.1852	0.8061	3.0734	Accept
phen 6 - o3phen 6	6.0178	0.1852	32.4987	3.0734	Reject

Homogeneous Subsets:

Group 1:	con 6 ozone 6 phen 6		Group 2:	o3phen 6	
Pooled mean =	0.222		Pooled mean =	6.406	
95% Confidence Interval =	-0.011	0.455	95% Confidence Interval =	6.003	6.810

Appendix 3.12 (Figure 5.9) Effects of ozone and phenmedipham on phosphate leakage of sugarbeet cv Saxon, where n=4-6 on days 0, 1, 2, 3, 4 and 6. Results of three-way ANOVA, classified by ozone, phenmedipham and time.

Dependent Variable: **PHOSPHATE**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
time	153.607	5	30.721	2.558	0.0329
ozone	6.057	1	6.057	0.504	0.4795
phen	98.415	1	98.415	8.194	0.0053
time × ozone	59.875	5	11.975	0.997	0.4244
time × phen	68.439	5	13.688	1.140	0.3456
ozone × phen	10.073	1	10.073	0.839	0.3623
time × ozone × phen	43.786	5	8.757	0.729	0.6035
Error	1056.984	88	12.011		
Total	1497.236	111	13.489		

Appendix 3.12.1 Effects of ozone and phenmedipham on phosphate leakage of sugarbeet cv Saxon, where n=4-6 on days 0, 1, 2, 3, 4 and 6. Results of two-way ANOVA, classified by ozone and/or phenmedipham.

Dependent Variable: **PHOSPHATE 0**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	2.412	1	2.412	1.826	0.2064
Error	13.207	10	1.321		
Total	15.619	11	1.420		

Dependent Variable: **PHOSPHATE 1**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.931	1	0.931	0.166	0.6876
phen	17.570	1	17.570	3.142	0.0915
ozone × phen	0.453	1	0.453	0.081	0.7790
Error	111.823	20	5.591		
Total	130.777	23	5.686		

Dependent Variable: **PHOSPHATE 2**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	17.191	1	17.191	1.078	0.3197
phen	15.184	1	15.184	0.952	0.3485
ozone × phen	30.231	1	30.231	1.895	0.1938
Error	191.448	12	15.954		
Total	254.054	15	16.937		

Dependent Variable: **PHOSPHATE 3**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	38.353	1	38.353	0.689	0.4226
phen	111.733	1	111.733	2.008	0.1819
ozone × phen	18.126	1	18.126	0.326	0.5787
Error	667.626	12	55.636		
Total	835.838	15	55.723		

Dependent Variable: **PHOSPHATE 4**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	4.604	1	4.604	1.001	0.3367
phen	13.675	1	13.675	2.975	0.1102
ozone × phen	5.045	1	5.045	1.097	0.3155
Error	55.167	12	4.597		
Total	78.490	15	5.233		

Dependent Variable: **PHOSPHATE 6**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	0.030	1	0.030	0.080	0.7818
phen	8.693	1	8.693	23.154	0.0004
ozone × phen	0.004	1	0.004	0.011	0.9195
Error	4.505	12	0.375		
Total	13.232	15	0.882		

Appendix 3.13 (Table 5.2) Effects of ozone and phenmedipham on starch grain content per 100 chloroplasts of sugarbeet cv Saxon, where n=5 on days 3 and 7. Results of three-way ANOVA, classified by ozone, phenmedipham and time.

Dependent Variable: **STARCH GRAINS PER 100 CHLOROPLASTS**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	330.6	1	330.6	0.314	0.579
phen	100500.6	1	100500.6	95.367	0.000
time	469.2	1	469.2	0.445	0.509
ozone × phen	354.0	1	354.0	0.336	0.566
ozone × time	8439.0	1	8439.0	8.008	0.008
phen × time	648.0	1	648.0	0.615	0.439
ozone × phen × time	9333.0	1	9333.0	8.856	0.006
Error	33722.4	32	1053.8		
Total	153797.0	39	3943.5		

Appendix 3.13.1 Effects of ozone and phenmedipham on starch grain content per 100 chloroplasts of sugarbeet cv Saxon, where n=5 on days 3 and 7. Results of Duncan's Multiple Range Test, classified by ozone, phenmedipham and time. In the comparisons table, accept indicates that the two treatments are not significantly different at $p < 0.05$.

For **STARCH GRAINS PER 100 CHLOROPLASTS**, classified by treatment

Comparison	Difference	Std Error	q Stat	Table q	Result
phen7 - control7	144.8	14.52	9.974	3.320	Reject
phen7 - ozone7	131.6	14.52	9.065	3.286	Reject
phen7 - control3	128.4	14.52	8.844	3.244	Reject
phen7 - ozone3	118.2	14.52	8.142	3.191	Reject
phen7 - phen3	60.8	14.52	4.188	3.127	Reject
phen7 - o3phen7	59.8	14.52	4.119	3.027	Reject
phen7 - o3phen3	1.4	14.52	0.096	2.879	Accept
o3phen3 - control7	143.4	14.52	9.878	3.286	Reject
o3phen3 - ozone7	130.2	14.52	8.968	3.244	Reject
o3phen3 - control3	127.0	14.52	8.748	3.191	Reject
o3phen3 - ozone3	116.8	14.52	8.045	3.127	Reject
o3phen3 - phen3	59.4	14.52	4.092	3.027	Reject
o3phen3 - o3phen7	58.4	14.52	4.023	2.879	Reject
o3phen7 - control7	85.0	14.52	5.855	3.244	Reject
o3phen7 - ozone7	71.8	14.52	4.946	3.191	Reject
o3phen7 - control3	68.6	14.52	4.725	3.127	Reject
o3phen7 - ozone3	58.4	14.52	4.023	3.027	Reject
o3phen7 - phen3	1.0	14.52	0.069	2.879	Accept
phen3 - control7	84.0	14.52	5.786	3.191	Reject
phen3 - ozone7	70.8	14.52	4.877	3.127	Reject
phen3 - control3	67.6	14.52	4.656	3.027	Reject
phen3 - ozone3	57.4	14.52	3.954	2.879	Reject
ozone3 - control7	26.6	14.52	1.832	3.127	Accept
ozone3 - ozone7	13.4	14.52	0.923	3.027	Accept
ozone3 - control3	10.2	14.52	0.703	2.879	Accept
control3 - control7	16.4	14.52	1.130	3.027	Accept
control3 - ozone7	3.2	14.52	0.220	2.879	Accept
ozone7 - control7	13.2	14.52	0.909	2.879	Accept

Homogeneous Subsets:

Group 1: phen7 o3phen3

Pooled mean = 59.30

95% Confidence Interval = 38.39 80.21

Group 2: o3phen7 phen3

Pooled mean = 118.90

95% Confidence Interval = 97.99 139.81

Group 3: ozone3 control3 ozone7 control7

Pooled mean = 189.35

95% Confidence Interval = 174.56 204.14

Appendix 3.14 (Table 5.3) Effects of ozone and phenmedipham on thylakoid appression in chloroplasts of sugarbeet cv Saxon, where n=5 on days 3 and 7. Results of three-way ANOVA, classified by ozone, phenmedipham and time.

Dependent Variable: **THYLAKOID APPRESSION**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
ozone	6.241	1	6.241	24.902	0.0000
phen	19.321	1	19.321	77.091	0.0000
time	0.841	1	0.841	3.356	0.0763
ozone × phen	10.000	1	10.000	39.900	0.0000
ozone × time	1.296	1	1.296	5.171	0.0298
phen × time	1.600	1	1.600	6.384	0.0167
ozone × phen × time	0.841	1	0.841	3.356	0.0763
Error	8.020	32	0.251		
Total	48.160	39	1.235		

Appendix 3.14.1 Effects of ozone and phenmedipham on thylakoid appression in chloroplasts of sugarbeet cv Saxon, where n=5 on days 3 and 7. Results of Duncan's Multiple Range Test, classified by ozone, phenmedipham and time. In the comparisons table, accept indicates that the two treatments are not significantly different at $p < 0.05$.

For **THYLAKOID APPRESSION**, classified by treatment

Comparison	Difference	Std Error	q Stat	Table q	Result
ozone7 - o3phen7	3.08	0.22	13.76	3.32	Reject
ozone7 - o3phen3	2.46	0.22	10.99	3.29	Reject
ozone7 - phen7	1.36	0.22	6.07	3.24	Reject
ozone7 - control7	0.86	0.22	3.84	3.19	Reject
ozone7 - ozone3	0.76	0.22	3.39	3.13	Reject
ozone7 - phen3	0.60	0.22	2.68	3.03	Accept
ozone7 - control3	0.32	0.22	1.43	2.88	Accept
control3 - o3phen7	2.76	0.22	12.33	3.29	Reject
control3 - o3phen3	2.14	0.22	9.56	3.24	Reject
control3 - phen7	1.04	0.22	4.65	3.19	Reject
control3 - control7	0.54	0.22	2.41	3.13	Accept
control3 - ozone3	0.44	0.22	1.97	3.03	Accept
control3 - phen3	0.28	0.22	1.25	2.88	Accept
phen3 - o3phen7	2.48	0.22	11.08	3.24	Reject
phen3 - o3phen3	1.86	0.22	8.31	3.19	Reject
phen3 - phen7	0.76	0.22	3.39	3.13	Reject
phen3 - control7	0.26	0.22	1.16	3.03	Accept
phen3 - ozone3	0.16	0.22	0.71	2.88	Accept
ozone3 - o3phen7	2.32	0.22	10.36	3.19	Reject
ozone3 - o3phen3	1.70	0.22	7.59	3.13	Reject
ozone3 - phen7	0.60	0.22	2.68	3.03	Accept
ozone3 - control7	0.10	0.22	0.45	2.88	Accept
control7 - o3phen7	2.22	0.22	9.92	3.13	Reject
control7 - o3phen3	1.60	0.22	7.15	3.03	Reject
control7 - phen7	0.50	0.22	2.23	2.88	Accept
phen7 - o3phen7	1.72	0.22	7.68	3.03	Reject
phen7 - o3phen3	1.10	0.22	4.91	2.88	Reject
o3phen3 - o3phen7	0.62	0.22	2.77	2.88	Accept

Homogeneous Subsets:

Group 1:	ozone7 control3 phen3		Group 2:	control3 phen3 ozone3 control7	
Pooled mean =	4.63		Pooled mean =	4.96	
95% Confidence Interval =	4.36	4.89	95% Confidence Interval =	4.73	5.18
Group 3:	ozone3 control7 phen7		Group 4:	o3phen3 o3phen7	
Pooled mean =	5.31		Pooled mean =	7.09	
95% Confidence Interval =	5.05	5.58	95% Confidence Interval =	6.77	7.41

Appendix 4.1.1 (Table 6.1) Effects of ozone on protein content, expressed on a fresh weight basis, of sugarbeet cv Saxon, where n=4 on days -2 and -1. Results of one-way ANOVA, classified by ozone.

Dependent Variable: **PROTEIN** -2

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	6.174	1	6.174	2.709	0.1509
Error	13.676	6	2.279		
Total	19.851	7	2.836		

Dependent Variable: **PROTEIN** -1

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Treatment	1.555	1	1.555	0.784	0.4100
Error	11.899	6	1.983		
Total	13.453	7	1.922		

Appendix 4.1.2 (Table 6.1) Effects of ozone on superoxide dismutase activity, expressed on a fresh weight basis, of sugarbeet cv Saxon, where n=4 on days -2 and -1. Results of one-way ANOVA, classified by ozone.

Dependent Variable: **SUPEROXIDE DISMUTASE** d-2

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	303402.6	1	303402.6	1.833	0.2246
Error	993374.5	6	165562.4		
Total	1296777.1	7	185253.9		

Dependent Variable: **SUPEROXIDE DISMUTASE** d-1

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	1292175.3	1	1292175.3	5.437	0.0585
Error	1425983.4	6	237663.9		
Total	2718158.7	7	388308.4		

Appendix 4.1.3 (Table 6.1) Effects of ozone on ascorbate peroxidase activity, expressed on a fresh weight basis, of sugarbeet cv Saxon, where n=4 on days -2 and -1. Results of one-way ANOVA, classified by ozone.

Dependent Variable: **ASCORBATE PEROXIDASE** -2

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	203.543	1	203.543	3.283	0.1200
Error	372.005	6	62.001		
Total	575.548	7	82.221		

Dependent Variable: **ASCORBATE PEROXIDASE** -1

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Treatment	745.563	1	745.563	2.829	0.1436
Error	1581.314	6	263.552		
Total	2326.877	7	332.411		

Appendix 4.1.4 (Table 6.1) Effects of ozone on monodehydroascorbate reductase activity, expressed on a fresh weight basis, of sugarbeet cv Saxon, where n=4 on days -2 and -1. Results of one-way ANOVA, classified by ozone.

Dependent Variable: **MONODEHYDROASCORBATE REDUCTASE** -2

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.058	1	0.058	0.020	0.8909
Error	17.057	6	2.843		
Total	17.115	7	2.445		

Dependent Variable: **MONODEHYDROASCORBATE REDUCTASE** -1

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Treatment	0.306	1	0.306	0.314	0.5957
Error	5.855	6	0.976		
Total	6.161	7	0.880		

Appendix 4.1.5 (Table 6.1) Effects of ozone on glutathione reductase activity, expressed on a fresh weight basis, of sugarbeet cv Saxon, where n=4 on days -2 and -1. Results of one-way ANOVA, classified by ozone.

Dependent Variable: **GLUTATHIONE REDUCTASE** -2

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.768	1	0.768	2.669	0.1534
Error	1.726	6	0.288		
Total	2.494	7	0.356		

Dependent Variable: **GLUTATHIONE REDUCTASE** -1

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	2.099	1	2.099	3.917	0.0951
Error	3.216	6	0.536		
Total	5.315	7	0.759		

Appendix 4.1.6 (Table 6.1) Effects of ozone on catalase activity, expressed on a fresh weight basis, of sugarbeet cv Saxon, where n=4 on days -2 and -1. Results of one-way ANOVA, classified by ozone.

Dependent Variable: **CATALASE** -2

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.055	1	0.055	1.290	0.2994
Error	0.254	6	0.042		
Total	0.309	7	0.044		

Dependent Variable: **CATALASE** -1

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.000	1	0.000	0.001	0.9704
Error	0.112	6	0.019		
Total	0.112	7	0.016		

Appendix 4.1.7 (Table 6.1) Effects of ozone on general peroxidase activity, expressed on a fresh weight basis, of sugarbeet cv Saxon, where n=4 on days -2 and -1. Results of one-way ANOVA, classified by ozone.

Dependent Variable: **GENERAL PEROXIDASE** -2

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.188	1	0.188	0.447	0.5285
Error	2.527	6	0.421		
Total	2.715	7	0.388		

Dependent Variable: **GENERAL PEROXIDASE** -1

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.830	1	0.830	1.480	0.2695
Error	3.368	6	0.561		
Total	4.198	7	0.600		

Appendix 4.1.8 (Table 6.1) Effects of ozone on glutathione s-transferase activity, expressed on a fresh weight basis, of sugarbeet cv Saxon, where n=4 on days -2 and -1. Results of one-way ANOVA, classified by ozone.

Dependent Variable: **GLUTATHIONE S-TRANSFERASE** -2

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.242	1	0.242	3.450	0.1126
Error	0.421	6	0.070		
Total	0.664	7	0.095		

Dependent Variable: **GLUTATHIONE S-TRANSFERASE** -1

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.460	1	0.460	3.375	0.1159
Error	0.817	6	0.136		
Total	1.277	7	0.182		

Appendix 4.2 (Figure 6.8) Effects of ozone and phenmedipham on protein content, expressed on a fresh weight basis, of sugarbeet cv Saxon, where n=4 on days 0, 1, 2, 3, 4 and 6. Results of three-way ANOVA, classified by ozone, phenmedipham and time.

Dependent Variable: **PROTEIN** d0-6

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	5.848	1	5.848	1.871	0.1756
Phen	44.443	1	44.443	14.217	0.0003
Time	195.503	5	39.101	12.508	0.0000
Ozone × Phen	44.151	1	44.151	14.123	0.0003
Ozone × Time	41.385	5	8.277	2.648	0.0297
Phen × Time	37.551	5	7.510	2.402	0.0451
Ozone × Phen × Time	21.186	5	4.237	1.355	0.2513
Error	225.082	72	3.126		
Total	615.149	95	6.475		

Appendix 4.2.1 (Figure 6.8) Effects of ozone and phenmedipham on protein content of sugarbeet cv Saxon, where n=4 on days 0, 1, 2, 3, 4 and 6. Results of two-way ANOVA and, where appropriate, Duncan's Multiple Range Tests for protein content, classified by ozone and/or phenmedipham. In the DMRT comparisons table, accept indicates that the two means are not significantly different at $p < 0.05$ and * denotes significantly different pairs.

Dependent Variable: **PROTEIN d0**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	18.581	1	18.581	2.045	0.1747
Error	127.216	14	9.087		
Total	145.797	15	9.720		

Dependent Variable: **PROTEIN d1**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	2.996	1	2.996	1.283	0.2795
Phen	23.767	1	23.767	10.178	0.0078
Ozone × Phen	1.249	1	1.249	0.535	0.4786
Error	28.021	12	2.335		
Total	56.033	15	3.736		

Dependent Variable: **PROTEIN d2**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	8.721	1	8.721	6.031	0.0303
Phen	0.073	1	0.073	0.050	0.8260
Ozone × Phen	18.939	1	18.939	13.098	0.0035
Error	17.351	12	1.446		
Total	45.085	15	3.006		

For **PROTEIN d2**, classified by Treatment

Group	Cases	Mean	c2	op2	p2	o2
c2	4	9.6207			*	*
op2	4	10.9622				*
p2	4	11.6616	*			
o2	4	13.2733	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
c2 - o2	3.6525	0.6012	6.0750	3.3193	Reject

c2 - p2	2.0409	0.6012	3.3944	3.2216	Reject
c2 - op2	1.3415	0.6012	2.2312	3.0734	Accept
op2 - o2	2.3111	0.6012	3.8438	3.2216	Reject
op2 - p2	0.6994	0.6012	1.1633	3.0734	Accept
p2 - o2	1.6117	0.6012	2.6806	3.0734	Accept

Homogeneous Subsets:

Group 1:	c2 op2		Group 2:	op2 p2	
Pooled mean =	10.2915		Pooled mean =	11.3119	
95% Confidence Interval =	9.3652	11.2178	95% Confidence Interval =	10.3856	12.2382
Group 3:	p2 o2				
Pooled mean =	12.4674				
95% Confidence Interval =	11.5411	13.3937			

Dependent Variable: **PROTEIN d3**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	6.021	1	6.021	1.743	0.2114
Phen	4.159	1	4.159	1.204	0.2941
Ozone × Phen	24.886	1	24.886	7.205	0.0199
Error	41.450	12	3.454		
Total	76.515	15	5.101		

For **PROTEIN d3**, classified by Treatment

Group	Cases	Mean	op3	c3	o3	p3
op3	4	7.9650			*	*
c3	4	10.2115				
o3	4	11.4790	*			
p3	4	11.6862	*			

Comparison	Difference	Std Error	q Stat	Table q	Result
op3 - p3	3.7211	0.9293	4.0044	3.3193	Reject
op3 - o3	3.5139	0.9293	3.7814	3.2216	Reject
op3 - c3	2.2465	0.9293	2.4175	3.0734	Accept
c3 - p3	1.4746	0.9293	1.5869	3.2216	Accept
c3 - o3	1.2674	0.9293	1.3639	3.0734	Accept
o3 - p3	0.2072	0.9293	0.2230	3.0734	Accept

Homogeneous Subsets:

Group 1:	op3 c3		Group 2:	c3 o3 p3	
Pooled mean =	9.0883		Pooled mean =	11.1256	
95% Confidence Interval =	7.6566	10.5200	95% Confidence Interval =	9.9566	12.2945

Dependent Variable: **PROTEIN d4**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	10.562	1	10.562	3.551	0.0839
Phen	49.566	1	49.566	16.665	0.0015
Ozone × Phen	17.649	1	17.649	5.934	0.0314
Error	35.690	12	2.974		
Total	113.467	15	7.564		

For **PROTEIN d4**, classified by Treatment

Group	Cases	Mean	op4	p4	c4	o4
op4	4	8.9053		*	*	*
p4	4	12.6307	*			
c4	4	14.0504	*			
o4	4	14.5259	*			

Comparison	Difference	Std Error	q Stat	Table q	Result
op4 - o4	5.6207	0.8623	6.5183	3.3193	Reject
op4 - c4	5.1451	0.8623	5.9668	3.2216	Reject
op4 - p4	3.7255	0.8623	4.3204	3.0734	Reject
p4 - o4	1.8952	0.8623	2.1979	3.2216	Accept
p4 - c4	1.4196	0.8623	1.6464	3.0734	Accept
c4 - o4	0.4756	0.8623	0.5515	3.0734	Accept

Homogeneous Subsets:

Group 1:	op4		Group 2:	p4 c4 o4	
Pooled mean =	8.9053		Pooled mean =	13.7357	
95% Confidence Interval =	7.0265	10.7840	95% Confidence Interval =	12.6510	14.8204

Dependent Variable: **PROTEIN d6**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.352	1	0.352	0.177	0.6814
Phen	4.430	1	4.430	2.227	0.1615
Ozone × Phen	2.614	1	2.614	1.314	0.2741
Error	23.877	12	1.990		
Total	31.273	15	2.085		

Appendix 4.3 (Figure 6.9) Effects of ozone and phenmedipham on superoxide dismutase activity, expressed on a fresh weight basis, of sugarbeet cv Saxon, where n=4 on days 0, 1, 2, 3, 4 and 6. Results of three-way ANOVA, classified by ozone, phenmedipham and time.

Dependent Variable: **SUPEROXIDE DISMUTASE d0-6**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	422624218.6	1	422624218.6	5.047	0.0272
Phen	55944333.2	1	55944333.2	0.668	0.4159
Time	11929428322.1	5	2385885664.4	28.492	0.0000
Ozone × Phen	177365274.0	1	177365274.0	2.118	0.1491
Ozone × Time	1211236935.5	5	242247387.1	2.893	0.0182
Phen × Time	1322508262.4	5	264501652.5	3.159	0.0114
Ozone × Phen × Time	2964947285.3	5	592989457.1	7.081	0.0000
Error	7369128387.7	88	83740095.3		
Total	25453183018.8	111	229307955.1		

Appendix 4.3.1 (Figure 6.9) Effects of ozone and phenmedipham on superoxide dismutase activity, expressed on a fresh weight basis, of sugarbeet cv Saxon, where n=4 on day 0, 1, 2, 3, 4 and 6. Results of two-way ANOVA and, where appropriate, Duncan's Multiple Range Tests, classified by ozone and/or phenmedipham. In the DMRT comparisons table, accept indicates that the two means are not significantly different at $p < 0.05$ and * denotes significantly different pairs.

Dependent Variable: **SUPEROXIDE DISMUTASE d0**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	727423514.3	1	727423514.3	8.298	0.0121
Error	1227260675.4	14	87661476.8		
Total	1954684189.7	15	130312279.3		

For **SUPEROXIDE DISMUTASE**, classified by Treatment

Group	Cases	Mean	o0	c0
o0	8	47592.4		*
c0	8	61077.8	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
o0 - c0	13485.4	3310.2	4.0738	3.0261	Reject

Homogeneous Subsets:

Group 1:	o0		Group 2:	c0	
Pooled mean =	47592.4		Pooled mean =	61077.8	
95% Confidence Interval =	40492.6	54692.1	95% Confidence Interval =	53978.0	68177.5

Dependent Variable: **SUPEROXIDE DISMUTASE d1**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	41289830.1	1	41289830.1	0.771	0.3970
Phen	122303730.6	1	122303730.6	2.285	0.1565
Ozone × Phen	3388492.4	1	3388492.4	0.063	0.8056
Error	642344440.6	12	53528703.4		
Total	809326493.6	15	53955099.6		

Dependent Variable: **SUPEROXIDE DISMUTASE d2**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	615742.3	1.0	615742.3	0.003	0.9583
Phen	812692281.8	1.0	812692281.8	3.761	0.0763
Ozone × Phen	1524465659.8	1.0	1524465659.8	7.055	0.0209
Error	2592909584.8	12.0	216075798.7		
Total	4930683268.7	15.0	328712217.9		

For **SUPEROXIDE DISMUTASE d2**, classified by Treatment

Group	Cases	Mean	p2	o2	op2	c2
p2	4	48041.7				*
o2	4	61903.3				
op2	4	67171.6				
c2	4	81817.8	*			

Comparison	Difference	Std Error	q Stat	Table q	Result
p2 - c2	33776.1	7349.8	4.5955	3.3193	Reject
p2 - op2	19129.9	7349.8	2.6028	3.2216	Accept
p2 - o2	13861.5	7349.8	1.8860	3.0734	Accept
o2 - c2	19914.5	7349.8	2.7096	3.2216	Accept
o2 - op2	5268.3	7349.8	0.7168	3.0734	Accept
op2 - c2	14646.2	7349.8	1.9927	3.0734	Accept

Homogeneous Subsets:

Group 1:	p2 o2 op2		Group 2:	o2 op2 c2	
Pooled mean =	59038.9		Pooled mean =	70297.6	
95% Confidence Interval =	49793.3	68284.4	95% Confidence Interval =	61052.0	79543.1

Dependent Variable: **SUPEROXIDE DISMUTASE d3**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	48877494.1	1	48877494.1	0.866	0.3704
Phen	306985142.2	1	306985142.2	5.440	0.0379
Ozone × Phen	942871759.8	1	942871759.8	16.708	0.0015
Error	677182147.7	12	56431845.6		
Total	1975916543.9	15	131727769.6		

For **SUPEROXIDE DISMUTASE d3**, classified by Treatment

Group	Cases	Mean	o3	p3	c3	op3
o3	4	56128.0				*
p3	4	61392.9				*
c3	4	67985.5				*
op3	4	80241.6	*	*	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
o3 - op3	24113.6	3756.1	6.4199	3.3193	Reject
o3 - c3	11857.5	3756.1	3.1569	3.2216	Accept
o3 - p3	5264.9	3756.1	1.4017	3.0734	Accept
p3 - op3	18848.7	3756.1	5.0182	3.2216	Reject
p3 - c3	6592.6	3756.1	1.7552	3.0734	Accept
c3 - op3	12256.1	3756.1	3.2630	3.0734	Reject

Homogeneous Subsets:

Group 1:	o3 p3 c3		Group 2:	op3	
Pooled mean =	61835.5		Pooled mean =	80241.6	
95% Confidence Interval =	57110.6	66560.4	95% Confidence Interval =	72057.9	88425.4

Dependent Variable: **SUPEROXIDE DISMUTASE d4**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	29008862.7	1	29008862.7	0.695	0.4208
Phen	49768824.5	1	49768824.5	1.192	0.2963
Ozone × Phen	251627473.0	1	251627473.0	6.027	0.0303
Error	501001518.6	12	41750126.5		
Total	831406678.8	15	55427111.9		

For **SUPEROXIDE DISMUTASE d4**, classified by Treatment

Group	Cases	Mean	op4	c4	p4	o4
op4	4	37319.0			*	*
c4	4	43539.3				
p4	4	47943.4	*			
o4	4	48777.7	*			

Comparison	Difference	Std Error	q Stat	Table q	Result
op4 - o4	11458.7	3230.7	3.5468	3.3193	Reject
op4 - p4	10624.4	3230.7	3.2886	3.2216	Reject
op4 - c4	6220.3	3230.7	1.9254	3.0734	Accept
c4 - o4	5238.4	3230.7	1.6214	3.2216	Accept
c4 - p4	4404.0	3230.7	1.3632	3.0734	Accept
p4 - o4	834.4	3230.7	0.2583	3.0734	Accept

Homogeneous Subsets:

Group 1:	op4 c4		Group 2:	c4 p4 o4	
Pooled mean =	40429.2		Pooled mean =	46753.5	
95% Confidence Interval =	35451.8	45406.6	95% Confidence Interval =	42689.4	50817.5

Dependent Variable: **SUPEROXIDE DISMUTASE d6**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	59222196.3	1	59222196.3	1.418	0.2568
Phen	86702616.5	1	86702616.5	2.076	0.1752
Ozone × Phen	419959174.2	1	419959174.2	10.056	0.0081
Error	501169345.2	12	41764112.1		
Total	1067053332.3	15	71136888.8		

For **SUPEROXIDE DISMUTASE d6**, classified by Treatment

Group	Cases	Mean	c6	op6	o6	p6
c6	4	38649.5				*
op6	4	39457.4				*
o6	4	45048.1				
p6	4	53551.7	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
c6 - p6	14902.2	3231.3	4.6119	3.3193	Reject
c6 - o6	6398.7	3231.3	1.9802	3.2216	Accept
c6 - op6	807.9	3231.3	0.2500	3.0734	Accept
op6 - p6	14094.3	3231.3	4.3618	3.2216	Reject
op6 - o6	5590.7	3231.3	1.7302	3.0734	Accept
o6 - p6	8503.5	3231.3	2.6316	3.0734	Accept

Homogeneous Subsets:

Group 1:	c6 op6 o6		Group 2:	o6 p6	
Pooled mean =	41051.7		Pooled mean =	49299.9	
95% Confidence Interval =	36987.0	45116.4	95% Confidence Interval =	44321.7	54278.2

Appendix 4.4 (Figure 6.10) Effects of ozone and phenmedipham on ascorbate peroxidase activity, expressed on a fresh weight basis, of sugarbeet cv Saxon, where n=4 on days 0, 1, 2, 3, 4 and 6. Results of three-way ANOVA, classified by ozone, phenmedipham and time.

Dependent Variable: **ASCORBATE PEROXIDASE** d0-6

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	203.116	1	203.116	1.058	0.3071
Phen	0.615	1	0.615	0.003	0.9550
Time	19697.900	5	3939.580	20.518	0.0000
Ozone × Phen	800.358	1	800.358	4.168	0.0448
Ozone × Time	5735.913	5	1147.183	5.975	0.0001
Phen × Time	3783.210	5	756.642	3.941	0.0033
Ozone × Phen × Time	1972.754	5	394.551	2.055	0.0811
Error	13824.466	72	192.006		
Total	46018.333	95	484.404		

Appendix 4.4.1 (Figure 6.10) Effects of ozone and phenmedipham on ascorbate peroxidase activity expressed on a fresh weight basis, of sugarbeet cv Saxon, where n=4 on day 0, 1, 2, 3, 4 and 6. Results of two-way ANOVA and, where appropriate, Duncan's Multiple Range Tests, classified by ozone and/or phenmedipham. In the DMRT comparisons table, accept indicates that the two means are not significantly different at $p < 0.05$ and * denotes significantly different pairs.

Dependent Variable: **ASCORBATE PEROXIDASE d0**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	495.338	1	495.338	1.330	0.2681
Error	5214.000	14	372.429		
Total	5709.337	15	380.622		

Dependent Variable: **ASCORBATE PEROXIDASE** d1

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	24.561	1	24.561	0.121	0.7340
Phen	731.966	1	731.966	3.605	0.0819
Ozone × Phen	417.892	1	417.892	2.058	0.1769
Error	2436.287	12	203.024		
Total	3610.706	15	240.714		

Dependent Variable: **ASCORBATE PEROXIDASE d2**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	3789.144	1	3789.144	20.685	0.0007
Phen	220.831	1	220.831	1.206	0.2938
Ozone × Phen	229.945	1	229.945	1.255	0.2845
Error	2198.196	12	183.183		
Total	6438.117	15	429.208		

For **ASCORBATE PEROXIDASE d2**, classified by Treatment

Group	Cases	Mean	c2	p2	op2	o2
c2	4	64.3446			*	*
p2	4	79.3568			*	*
op2	4	102.5528	*	*		
o2	4	102.7046	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
c2 - o2	38.3600	6.7673	5.6685	3.3193	Reject

c2 - op2	38.2082	6.7673	5.6460	3.2216	Reject
c2 - p2	15.0122	6.7673	2.2184	3.0734	Accept
p2 - o2	23.3478	6.7673	3.4501	3.2216	Reject
p2 - op2	23.1960	6.7673	3.4277	3.0734	Reject
op2 - o2	0.1518	6.7673	0.0224	3.0734	Accept

Homogeneous Subsets:

Group 1:	c2 p2		Group 2:	op2 o2	
Pooled mean =	71.85		Pooled mean =	102.63	
95% Confidence Interval =	61.42	82.28	95% Confidence Interval =	92.20	113.05

Dependent Variable: **ASCORBATE PEROXIDASE d3**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	535.139	1	535.139	10.037	0.0081
Phen	206.394	1	206.394	3.871	0.0727
Ozone × Phen	481.296	1	481.296	9.027	0.0110
Error	639.788	12	53.316		
Total	1862.616	15	124.174		

For **ASCORBATE PEROXIDASE d3**, classified by Treatment

Group	Cases	Mean	op3	o3	c3	p3
op3	4	44.1914				*
o3	4	47.9775				*
c3	4	48.5748				*
p3	4	66.7272	*	*	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
op3 - p3	22.5358	3.6509	6.1727	3.3193	Reject
op3 - c3	4.3833	3.6509	1.2006	3.2216	Accept
op3 - o3	3.7860	3.6509	1.0370	3.0734	Accept
o3 - p3	18.7497	3.6509	5.1357	3.2216	Reject
o3 - c3	0.5973	3.6509	0.1636	3.0734	Accept
c3 - p3	18.1524	3.6509	4.9721	3.0734	Reject

Homogeneous Subsets:

Group 1:	op3 o3 c3		Group 2:	p3	
Pooled mean =	46.9146		Pooled mean =	66.7272	
95% Confidence Interval =	42.3220	51.5071	95% Confidence Interval =	58.7726	74.6818

Dependent Variable: **ASCORBATE PEROXIDASE d4**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	69.308	1	69.308	0.313	0.5863
Phen	10.438	1	10.438	0.047	0.8318
Ozone × Phen	223.420	1	223.420	1.008	0.3352
Error	2659.435	12	221.620		
Total	2962.601	15	197.507		

Dependent Variable: **ASCORBATE PEROXIDASE d6**

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	1025.539	1	1025.539	7.497	0.0180
Phen	2614.196	1	2614.196	19.110	0.0009
Ozone × Phen	1420.559	1	1420.559	10.384	0.0073
Error	1641.554	12	136.796		
Total	6701.849	15	446.790		

For **ASCORBATE PEROXIDASE d6**, classified by Treatment

Group	Cases	Mean	op6	p6	c6	o6
op6	4	32.7429				*
p6	4	35.5760				*
c6	4	42.2955				*
o6	4	77.1527	*	*	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
op6 - o6	44.4098	5.8480	7.5940	3.3193	Reject
op6 - c6	9.5526	5.8480	1.6335	3.2216	Accept
op6 - p6	2.8331	5.8480	0.4845	3.0734	Accept
p6 - o6	41.5766	5.8480	7.1096	3.2216	Reject
p6 - c6	6.7195	5.8480	1.1490	3.0734	Accept
c6 - o6	34.8572	5.8480	5.9605	3.0734	Reject

Homogeneous Subsets:

Group 1:	op6 p6 c6		Group 2:	o6	
Pooled mean =	36.8715		Pooled mean =	77.1527	
95% Confidence Interval =	29.5151	44.2279	95% Confidence Interval =	64.4110	89.8944

Appendix 4.5 (Figure 6.11) Effects of ozone and phenmedipham on monodehydroascorbate reductase activity, expressed on a fresh weight basis, of sugarbeet cv Saxon, where n=4 on days 0, 1, 2, 3, 4 and 6. Results of three-way ANOVA, classified by ozone, phenmedipham and time.

Dependent Variable: **MONODEHYDROASCORBATE REDUCTASE** d0-6

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	13.134	1	13.134	19.476	0.0000
Phen	0.805	1	0.805	1.194	0.2781
Time	310.230	5	62.046	92.002	0.0000
Ozone × Phen	0.107	1	0.107	0.159	0.6910
Ozone × Time	23.567	5	4.713	6.989	0.0000
Phen × Time	7.211	5	1.442	2.139	0.0705
Ozone × Phen × Time	16.426	5	3.285	4.871	0.0007
Error	48.557	72	0.674		
Total	420.038	95	4.421		

Appendix 4.5.1 (Figure 6.11) Effects of ozone and phenmedipham on monodehydroascorbate reductase activity expressed on a fresh weight basis, of sugarbeet cv Saxon, where n=4 on day 0, 1, 2, 3, 4 and 6. Results of two-way ANOVA and, where appropriate, Duncan's Multiple Range Tests, classified by ozone and/or phenmedipham. In the DMRT comparisons table, accept indicates that the two means are not significantly different at $p < 0.05$ and * denotes significantly different pairs.

Dependent Variable: **MONODEHYDROASCORBATE REDUCTASE** d0

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	24.653	1	24.653	5.308	0.0371
Error	65.021	14	4.644		
Total	89.674	15	5.978		

For **MONODEHYDROASCORBATE REDUCTASE** d0, classified by Treatment

Group	Cases	Mean	c0	o0
c0	8	4.5711		*
o0	8	7.0537	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
c0 - o0	2.4826	0.7619	3.2583	3.0261	Reject

Homogeneous Subsets:

Group 1:	c0		Group 2:	o0	
Pooled mean =	4.5711275		Pooled mean =	7.0537256	
95% Confidence Interval =	2.9369369	6.2053182	95% Confidence Interval =	5.4195349	8.6879162

Dependent Variable: **MONODEHYDROASCORBATE REDUCTASE** d1

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	4.528	1	4.528	10.228	0.0077
Phen	6.015	1	6.015	13.587	0.0031
Ozone × Phen	8.060	1	8.060	18.206	0.0011
Error	5.313	12	0.443		
Total	23.917	15	1.594		

For **MONODEHYDROASCORBATE REDUCTASE** d1, classified by Treatment

Group	Cases	Mean	o1	p1	c1	op1
o1	4.00	4.65				*
p1	4.00	4.81				*
c1	4.00	5.00				*
op1	4.00	7.29	*	*	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
o1 - op1	2.65	0.33	7.95	3.32	Reject
o1 - c1	0.36	0.33	1.07	3.22	Accept
o1 - p1	0.16	0.33	0.49	3.07	Accept
p1 - op1	2.48	0.33	7.47	3.22	Reject
p1 - c1	0.19	0.33	0.58	3.07	Accept
c1 - op1	2.29	0.33	6.88	3.07	Reject

Homogeneous Subsets:

Group 1:	o1 p1 c1		Group 2:	op1	
Pooled mean =	4.82		Pooled mean =	7.29	
95% Confidence Interval =	4.40	5.24	95% Confidence Interval =	6.57	8.02

Dependent Variable: **MONODEHYDROASCORBATE REDUCTASE** d2

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	6.986	1	6.986	10.450	0.0072
Phen	1.031	1	1.031	1.542	0.2380
Ozone × Phen	6.390	1	6.390	9.559	0.0093
Error	8.022	12	0.669		
Total	22.429	15	1.495		

For **MONODEHYDROASCORBATE REDUCTASE** d2, classified by Treatment

Group	Cases	Mean	c2	p2	op2	o2
c2	4	3.8339		*	*	*
p2	4	5.6055	*			
op2	4	5.6631	*			
o2	4	6.4194	*			

Comparison	Difference	Std Error	q Stat	Table q	Result
c2 - o2	2.5855	0.4088	6.3244	3.3193	Reject
c2 - op2	1.8292	0.4088	4.4745	3.2216	Reject
c2 - p2	1.7716	0.4088	4.3335	3.0734	Reject
p2 - o2	0.8139	0.4088	1.9909	3.2216	Accept
p2 - op2	0.0576	0.4088	0.1410	3.0734	Accept
op2 - o2	0.7563	0.4088	1.8499	3.0734	Accept

Homogeneous Subsets:

Group 1:	c2		Group 2:	p2 op2 o2	
Pooled mean =	3.8339		Pooled mean =	5.8960	
95% Confidence Interval =	2.9432	4.7246	95% Confidence Interval =	5.3817	6.4103

Dependent Variable: **MONODEHYDROASCORBATE REDUCTASE** d3

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.357	1	0.357	6.454	0.0259
Phen	0.028	1	0.028	0.513	0.4875
Ozone × Phen	0.012	1	0.012	0.224	0.6446
Error	0.664	12	0.055		
Total	1.062	15	0.071		

Dependent Variable: **MONODEHYDROASCORBATE REDUCTASE** d4

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.019	1	0.019	0.135	0.7195
Phen	0.020	1	0.020	0.144	0.7106

Ozone × Phen	0.040	1	0.040	0.290	0.5998
Error	1.647	12	0.137		
Total	1.725	15	0.115		

Dependent Variable: **MONODEHYDROASCORBATE REDUCTASE** d6

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.158	1	0.158	1.382	0.2626
Phen	0.922	1	0.922	8.066	0.0149
Ozone × Phen	2.030	1	2.030	17.755	0.0012
Error	1.372	12	0.114		
Total	4.483	15	0.299		

For **MONODEHYDROASCORBATE REDUCTASE** d6, classified by Treatment

Group	Cases	Mean	op6	c6	p6	o6
op6	4	3.2723		*	*	*
c6	4	3.9512	*			
p6	4	4.1835	*			
o6	4	4.4649	*			

Comparison	Difference	Std Error	q Stat	Table q	Result
op6 - o6	1.1927	0.1691	7.0538	3.3193	Reject
op6 - p6	0.9112	0.1691	5.3893	3.2216	Reject
op6 - c6	0.6790	0.1691	4.0157	3.0734	Reject
c6 - o6	0.5137	0.1691	3.0381	3.2216	Accept
c6 - p6	0.2322	0.1691	1.3736	3.0734	Accept
p6 - o6	0.2814	0.1691	1.6646	3.0734	Accept

Homogeneous Subsets:

Group 1:	op6		Group 2:	c6 p6 o6	
Pooled mean =	3.2723		Pooled mean =	4.1999	
95% Confidence Interval =	2.9039	3.6407	95% Confidence Interval =	3.9872	4.4126

Appendix 4.6 (Figure 6.12) Effects of ozone and phenmedipham on glutathione reductase activity, expressed on a fresh weight basis, of sugarbeet cv Saxon, where n=4 on days 0, 1, 2, 3, 4 and 6. Results of three-way ANOVA, classified by ozone, phenmedipham and time.

Dependent Variable: **GLUTATHIONE REDUCTASE** d0-6

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	10.157	1	10.157	13.210	0.0005
Phen	19.882	1	19.882	25.859	0.0000
Time	106.226	5	21.245	27.631	0.0000
Ozone × Phen	0.559	1	0.559	0.727	0.3966
Ozone × Time	4.619	5	0.924	1.202	0.3172
Phen × Time	12.662	5	2.532	3.294	0.0098
Ozone × Phen × Time	10.263	5	2.053	2.670	0.0286
Error	55.359	72	0.769		
Total	219.728	95	2.313		

Appendix 4.6.1 (Figure 6.12) Effects of ozone and phenmedipham on glutathione reductase activity expressed on a fresh weight basis, of sugarbeet cv Saxon, where n=4 on day 0, 1, 2, 3, 4 and 6. Results of two-way ANOVA and, where appropriate, Duncan's Multiple Range Tests, classified by ozone and/or phenmedipham. In the DMRT comparisons table, accept indicates that the two means are not significantly different at $p < 0.05$ and * denotes significantly different pairs.

Dependent Variable: **GLUTATHIONE REDUCTASE** d0

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.519	1	0.519	0.135	0.7183
Error	53.643	14	3.832		
Total	54.162	15	3.611		

Dependent Variable: **GLUTATHIONE REDUCTASE** d1

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	7.704	1	7.704	16.917	0.0014
Phen	15.782	1	15.782	34.653	0.0001
Ozone × Phen	5.750	1	5.750	12.626	0.0040
Error	5.465	12	0.455		
Total	34.701	15	2.313		

For **GLUTATHIONE REDUCTASE** d1, classified by Treatment

Group	Cases	Mean	c1	o1	p1	op1
c1	4	4.1781				*
o1	4	4.3670				*
p1	4	4.9655				*
op1	4	7.5523	*	*	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
c1 - op1	3.3742	0.3374	9.9997	3.3193	Reject
c1 - p1	0.7873	0.3374	2.3333	3.2216	Accept
c1 - o1	0.1889	0.3374	0.5597	3.0734	Accept
o1 - op1	3.1853	0.3374	9.4400	3.2216	Reject
o1 - p1	0.5985	0.3374	1.7736	3.0734	Accept
p1 - op1	2.5868	0.3374	7.6664	3.0734	Reject

Homogeneous Subsets:

Group 1:	c1 o1 p1		Group 2:	op1	
Pooled mean =	4.5035257		Pooled mean =	7.5522817	
95% Confidence Interval =	4.0790653	4.927861	95% Confidence Interval =	6.8170948	8.2874687

Dependent Variable: **GLUTATHIONE REDUCTASE** d2

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	3.546	1	3.546	7.201	0.0199
Phen	12.559	1	12.559	25.501	0.0003
Ozone × Phen	2.403	1	2.403	4.880	0.0474
Error	5.910	12	0.492		
Total	24.419	15	1.628		

For **GLUTATHIONE REDUCTASE** d2, classified by Treatment

Group	Cases	Mean	c2	o2	p2	op2
c2	4	3.5707		*	*	*
o2	4	5.2874	*			
p2	4	6.1177	*			
op2	4	6.2842	*			

Comparison	Difference	Std Error	q Stat	Table q	Result
c2 - op2	2.7135	0.3509	7.7333	3.3193	Reject
c2 - p2	2.5471	0.3509	7.2589	3.2216	Reject
c2 - o2	1.7167	0.3509	4.8925	3.0734	Reject
o2 - op2	0.9968	0.3509	2.8408	3.2216	Accept
o2 - p2	0.8304	0.3509	2.3665	3.0734	Accept
p2 - op2	0.1665	0.3509	0.4744	3.0734	Accept

Homogeneous Subsets:

Group 1:	c2		Group 2:	o2 p2 op2	
Pooled mean =	3.5707		Pooled mean =	5.8964	
95% Confidence Interval =	2.8062	4.3352	95% Confidence Interval =	5.4550	6.3378

Dependent Variable: **GLUTATHIONE REDUCTASE** d3

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	2.693	1	2.693	22.973	0.0004
Phen	2.182	1	2.182	18.617	0.0010
Ozone × Phen	0.230	1	0.230	1.963	0.1865
Error	1.407	12	0.117		
Total	6.512	15	0.434		

For Glutathione reductase d0-6, classified by Treatment

Group	Cases	Mean	c3	p3	o3	op3
c3	4	1.9415		*	*	*
p3	4	2.9200	*			*
o3	4	3.0019	*			
op3	4	3.5007	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
c3 - op3	1.5591	0.1712	9.1078	3.3193	Reject
c3 - o3	1.0604	0.1712	6.1942	3.2216	Reject
c3 - p3	0.9785	0.1712	5.7159	3.0734	Reject
p3 - op3	0.5807	0.1712	3.3919	3.2216	Reject
p3 - o3	0.0819	0.1712	0.4783	3.0734	Accept
o3 - op3	0.4988	0.1712	2.9136	3.0734	Accept

Homogeneous Subsets:

Group 1:	c3		Group 2:	p3 o3	
Pooled mean =	1.942		Pooled mean =	2.961	
95% Confidence Interval =	1.569	2.315	95% Confidence Interval =	2.697	3.225

Group 3:	o3 op3	
Pooled mean =	3.251	
95% Confidence Interval =	2.988	3.515

Dependent Variable: **GLUTATHIONE REDUCTASE** d4

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.121	1	0.121	1.057	0.3243
Phen	0.462	1	0.462	4.040	0.0675
Ozone × Phen	1.001	1	1.001	8.747	0.0120
Error	1.373	12	0.114		
Total	2.956	15	0.197		

For **GLUTATHIONE REDUCTASE** d4, classified by Treatment

Group	Cases	Mean	c4	op4	o4	p4
c4	4	2.4852			*	*
op4	4	2.9990				
o4	4	3.1592	*			
p4	4	3.3253	*			

Comparison	Difference	Std Error	q Stat	Table q	Result
c4 - p4	0.8400	0.1691	4.9674	3.3193	Reject
c4 - o4	0.6740	0.1691	3.9854	3.2216	Reject
c4 - op4	0.5137	0.1691	3.0378	3.0734	Accept
op4 - p4	0.3263	0.1691	1.9296	3.2216	Accept
op4 - o4	0.1602	0.1691	0.9476	3.0734	Accept
o4 - p4	0.1661	0.1691	0.9820	3.0734	Accept

Homogeneous Subsets:

Group 1:	c4 op4		Group 2:	op4 o4 p4	
Pooled mean =	2.7421		Pooled mean =	3.1612	
95% Confidence Interval =	2.4816	3.0027	95% Confidence Interval =	2.9484	3.3739

Dependent Variable: **GLUTATHIONE REDUCTASE** d6

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.192	1	0.192	0.703	0.4183
Phen	1.559	1	1.559	5.699	0.0343
Ozone × Phen	1.438	1	1.438	5.258	0.0407
Error	3.282	12	0.274		
Total	6.472	15	0.431		

For **GLUTATHIONE REDUCTASE** d6, classified by Treatment

Group	Cases	Mean	c6	o6	op6	p6
c6	4	2.3278		*	*	*
o6	4	3.1467	*			
op6	4	3.1713	*			
p6	4	3.5518	*			

Comparison	Difference	Std Error	q Stat	Table q	Result
c6 - p6	1.2240	0.2615	4.6804	3.3193	Reject
c6 - op6	0.8435	0.2615	3.2255	3.2216	Reject
c6 - o6	0.8188	0.2615	3.1313	3.0734	Reject
o6 - p6	0.4051	0.2615	1.5491	3.2216	Accept
o6 - op6	0.0246	0.2615	0.0942	3.0734	Accept
op6 - p6	0.3805	0.2615	1.4549	3.0734	Accept

Homogeneous Subsets:

Group 1:	c6			Group 2:	o6 op6 p6	
Pooled mean =	2.3278			Pooled mean =	3.2899	
95% Confidence Interval =	1.7580	2.8976		95% Confidence Interval =	2.9610	3.6189

Appendix 4.7 (Figure 6.13) Effects of ozone and phenmedipham on catalase activity, expressed on a fresh weight basis, of sugarbeet cv Saxon, where n=4 on days 0, 1, 2, 3, 4 and 6. Results of three-way ANOVA, classified by ozone, phenmedipham and time.

Dependent Variable: **CATALASE** d0-6

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.066	1	0.066	4.132	0.0458
Phen	0.017	1	0.017	1.073	0.3037
Time	1.548	5	0.310	19.343	0.0000
Ozone × Phen	0.207	1	0.207	12.947	0.0006
Ozone × Time	0.767	5	0.153	9.589	0.0000
Phen × Time	0.214	5	0.043	2.669	0.0287
Ozone × Phen × Time	0.406	5	0.081	5.068	0.0005
Error	1.153	72	0.016		
Total	4.378	95	0.046		

Appendix 4.7.1 (Figure 6.13) Effects of ozone and phenmedipham on catalase activity expressed on a fresh weight basis, of sugarbeet cv Saxon, where n=4 on day 0, 1, 2, 3, 4 and 6. Results of two-way ANOVA and, where appropriate, Duncan's Multiple Range Tests, classified by ozone and/or phenmedipham. In the DMRT comparisons table, accept indicates that the two means are not significantly different at $p < 0.05$ and * denotes significantly different pairs.

Dependent Variable: **CATALASE** d0

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.086	1	0.086	1.439	0.2502
Error	0.840	14	0.060		
Total	0.926	15	0.062		

Dependent Variable: **CATALASE** d1

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.000	1	0.000	0.030	0.8661
Phen	0.063	1	0.063	6.044	0.0301
Ozone × Phen	0.078	1	0.078	7.489	0.0180
Error	0.125	12	0.010		
Total	0.265	15	0.018		

For **CATALASE** d1, classified by Treatment

Group	Cases	Mean	p1	o1	op1	c1
p1	4	0.5733				*
o1	4	0.6898				
op1	4	0.7039				
c1	4	0.8379	*			

Comparison	Difference	Std Error	q Stat	Table q	Result
p1 - c1	0.2646	0.0509	5.1949	3.3193	Reject
p1 - op1	0.1306	0.0509	2.5644	3.2216	Accept
p1 - o1	0.1165	0.0509	2.2861	3.0734	Accept
o1 - c1	0.1482	0.0509	2.9088	3.2216	Accept
o1 - op1	0.0142	0.0509	0.2782	3.0734	Accept
op1 - c1	0.1340	0.0509	2.6306	3.0734	Accept

Homogeneous Subsets:

Group 1:	p1 o1 op1		Group 2:	o1 op1 c1	
Pooled mean =	0.6557		Pooled mean =	0.7439	
95% Confidence Interval =	0.5916	0.7197	95% Confidence Interval =	0.6798	0.8080

Dependent Variable: **CATALASE** d2

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.627	1	0.627	174.231	0.0000
Phen	0.064	1	0.064	17.844	0.0012
Ozone × Phen	0.171	1	0.171	47.455	0.0000
Error	0.043	12	0.004		
Total	0.905	15	0.060		

For **CATALASE** d2, classified by Treatment

Group	Cases	Mean	c2	p2	op2	o2
c2	4	0.2623			*	*
p2	4	0.3422			*	*
op2	4	0.5314	*	*		*
o2	4	0.8647	*	*	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
c2 - o2	0.6024	0.0300	20.0885	3.3193	Reject
c2 - op2	0.2691	0.0300	8.9754	3.2216	Reject
c2 - p2	0.0799	0.0300	2.6645	3.0734	Accept
p2 - o2	0.5225	0.0300	17.4239	3.2216	Reject
p2 - op2	0.1892	0.0300	6.3109	3.0734	Reject
op2 - o2	0.3332	0.0300	11.1130	3.0734	Reject

Homogeneous Subsets:

Group 1:	c2 p2		Group 2:	op2	
Pooled mean =	0.3022		Pooled mean =	0.5314	
95% Confidence Interval =	0.2561	0.3484	95% Confidence Interval =	0.4661	0.5968
Group 3:	o2				
Pooled mean =	0.8647				
95% Confidence Interval =	0.7993	0.9300			

Dependent Variable: **CATALASE** d3

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.002	1	0.002	0.387	0.5456
Phen	0.084	1	0.084	14.302	0.0026
Ozone × Phen	0.125	1	0.125	21.273	0.0006
Error	0.071	12	0.006		
Total	0.283	15	0.019		

For **CATALASE** d3, classified by Treatment

Group	Cases	Mean	c3	op3	o3	p3
c3	4	0.1271		*	*	*
op3	4	0.2961	*			*
o3	4	0.3279	*			*
p3	4	0.4492	*	*	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
c3 - p3	0.3221	0.0384	8.3941	3.3193	Reject
c3 - o3	0.2009	0.0384	5.2343	3.2216	Reject
c3 - op3	0.1690	0.0384	4.4038	3.0734	Reject
op3 - p3	0.1531	0.0384	3.9903	3.2216	Reject
op3 - o3	0.0319	0.0384	0.8305	3.0734	Accept
o3 - p3	0.1213	0.0384	3.1599	3.0734	Reject

Homogeneous Subsets:

Group 1:	c3		Group 2:	op3 o3	
Pooled mean =	0.1271		Pooled mean =	0.3120	
95% Confidence Interval =	0.0435	0.2107	95% Confidence Interval =	0.2529	0.3711
Group 3:	p3				
Pooled mean =	0.4492				
95% Confidence Interval =	0.3656	0.5328			

Dependent Variable: **CATALASE** d4

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.063	1	0.063	9.699	0.0089
Phen	0.019	1	0.019	2.872	0.1159
Ozone × Phen	0.211	1	0.211	32.353	0.0001
Error	0.078	12	0.007		
Total	0.371	15	0.025		

For **CATALASE** d4, classified by Treatment

Group	Cases	Mean	op4	c4	o4	p4
op4	4	0.2501		*	*	*
c4	4	0.4444	*			*
o4	4	0.5483	*			
p4	4	0.6057	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
op4 - p4	0.3555	0.0404	8.8023	3.3193	Reject
op4 - o4	0.2982	0.0404	7.3827	3.2216	Reject
op4 - c4	0.1942	0.0404	4.8090	3.0734	Reject
c4 - p4	0.1613	0.0404	3.9932	3.2216	Reject
c4 - o4	0.1040	0.0404	2.5737	3.0734	Accept
o4 - p4	0.0573	0.0404	1.4196	3.0734	Accept

Homogeneous Subsets:

Group 1:	op4		Group 2:	c4 o4	
Pooled mean =	0.2501		Pooled mean =	0.4964	
95% Confidence Interval =	0.1621	0.3381	95% Confidence Interval =	0.4341	0.5586
Group 3:	o4 p4				
Pooled mean =	0.5770				
95% Confidence Interval =	0.5148	0.6392			

Appendix 4.8 (Figure 6.14) Effects of ozone and phenmedipham on general peroxidase activity, expressed on a fresh weight basis, of sugarbeet cv Saxon, where n=4 on days 0, 1, 2, 3, 4 and 6. Results of three-way ANOVA, classified by ozone, phenmedipham and time.

Dependent Variable: **GENERAL PEROXIDASE** d0-6

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	24.714	1	24.714	38.797	0.0000
Phen	52.516	1	52.516	82.439	0.0000
Time	25.605	5	5.121	8.039	0.0000
Ozone × Phen	8.071	1	8.071	12.669	0.0007
Ozone × Time	16.535	5	3.307	5.191	0.0004
Phen × Time	15.304	5	3.061	4.805	0.0008
Ozone × Phen × Time	27.088	5	5.418	8.505	0.0000
Error	45.865	72	0.637		
Total	215.698	95	2.271		

Appendix 4.8.1 (Figure 6.14) Effects of ozone and phenmedipham on general peroxidase activity expressed on a fresh weight basis, of sugarbeet cv Saxon, where n=4 on day 0, 1, 2, 3, 4 and 6. Results of two-way ANOVA and, where appropriate, Duncan's Multiple Range Tests, classified by ozone and/or phenmedipham. In the DMRT comparisons table, accept indicates that the two means are not significantly different at $p < 0.05$ and * denotes significantly different pairs.

Dependent Variable: **GENERAL PEROXIDASE** d0

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	30.975	1	30.975	8.983	0.0096
Error	48.276	14	3.448		
Total	79.250	15	5.283		

For **GENERAL PEROXIDASE** d0, classified by Treatment

Group	Cases	Mean	c0	o0
c0	8	2.8311		*
o0	8	5.6138	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
c0 - o0	2.7828	0.6565	4.2386	3.0261	Reject

Homogeneous Subsets:

Group 1:	c0		Group 2:	o0	
Pooled mean =	2.8311		Pooled mean =	5.6138	
95% Confidence Interval =	1.4229	4.2392	95% Confidence Interval =	4.2057	7.0219

Dependent Variable: **GENERAL PEROXIDASE** d1

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	4.705	1	4.705	5.913	0.0316
Phen	7.157	1	7.157	8.995	0.0111
Ozone × Phen	7.980	1	7.980	10.030	0.0081
Error	9.548	12	0.796		
Total	29.390	15	1.959		

For **GENERAL PEROXIDASE** d1, classified by Treatment

Group	Cases	Mean	o1	p1	c1	op1
o1	4	2.6290				*
p1	4	2.8821				*
c1	4	2.9570				*
op1	4	5.3791	*	*	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
o1 - op1	2.7501	0.4460	6.1661	3.3193	Reject
o1 - c1	0.3280	0.4460	0.7353	3.2216	Accept
o1 - p1	0.2531	0.4460	0.5675	3.0734	Accept
p1 - op1	2.4970	0.4460	5.5986	3.2216	Reject
p1 - c1	0.0748	0.4460	0.1678	3.0734	Accept
c1 - op1	2.4222	0.4460	5.4308	3.0734	Reject

Homogeneous Subsets:

Group 1:	o1 p1 c1		Group 2:	op1	
Pooled mean =	2.8227		Pooled mean =	5.3791	
95% Confidence Interval =	2.2616	3.3837	95% Confidence Interval =	4.4074	6.3509

Dependent Variable: **GENERAL PEROXIDASE** d2

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	1.464	1	1.464	3.789	0.0754
Phen	13.780	1	13.780	35.667	0.0001
Ozone × Phen	1.890	1	1.890	4.892	0.0471
Error	4.636	12	0.386		
Total	21.770	15	1.451		

For **GENERAL PEROXIDASE** d2, classified by Treatment

Group	Cases	Mean	c2	o2	op2	p2
c2	4	1.7911		*	*	*
o2	4	3.0834	*		*	*
op2	4	4.2521	*	*		
p2	4	4.3345	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
c2 - p2	2.5434	0.3108	8.1840	3.3193	Reject
c2 - op2	2.4610	0.3108	7.9187	3.2216	Reject
c2 - o2	1.2923	0.3108	4.1583	3.0734	Reject
o2 - p2	1.2511	0.3108	4.0256	3.2216	Reject
o2 - op2	1.1687	0.3108	3.7604	3.0734	Reject
op2 - p2	0.0824	0.3108	0.2652	3.0734	Accept

Homogeneous Subsets:

Group 1:	c2		Group 2:	o2	
Pooled mean =	1.7911		Pooled mean =	3.0834	
95% Confidence Interval =	1.1139	2.4682	95% Confidence Interval =	2.4063	3.7605
Group 3:	op2 p2				
Pooled mean =	4.2933				
95% Confidence Interval =	3.8145	4.7721			

Dependent Variable: **GENERAL PEROXIDASE** d3

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.588	1	0.588	3.270	0.0957
Phen	18.865	1	18.865	104.852	0.0000
Ozone × Phen	6.313	1	6.313	35.087	0.0001
Error	2.159	12	0.180		
Total	27.925	15	1.862		

For **GENERAL PEROXIDASE** d3, classified by Treatment

Group	Cases	Mean	c3	o3	op3	p3
c3	4	0.6679		*	*	*

o3	4	2.3077	*		*	*
op3	4	3.2231	*	*		*
p3	4	4.0958	*	*	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
c3 - p3	3.4280	0.2121	16.1631	3.3193	Reject
c3 - op3	2.5552	0.2121	12.0480	3.2216	Reject
c3 - o3	1.6398	0.2121	7.7317	3.0734	Reject
o3 - p3	1.7882	0.2121	8.4314	3.2216	Reject
o3 - op3	0.9154	0.2121	4.3163	3.0734	Reject
op3 - p3	0.8728	0.2121	4.1151	3.0734	Reject

Homogeneous Subsets:

Group 1:	c3		Group 2:	o3	
Pooled mean =	0.6679		Pooled mean =	2.3077	
95% Confidence Interval =	0.2058	1.1300	95% Confidence Interval =	1.8456	2.7697
Group 3:	op3		Group 4:	p3	
Pooled mean =	3.2231		Pooled mean =	4.0958	
95% Confidence Interval =	2.7610	3.6852	95% Confidence Interval =	3.6337	4.5579

Dependent Variable: **GENERAL PEROXIDASE** d4

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.554	1	0.554	1.797	0.2049
Phen	4.887	1	4.887	15.847	0.0018
Ozone × Phen	4.227	1	4.227	13.706	0.0030
Error	3.701	12	0.308		
Total	13.369	15	0.891		

For **GENERAL PEROXIDASE** d4, classified by Treatment

Group	Cases	Mean	c4	o4	op4	p4
c4	4	1.6613		*	*	*
o4	4	3.0615	*			
op4	4	3.1388	*			
p4	4	3.7946	*			

Comparison	Difference	Std Error	q Stat	Table q	Result
c4 - p4	2.1333	0.2777	7.6830	3.3193	Reject
c4 - op4	1.4776	0.2777	5.3214	3.2216	Reject
c4 - o4	1.4002	0.2777	5.0428	3.0734	Reject
o4 - p4	0.7331	0.2777	2.6402	3.2216	Accept
o4 - op4	0.0774	0.2777	0.2786	3.0734	Accept
op4 - p4	0.6557	0.2777	2.3616	3.0734	Accept

Homogeneous Subsets:

Group 1:	c4		Group 2:	o4 op4 p4	
Pooled mean =	1.6613		Pooled mean =	3.3316	
95% Confidence Interval =	1.0563	2.2663	95% Confidence Interval =	2.9824	3.6809

Dependent Variable: **GENERAL PEROXIDASE** d6

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	2.963	1	2.963	2.662	0.1287
Phen	23.131	1	23.131	20.784	0.0007
Ozone × Phen	14.749	1	14.749	13.252	0.0034
Error	13.355	12	1.113		
Total	54.198	15	3.613		

For **GENERAL PEROXIDASE** d6, classified by Treatment

Group	Cases	Mean	c6	o6	op6	p6
c6	4	0.9409		*	*	*
o6	4	3.7218	*			
op6	4	4.2063	*			
p6	4	5.2658	*			

Comparison	Difference	Std Error	q Stat	Table q	Result
c6 - p6	4.3249	0.5275	8.1993	3.3193	Reject
c6 - op6	3.2654	0.5275	6.1906	3.2216	Reject
c6 - o6	2.7809	0.5275	5.2720	3.0734	Reject
o6 - p6	1.5441	0.5275	2.9273	3.2216	Accept
o6 - op6	0.4845	0.5275	0.9186	3.0734	Accept
op6 - p6	1.0595	0.5275	2.0087	3.0734	Accept

Homogeneous Subsets:

Group 1:	c6		Group 2:	o6 op6 p6	
Pooled mean =	0.9409		Pooled mean =	4.3980	
95% Confidence Interval =	-0.2084	2.0901	95% Confidence Interval =	3.7344	5.0615

Appendix 4.9 (Figure 6.15) Effects of ozone and phenmedipham on glutathione s-transferase activity, expressed on a fresh weight basis, of sugarbeet cv Saxon, where n=4 on days 0, 1, 2, 3, 4 and 6. Results of three-way ANOVA, classified by ozone, phenmedipham and time.

Dependent Variable: **GLUTATHIONE S-TRANSFERASE** d0-6

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.731	1	0.731	17.734	0.0001
Phen	0.271	1	0.271	6.566	0.0125
Time	11.121	5	2.224	53.923	0.0000
Ozone × Phen	0.027	1	0.027	0.657	0.4201
Ozone × Time	0.382	5	0.076	1.852	0.1136
Phen × Time	1.101	5	0.220	5.340	0.0003
Ozone × Phen × Time	0.168	5	0.034	0.814	0.5438
Error	2.970	72	0.041		
Total	16.772	95	0.177		

Appendix 4.9.1 (Figure 6.15) Effects of ozone and phenmedipham on glutathione s-transferase activity expressed on a fresh weight basis, of sugarbeet cv Saxon, where n=4 on day 0, 1, 2, 3, 4 and 6. Results of two-way ANOVA and, where appropriate, Duncan's Multiple Range Tests, classified by ozone and/or phenmedipham. In the DMRT comparisons table, accept indicates that the two means are not significantly different at $p < 0.05$ and * denotes significantly different pairs.

Dependent Variable: **GLUTATHIONE S-TRANSFERASE** d0

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.398	1	0.398	3.660	0.0764
Error	1.522	14	0.109		
Total	1.920	15	0.128		

Dependent Variable: **GLUTATHIONE S-TRANSFERASE** d1

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.185	1	0.185	5.384	0.0387
Phen	0.209	1	0.209	6.070	0.0298
Ozone × Phen	0.029	1	0.029	0.843	0.3767
Error	0.413	12	0.034		
Total	0.836	15	0.056		

For **GLUTATHIONE S-TRANSFERASE** d1, classified by Treatment

Group	Cases	Mean	c1	o1	p1	op1
c1	4	1.4349				*
o1	4	1.5649				*
p1	4	1.5782				*
op1	4	1.8785	*	*	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
c1 - op1	0.4436	0.0927	4.7841	3.3193	Reject
c1 - p1	0.1433	0.0927	1.5456	3.2216	Accept
c1 - o1	0.1300	0.0927	1.4022	3.0734	Accept
o1 - op1	0.3136	0.0927	3.3818	3.2216	Reject
o1 - p1	0.0133	0.0927	0.1434	3.0734	Accept
p1 - op1	0.3003	0.0927	3.2384	3.0734	Reject

Homogeneous Subsets:

Group 1:	c1 o1 p1		Group 2:	op1	
Pooled mean =	1.526		Pooled mean =	1.879	
95% Confidence Interval =	1.409	1.643	95% Confidence Interval =	1.676	2.081

Dependent Variable: **GLUTATHIONE S-TRANSFERASE** d2

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.259	1	0.259	6.474	0.0257
Phen	0.583	1	0.583	14.577	0.0024
Ozone × Phen	0.016	1	0.016	0.403	0.5377
Error	0.480	12	0.040		
Total	1.337	15	0.089		

For **GLUTATHIONE S-TRANSFERASE** d2, classified by Treatment

Group	Cases	Mean	c2	o2	p2	op2
c2	4	1.4441		*	*	*
o2	4	1.7618	*			
p2	4	1.8891	*			
op2	4	2.0800	*			

Comparison	Difference	Std Error	q Stat	Table q	Result
c2 - op2	0.6359	0.0999	6.3625	3.3193	Reject
c2 - p2	0.4450	0.0999	4.4525	3.2216	Reject
c2 - o2	0.3177	0.0999	3.1790	3.0734	Reject
o2 - op2	0.3182	0.0999	3.1835	3.2216	Accept
o2 - p2	0.1273	0.0999	1.2735	3.0734	Accept
p2 - op2	0.1909	0.0999	1.9100	3.0734	Accept

Homogeneous Subsets:

Group 1:	c2		Group 2:	o2 p2 op2	
Pooled mean =	1.444		Pooled mean =	1.910	
95% Confidence Interval =	1.226	1.662	95% Confidence Interval =	1.785	2.036

Dependent Variable: **GLUTATHIONE S-TRANSFERASE** d3

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.000	1	0.000	0.037	0.8508
Phen	0.296	1	0.296	35.601	0.0001
Ozone × Phen	0.144	1	0.144	17.324	0.0013
Error	0.100	12	0.008		
Total	0.540	15	0.036		

For **GLUTATHIONE S-TRANSFERASE** d3, classified by Treatment

Group	Cases	Mean	c3	o3	op3	p3
c3	4	0.6629		*	*	*
o3	4	0.8614	*			*
op3	4	0.9437	*			*
p3	4	1.1247	*	*	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
c3 - p3	0.4617	0.0456	10.1290	3.3193	Reject
c3 - op3	0.2808	0.0456	6.1589	3.2216	Reject
c3 - o3	0.1985	0.0456	4.3544	3.0734	Reject
o3 - p3	0.2632	0.0456	5.7745	3.2216	Reject
o3 - op3	0.0823	0.0456	1.8044	3.0734	Accept
op3 - p3	0.1810	0.0456	3.9701	3.0734	Reject

Homogeneous Subsets:

Group 1:	c3	Group 2:	o3 op3
Pooled mean =	0.6629	Pooled mean =	0.9026

95% Confidence Interval =	0.5636	0.7623	95% Confidence Interval =	0.8323	0.9728
Group 3:	p3				
Pooled mean =	1.1247				
95% Confidence Interval =	1.0254	1.2240			

Dependent Variable: **GLUTATHIONE S-TRANSFERASE** d4

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.000	1	0.000	0.007	0.9327
Phen	0.002	1	0.002	0.081	0.7814
Ozone × Phen	0.006	1	0.006	0.254	0.6233
Error	0.273	12	0.023		
Total	0.281	15	0.019		

Dependent Variable: **GLUTATHIONE S-TRANSFERASE** d6

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.271	1	0.271	2.106	0.1723
Phen	0.283	1	0.283	2.200	0.1637
Ozone × Phen	0.000	1	0.000	0.001	0.9809
Error	1.545	12	0.129		
Total	2.099	15	0.140		

Appendix 4.10 (Figure 6.16) Effects of ozone and phenmedipham on total glutathione content, expressed on a fresh weight basis, of sugarbeet cv Saxon, where n=4 on days 0, 1, 2, 3, 4 and 6. Results of three-way ANOVA, classified by ozone, phenmedipham and time.

Dependent Variable: **GLUTATHIONE** d0-6

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	121173.254	1	121173.254	33.428	0.0000
Phen	100006.671	1	100006.671	27.589	0.0000
Time	463691.423	5	92738.285	25.584	0.0000
Ozone × Phen	8478.975	1	8478.975	2.339	0.1305
Ozone × Time	156996.818	5	31399.364	8.662	0.0000
Phen × Time	109151.784	5	21830.357	6.022	0.0001
Ozone × Phen × Time	64555.775	5	12911.155	3.562	0.0062
Error	260991.017	72	3624.875		
Total	1285045.718	95	13526.797		

Appendix 4.10.1 (Figure 6.16) Effects of ozone and phenmedipham on total glutathione content expressed on a fresh weight basis, of sugarbeet cv Saxon, where n=4 on day 0, 1, 2, 3, 4 and 6. Results of two-way ANOVA and, where appropriate, Duncan's Multiple Range Tests, classified by ozone and/or phenmedipham. In the DMRT comparisons table, accept indicates that the two means are not significantly different at $p < 0.05$ and * denotes significantly different pairs.

Dependent Variable: **GLUTATHIONE** d0

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	182118.530	1	182118.530	53.040	0.0000
Error	48070.392	14	3433.599		
Total	230188.922	15	15345.928		

For **GLUTATHIONE** d0, classified by Treatment

Group	Cases	Mean	o0	c0
o0	8	228.7965		*
c0	8	442.1732	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
o0 - c0	213.3767	20.7171	10.2995	3.0261	Reject

Homogeneous Subsets:

Group 1:	o0		Group 2:	c0	
Pooled mean =	228.80		Pooled mean =	442.17	
95% Confidence Interval =	184.36	273.23	95% Confidence Interval =	397.74	486.61

Dependent Variable: **GLUTATHIONE** d1

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	53813.296	1	53813.296	23.827	0.0004
Phen	127573.726	1	127573.726	56.486	0.0000
Ozone × Phen	10626.999	1	10626.999	4.705	0.0509
Error	27102.249	12	2258.521		
Total	219116.271	15	14607.751		

For **GLUTATHIONE** d1, classified by Treatment

Group	Cases	Mean	op1	p1	o1	c1
op1	4	176.9680		*	*	*
p1	4	344.5002	*			*
o1	4	407.0990	*			
c1	4	471.5438	*	*		

Comparison	Difference	Std Error	q Stat	Table q	Result
op1 - c1	294.5758	23.7619	12.3970	3.3193	Reject
op1 - o1	230.1310	23.7619	9.6849	3.2216	Reject
op1 - p1	167.5321	23.7619	7.0504	3.0734	Reject
p1 - c1	127.0437	23.7619	5.3465	3.2216	Reject
p1 - o1	62.5989	23.7619	2.6344	3.0734	Accept
o1 - c1	64.4448	23.7619	2.7121	3.0734	Accept

Homogeneous Subsets:

Group 1:	op1		Group 2:	p1 o1	
Pooled mean =	177.0		Pooled mean =	375.8	
95% Confidence Interval =	125.2	228.7	95% Confidence Interval =	339.2	412.4
Group 3:	o1 c1				
Pooled mean =	439.3				
95% Confidence Interval =	402.7	475.9			

Dependent Variable: **GLUTATHIONE** d2

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	125292.235	1	125292.235	10.508	0.0071
Phen	174589.983	1	174589.983	14.642	0.0024
Ozone × Phen	144764.880	1	144764.880	12.141	0.0045
Error	143088.403	12	11924.034		
Total	587735.501	15	39182.367		

For **GLUTATHIONE** d2, classified by Treatment

Group	Cases	Mean	p2	op2	o2	c2
p2	4	234.6446				*
op2	4	247.9013				*
o2	4	266.5813				*
c2	4	633.8044	*	*	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
p2 - c2	399.1597	54.5986	7.3108	3.3193	Reject
p2 - o2	31.9366	54.5986	0.5849	3.2216	Accept
p2 - op2	13.2567	54.5986	0.2428	3.0734	Accept
op2 - c2	385.9030	54.5986	7.0680	3.2216	Reject
op2 - o2	18.6799	54.5986	0.3421	3.0734	Accept
o2 - c2	367.2231	54.5986	6.7259	3.0734	Reject

Homogeneous Subsets:

Group 1:	p2 op2 o2		Group 2:	c2	
Pooled mean =	249.71		Pooled mean =	633.80	
95% Confidence Interval =	181.03	318.39	95% Confidence Interval =	514.84	752.76

Dependent Variable: **GLUTATHIONE** d3

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	270.737	1	270.737	0.127	0.7280
Phen	5617.990	1	5617.990	2.631	0.1308
Ozone × Phen	91.754	1	91.754	0.043	0.8393
Error	25623.503	12	2135.292		
Total	31603.984	15	2106.932		

Dependent Variable: **GLUTATHIONE** d4

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif

Ozone	98.290	1	98.290	0.021	0.8872
Phen	109.752	1	109.752	0.023	0.8808
Ozone × Phen	161.072	1	161.072	0.034	0.8559
Error	56154.892	12	4679.574		
Total	56524.006	15	3768.267		

Dependent Variable: **GLUTATHIONE** d6

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	266.914	1	266.914	0.186	0.6736
Phen	4118.903	1	4118.903	2.876	0.1157
Ozone × Phen	9033.985	1	9033.985	6.309	0.0273
Error	17184.282	12	1432.024		
Total	30604.085	15	2040.272		

For **GLUTATHIONE** d6, classified by Treatment

Group	Cases	Mean	p6	o6	op6	c6
p6	4	107.3725				*
o6	4	131.2931				
op6	4	146.7274				
c6	4	186.9854	*			

Comparison	Difference	Std Error	q Stat	Table q	Result
p6 - c6	79.6130	18.9210	4.2076	3.3193	Reject
p6 - op6	39.3549	18.9210	2.0800	3.2216	Accept
p6 - o6	23.9206	18.9210	1.2642	3.0734	Accept
o6 - c6	55.6924	18.9210	2.9434	3.2216	Accept
o6 - op6	15.4343	18.9210	0.8157	3.0734	Accept
op6 - c6	40.2581	18.9210	2.1277	3.0734	Accept

Homogeneous Subsets:

Group 1:	p6 o6 op6		Group 2:	o6 op6 c6	
Pooled mean =	128.46		Pooled mean =	155.00	
95% Confidence Interval =	104.66	152.27	95% Confidence Interval =	131.20	178.80

Appendix 5

Table A5.1 Effects of ozone on the activities, on a protein basis, of several antioxidant enzymes in sugarbeet 1 and 2 days after the end of exposure (d-2 & d-1, respectively). Values are means ± standard error, where n=4. Statistical analyses are presented in Appendix 5.1.

	1 day after ozone exposure		2 days after ozone exposure	
	Control	Ozone	Control	Ozone
Superoxide dismutase (units SOD mg^{-1} protein)	3.78 ± 0.20	4.03 ± 0.19	5.13 ± 0.24	4.42 ± 0.27
Ascorbate peroxidase (nmol mg^{-1} protein min^{-1})	425.8 ± 34.9	609.4 ± 56.7*	619.3 ± 47.2	747.9 ± 116.9
Monodehydroascorbate reductase (nmol mg^{-1} protein min^{-1})	30.1 ± 6.5	40.5 ± 11.5	45.2 ± 3.3	45.2 ± 6.4
Glutathione reductase (nmol mg^{-1} protein min^{-1})	49.3 ± 1.3	71.2 ± 9.6	72.7 ± 4.8	87.6 ± 10.8
Catalase (ΔA_{450} mg^{-1} protein min^{-1})	13.26 ± 2.17	13.36 ± 0.63	11.06 ± 2.31	10.16 ± 0.97
Guaiacol peroxidase (nmol mg^{-1} protein min^{-1})	32.6 ± 5.9	37.4 ± 5.8	43.1 ± 4.8	53.2 ± 8.4
Glutathione s-transferase (nmol mg^{-1} protein min^{-1})	26.0 ± 2.3	38.3 ± 6.6	31.2 ± 1.7	39.0 ± 4.0

* indicates significant difference from the control ($p \leq 0.05$).

Appendix 5.1.1 (Table A5.1) Effects of ozone on superoxide dismutase activity, expressed on a protein basis, of sugarbeet cv Saxon, where n=4 on days -2 and -1. Results of one-way ANOVA, classified by ozone.

Dependent Variable: **SUPEROXIDE DISMUTASE** d-2

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.132	1	0.132	0.855	0.3908
Error	0.924	6	0.154		
Total	1.055	7	0.151		

Dependent Variable: **SUPEROXIDE DISMUTASE** d-1

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	1.008	1	1.008	3.840	0.0978
Error	1.576	6	0.263		
Total	2.584	7	0.369		

Appendix 5.1.2 (Table A5.1) Effects of ozone on ascorbate peroxidase activity, expressed on a protein basis, of sugarbeet cv Saxon, where n=4 on days -2 and -1. Results of one-way ANOVA, classified by ozone.

Dependent Variable: **ASCORBATE PEROXIDASE** d-2

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.067	1	0.067	7.598	0.0330
Error	0.053	6	0.009		
Total	0.121	7	0.017		

Dependent Variable: **ASCORBATE PEROXIDASE** d-1

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.033	1	0.033	1.040	0.3471
Error	0.191	6	0.032		
Total	0.224	7	0.032		

Appendix 5.1.3 (Table A5.1) Effects of ozone on monodehydroascorbate reductase activity, expressed on a protein basis, of sugarbeet cv Saxon, where n=4 on days -2 and -1. Results of one-way ANOVA, classified by ozone.

Dependent Variable: **MONODEHYDROASCORBATE REDUCTASE** d-2

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.000	1	0.000	0.618	0.4617
Error	0.002	6	0.000		
Total	0.002	7	0.000		

Dependent Variable: **MONODEHYDROASCORBATE REDUCTASE** d-1

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.000	1	0.000	0.000	0.9935
Error	0.001	6	0.000		
Total	0.001	7	0.000		

Appendix 5.1.4 (Table A5.1) Effects of ozone on glutathione reductase activity, expressed on a protein basis, of sugarbeet cv Saxon, where n=4 on days -2 and -1. Results of one-way ANOVA, classified by ozone.

Dependent Variable: **GLUTATHIONE REDUCTASE** d-2

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.001	1	0.001	5.068	0.0653
Error	0.001	6	0.000		
Total	0.002	7	0.000		

Dependent Variable: **GLUTATHIONE REDUCTASE** d-1

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.000	1	0.000	1.571	0.2567
Error	0.002	6	0.000		
Total	0.002	7	0.000		

Appendix 5.1.5 (Table A5.1) Effects of ozone on catalase activity, expressed on a protein basis, of sugarbeet cv Saxon, where n=4 on days -2 and -1. Results of one-way ANOVA, classified by ozone.

Dependent Variable: **CATALASE** d-2

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.018	1	0.018	0.002	0.9679
Error	61.295	6	10.216		
Total	61.313	7	8.759		

Dependent Variable: **CATALASE** d-1

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	1.640	1	1.640	0.130	0.7306
Error	75.590	6	12.598		
Total	77.230	7	11.033		

Appendix 5.1.6 (Table A5.1) Effects of ozone on general peroxidase activity, expressed on a protein basis, of sugarbeet cv Saxon, where n=4 on days -2 and -1. Results of one-way ANOVA, classified by ozone.

Dependent Variable: **GENERAL PEROXIDASE** d-2

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.000	1	0.000	0.350	0.5756
Error	0.001	6	0.000		
Total	0.001	7	0.000		

Dependent Variable: **GENERAL PEROXIDASE** d-1

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.000	1	0.000	1.108	0.3331
Error	0.001	6	0.000		
Total	0.001	7	0.000		

Appendix 5.1.7 (Table A5.1) Effects of ozone on glutathione s-transferase activity, expressed on a protein basis, of sugarbeet cv Saxon, where n=4 on days -2 and -1. Results of one-way ANOVA, classified by ozone.

Dependent Variable: **GLUTATHIONE S-TRANSFERASE** d-2

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.000	1	0.000	3.053	0.1312
Error	0.001	6	0.000		
Total	0.001	7	0.000		

Dependent Variable: **GLUTATHIONE S-TRANSFERASE** d-1

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.000	1	0.000	3.234	0.1222
Error	0.000	6	0.000		
Total	0.000	7	0.000		

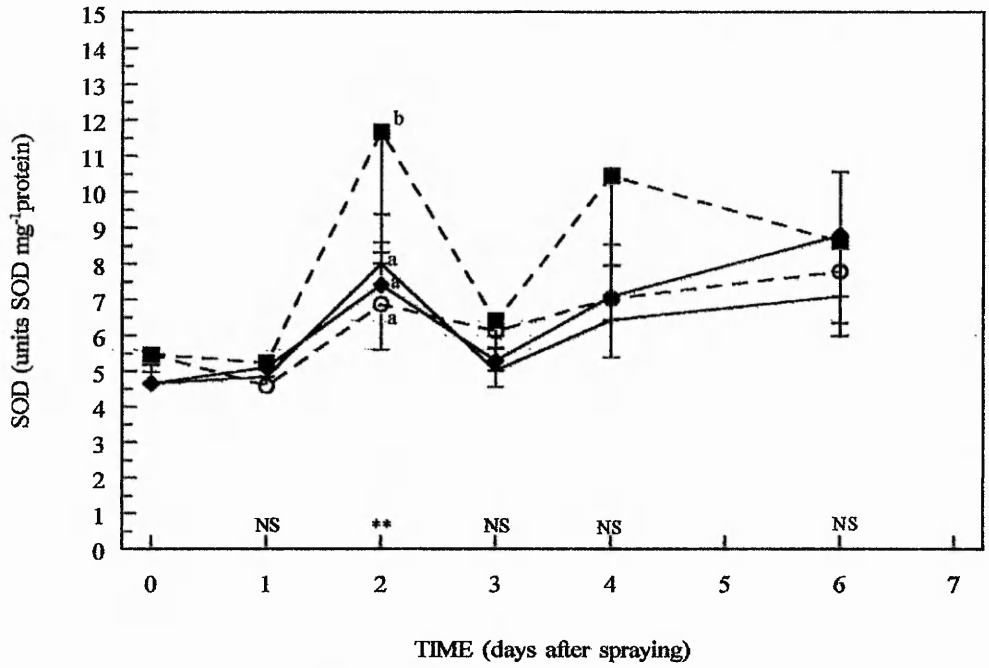

Figure A5.1 Effects of ozone and/or phenmedipham on superoxide dismutase activity in sugarbeet cv. Saxon. For statistical analysis see Appendix 5.2.
Different letters indicate significanc within that day. significant interactions are indicated by * ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$) and NS non-significant.
Key: control (+); ozone alone (O), phenmedipham alone (◆); ozone and phenmedipham (■).

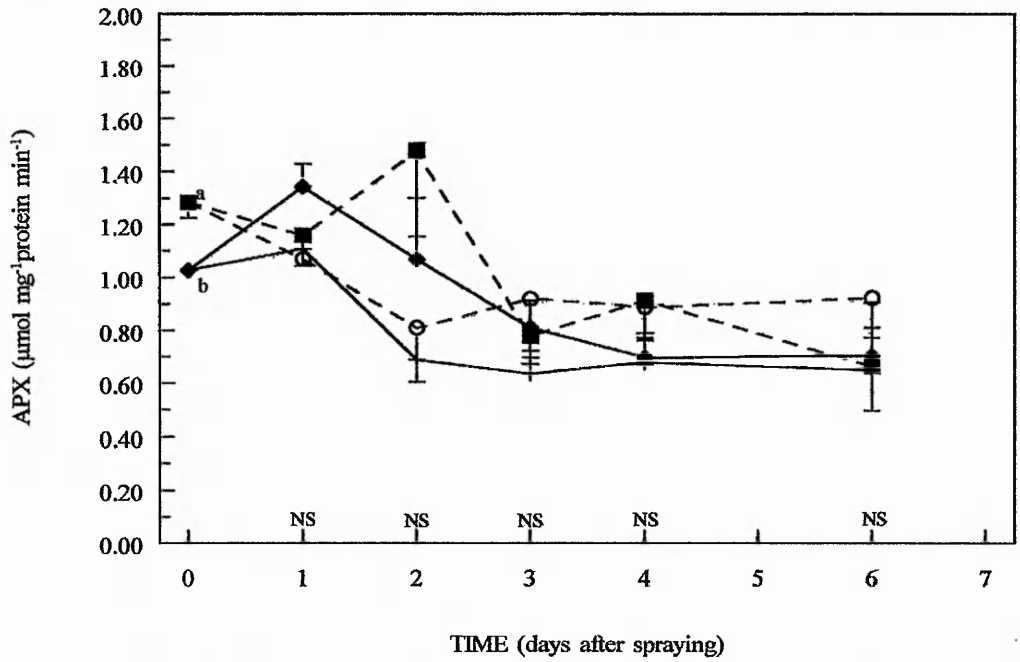

Figure A5.2 Effects of ozone and/or phenmedipham on ascorbate peroxidase activity in sugarbeet cv. Saxon. For key and statistical analysis see Figure A5.1 and Appendix 5.3.

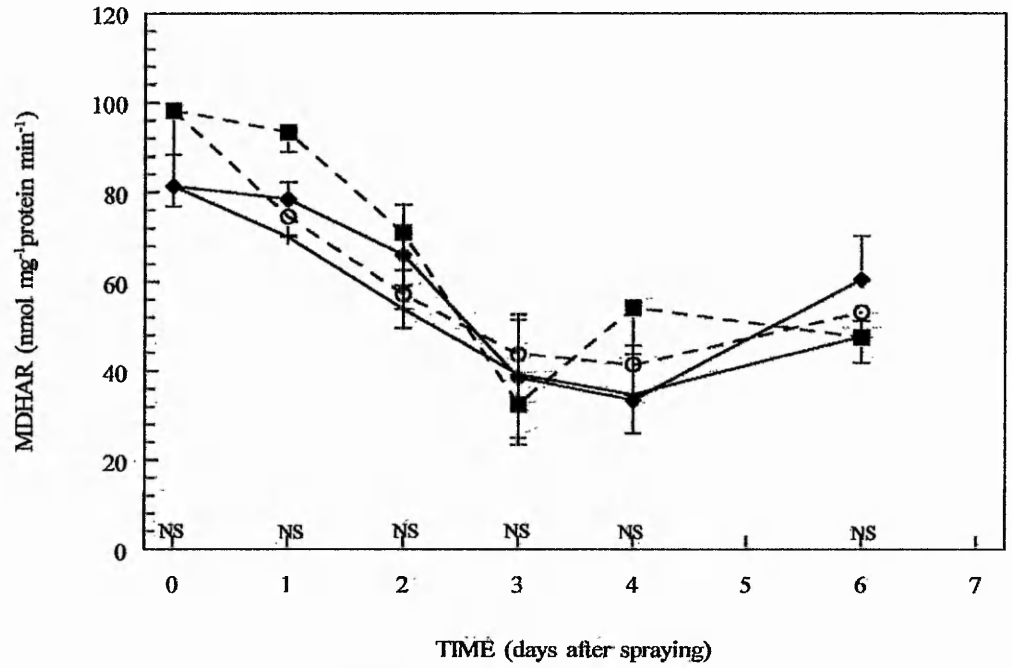

Figure A5.3 Effects of ozone and/or phenmedipham on monodehydroascorbate reductase in sugarbeet cv. Saxon. For key and statistical analysis see Figure A5.1 and Appnedix 5.4.

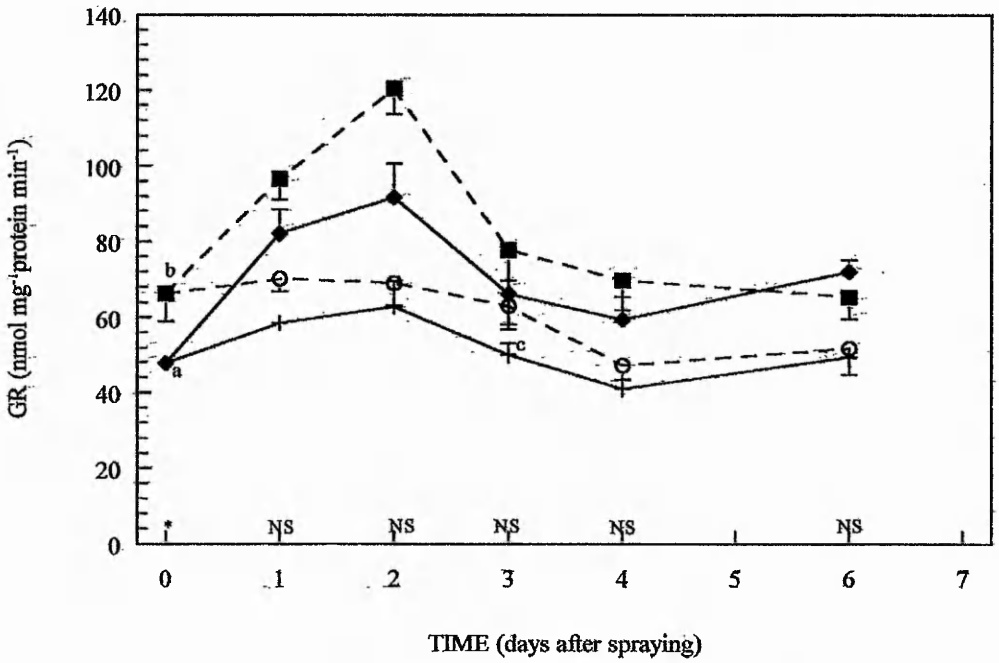

Figure A5.4 Effects of ozone and/or phenmedipham on glutathione reductase activity in sugarbeet cv. Saxon. For key and statistical analysis see Figure A5.1 and Appendix 5.5.

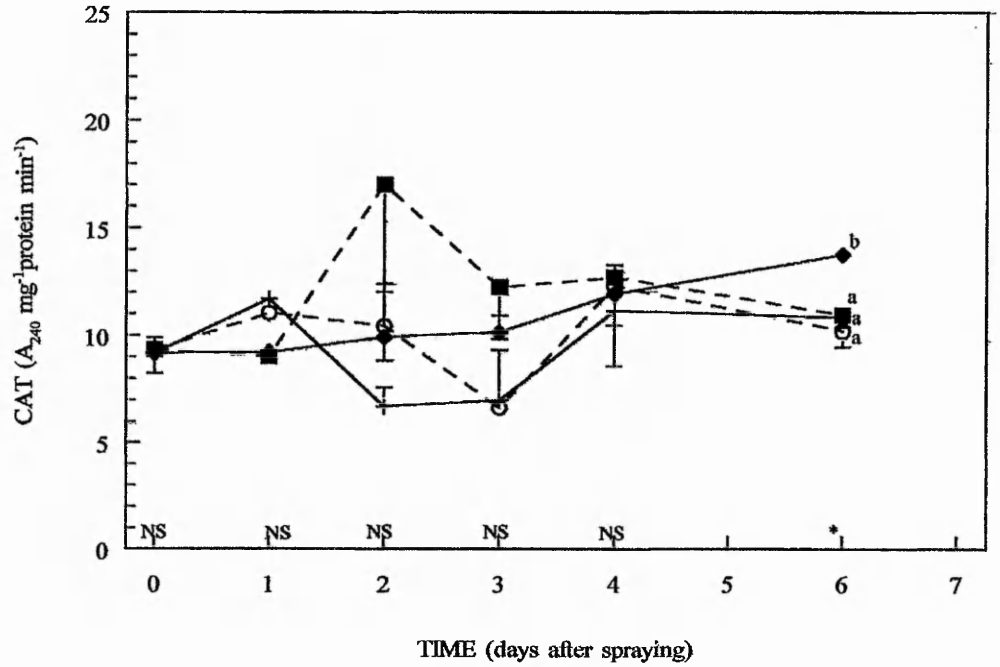

Figure A5.5 Effects of ozone and/or phenmedipham on catalase activity in sugarbeet cv. Saxon. For key and statistical analysis see Figure A5.1 and Appendix 5.6.

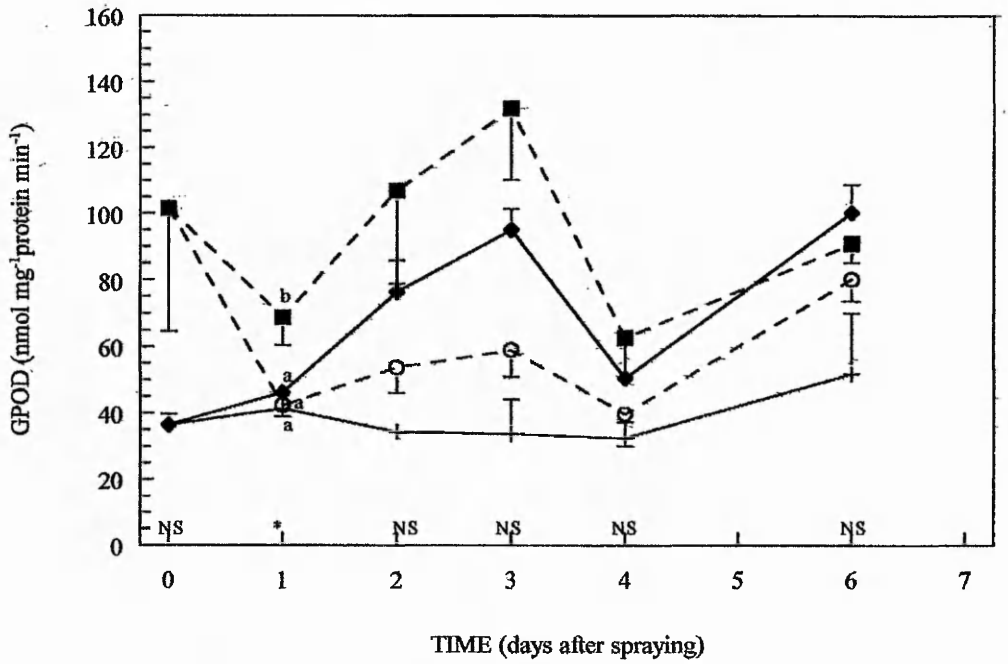

Figure A5.6 Effects of ozone and/or phenmedipham on guaiacol peroxidase activity in sugarbeet cv. Saxon. For key and statistical analysis see Figure A5.1 and Appendix 5.7.

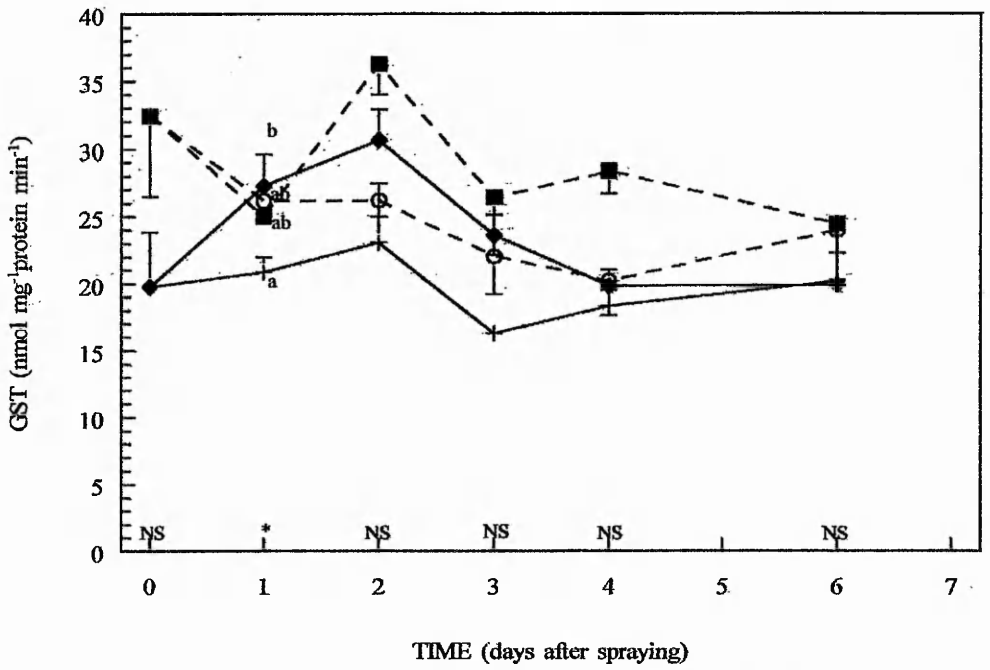

Figure A5.7 Effects of ozone and/or phenmedipham on glutathione transferase activity in sugarbeet cv Saxon. For key and statistical analysis see Figure A5.1 and Appendix 5.7.

Appendix 5.2 (Figure A5.1) Effects of ozone and phenmedipham on superoxide dismutase activity, expressed on a protein basis, of sugarbeet cv Saxon, where n=4 on days 0- 6. Results of three-way ANOVA, classified by ozone, phenmedipham and time.

Dependent Variable: **SUPEROXIDE DISMUTASE** per mg protein d 0-6

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	16.146	1	16.146	5.470	0.0215
Phen	51.224	1	51.224	17.354	0.0001
Time	487.432	6	81.239	27.523	0.0000
Ozone × Phen	17.297	1	17.297	5.860	0.0175
Ozone × Time	31.353	6	5.225	1.770	0.1138
Phen × Time	29.201	6	4.867	1.649	0.1427
Ozone × Phen × Time	41.646	6	6.941	2.352	0.0370
Error	271.553	92	2.952		
Total	945.852	119	7.948		

Appendix 5.2.1 (Figure A5.1) Effects of ozone and phenmedipham on superoxide dismutase activity, expressed on a protein basis, of sugarbeet cv Saxon, where n=4 on days 0, 1, 2, 3, 4 and 6. Results of two-way ANOVA and Duncan's Multiple Range Tests for superoxide dismutase activity, classified by ozone and/or phenmedipham. In the comparisons table, accept indicates that the two means are not significantly different at $p \leq 0.05$ and * denotes significantly different pairs.

Dependent Variable: **SUPEROXIDE DISMUTASE** per mg protein d0

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.471	1	0.471	1.028	0.3345
Error	4.579	10	0.458		
Total	5.050	11	0.459		

Dependent Variable: **SUPEROXIDE DISMUTASE** per mg protein d1

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.019	1	0.019	0.176	0.6821
Phen	0.829	1	0.829	7.892	0.0158
Ozone × Phen	0.152	1	0.152	1.445	0.2524
Error	1.260	12	0.105		
Total	2.260	15	0.151		

Dependent Variable: **SUPEROXIDE DISMUTASE** per mg protein d2

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	14.845	1	14.845	3.516	0.0853
Phen	29.530	1	29.530	6.994	0.0214
Ozone × Phen	43.679	1	43.679	10.345	0.0074
Error	50.664	12	4.222		
Total	138.718	15	9.248		

For **SUPEROXIDE DISMUTASE** per mg protein d2, classified by Treatment

Group	Cases	Mean	o2	p2	c2	op2
o2	4	6.8741				*
p2	4	7.6647				*
c2	4	8.2521				*
op2	4	12.8956	*	*	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
o2 - op2	6.0216	1.0274	5.8611	3.3193	Reject
o2 - c2	1.3780	1.0274	1.3413	3.2216	Accept
o2 - p2	0.7906	1.0274	0.7695	3.0734	Accept
p2 - op2	5.2310	1.0274	5.0916	3.2216	Reject
p2 - c2	0.5874	1.0274	0.5717	3.0734	Accept
c2 - op2	4.6436	1.0274	4.5198	3.0734	Reject

Homogeneous Subsets:

Group 1:	o2 p2 c2		Group 2:	op2	
Pooled mean =	7.60		Pooled mean =	12.9	
95% Confidence Interval =	6.30	8.89	95% Confidence Interval =	10.66	15.13

Dependent Variable: **SUPEROXIDE DISMUTASE** per mg protein d3

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	12.786	1	12.786	3.139	0.1018
Phen	3.159	1	3.159	0.776	0.3958
Ozone × Phen	7.045	1	7.045	1.730	0.2130
Error	48.879	12	4.073		
Total	71.870	15	4.791		

Dependent Variable: **SUPEROXIDE DISMUTASE** per mg protein d4

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	14.898	1	14.898	5.890	0.0319
Phen	16.831	1	16.831	6.654	0.0241
Ozone × Phen	7.451	1	7.451	2.946	0.1118
Error	30.352	12	2.529		
Total	69.532	15	4.635		

Dependent Variable: **SUPEROXIDE DISMUTASE** per mg protein d6

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.336	1	0.336	0.036	0.8523
Phen	6.197	1	6.197	0.667	0.4301
Ozone × Phen	0.556	1	0.556	0.060	0.8110
Error	111.509	12	9.292		
Total	118.598	15	7.907		

Appendix 5.3 (Figure A5.2) Effects of ozone and phenmedipham on ascorbate peroxidase activity, expressed on a protein basis, of sugarbeet cv Saxon, where n=4 on days 0-6. Results of three-way ANOVA, classified by ozone, phenmedipham and time.

Dependent Variable: **ASCORBATE PEROXIDASE** d 0, 1, 2, 3, 4 and 6

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Time	3.042	5	0.608	7.333	0.0000
Ozone	0.497	1	0.497	5.988	0.0168
Phen	0.259	1	0.259	3.125	0.0813
Time × Ozone	0.393	5	0.079	0.948	0.4555
Time × Phen	0.993	5	0.199	2.395	0.0457
Ozone × Phen	0.039	1	0.039	0.464	0.4978
Time × Ozone × Phen	0.265	5	0.053	0.640	0.6702
Error	5.974	72	0.083		
Total	11.462	95	0.121		

Appendix 5.3.1 (Figure A5.2) Effects of ozone and phenmedipham on ascorbate peroxidase activity, expressed on a protein basis, of sugarbeet cv Saxon, where n=4 on days 0, 1, 2, 3, 4 and 6. Results of two-way ANOVA and Duncan's Multiple Range Tests for superoxide dismutase activity, classified by ozone and/or phenmedipham. In the comparisons table, accept indicates that the two means are not significantly different at $p \leq 0.05$ and * denotes significantly different pairs.

Dependent Variable: **ASCORBATE PEROXIDASE** d0

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.131	1	0.131	16.319	0.0068
Error	0.048	6	0.008		
Total	0.180	7	0.026		

For **ASCORBATE PEROXIDASE** , classified by Treatment

Group	Cases	Mean	c0	o0
c0	4	1.0268		*
o0	4	1.2832	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
c0 - o0	0.2564	0.0449	5.7129	3.4523	Reject

Homogeneous Subsets:

Group 1:	c0		Group 2:	o0	
Pooled mean =	1.0268		Pooled mean =	1.2832	
95% Confidence Interval =	0.9170	1.1366	95% Confidence Interval =	1.1734	1.3931

Dependent Variable: **ASCORBATE PEROXIDASE** d1

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.050	1	0.050	2.268	0.1579
Phen	0.106	1	0.106	4.871	0.0475
Ozone × Phen	0.022	1	0.022	0.993	0.3388
Error	0.262	12	0.022		
Total	0.440	15	0.029		

Dependent Variable: **ASCORBATE PEROXIDASE** d2

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.283	1	0.283	1.389	0.2614
Phen	1.101	1	1.101	5.405	0.0384
Ozone × Phen	0.084	1	0.084	0.412	0.5329
Error	2.445	12	0.204		
Total	3.914	15	0.261		

Dependent Variable: **ASCORBATE PEROXIDASE** d3

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.063	1	0.063	0.737	0.4074
Phen	0.001	1	0.001	0.014	0.9084
Ozone × Phen	0.099	1	0.099	1.171	0.3005
Error	1.020	12	0.085		
Total	1.183	15	0.079		

Dependent Variable: **ASCORBATE PEROXIDASE** d4

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.178	1	0.178	2.157	0.1676
Phen	0.002	1	0.002	0.022	0.8857
Ozone × Phen	0.000	1	0.000	0.000	0.9832
Error	0.991	12	0.083		
Total	1.170	15	0.078		

Dependent Variable: **ASCORBATE PEROXIDASE** d6

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.054	1	0.054	0.557	0.4697
Phen	0.042	1	0.042	0.434	0.5224
Ozone × Phen	0.099	1	0.099	1.021	0.3322
Error	1.159	12	0.097		
Total	1.354	15	0.090		

Appendix 5.4 (Figure A5.3) Effects of ozone and phenmedipham on monodehydroascorbate reductase activity, expressed on a protein basis, of sugarbeet cv Saxon, where n=4 on days 0-6. Results of three-way ANOVA, classified by ozone, phenmedipham and time.

Dependent Variable: **MONODEHYDROASCORBATE REDUCTASE** d 0, 1, 2, 3, 4 and 6

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Time	0.034	5	0.007	12.588	0.0000
Ozone	0.001	1	0.001	1.958	0.1660
Phen	0.001	1	0.001	1.091	0.2997
Time × Ozone	0.001	5	0.000	0.486	0.7855
Time × Phen	0.001	5	0.000	0.417	0.8351
Ozone × Phen	0.000	1	0.000	0.002	0.9611
Time × Ozone × Phen	0.001	5	0.000	0.281	0.9221
Error	0.039	72	0.001		
Total	0.078	95	0.001		

Appendix 5.4.1 (Figure A5.3) Effects of ozone and phenmedipham on monodehydroascorbate reductase activity, expressed on a protein basis, of sugarbeet cv Saxon, where n=4 on days 0, 1, 2, 3, 4 and 6. Results of two-way ANOVA and Duncan's Multiple Range Tests for superoxide dismutase activity, classified by ozone and/or phenmedipham. In the comparisons table, accept indicates that the two means are not significantly different at $p \leqslant 0.05$ and * denotes significantly different pairs.

Dependent Variable: **MONODEHYDROASCORBATE REDUCTASE** d0

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.001	1	0.001	0.556	0.4839
Error	0.006	6	0.001		
Total	0.007	7	0.001		

Dependent Variable: **MONODEHYDROASCORBATE REDUCTASE** d1

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.000	1	0.000	7.084	0.0207
Phen	0.001	1	0.001	13.731	0.0030
Ozone × Phen	0.000	1	0.000	2.022	0.1805
Error	0.001	12	0.000		
Total	0.002	15	0.000		

Dependent Variable: **MONODEHYDROASCORBATE REDUCTASE** d2

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.000	1	0.000	0.166	0.6913
Phen	0.001	1	0.001	1.670	0.2206
Ozone × Phen	0.000	1	0.000	0.006	0.9373
Error	0.005	12	0.000		
Total	0.006	15	0.000		

Dependent Variable: **MONODEHYDROASCORBATE REDUCTASE** d3

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.000	1	0.000	0.002	0.9631
Phen	0.000	1	0.000	0.181	0.6778
Ozone × Phen	0.000	1	0.000	0.141	0.7140
Error	0.009	12	0.001		
Total	0.010	15	0.001		

Dependent Variable: **MONODEHYDROASCORBATE REDUCTASE** d4

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.001	1	0.001	0.889	0.3643
Phen	0.000	1	0.000	0.155	0.7004
Ozone × Phen	0.000	1	0.000	0.238	0.6343
Error	0.010	12	0.001		
Total	0.011	15	0.001		

Dependent Variable: **MONODEHYDROASCORBATE REDUCTASE** d6

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.000	1	0.000	0.340	0.5705
Phen	0.000	1	0.000	0.320	0.5822
Ozone × Phen	0.000	1	0.000	2.110	0.1720
Error	0.002	12	0.000		
Total	0.002	15	0.000		

Appendix 5.5 (Figure A5.4) Effects of ozone and phenmedipham on glutathione reductase activity, expressed on a protein basis, of sugarbeet cv Saxon, where n=4 on days 0-6. Results of three-way ANOVA, classified by ozone, phenmedipham and time.

Dependent Variable: **GLUTATHIONE REDUCTASE** d 0, 1, 2, 3, 4 and 6

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Time	0.012	5	0.002	13.718	0.0000
Ozone	0.003	1	0.003	16.834	0.0001
Phen	0.009	1	0.009	52.503	0.0000
Time × Ozone	0.001	5	0.000	1.261	0.2902
Time × Phen	0.003	5	0.001	3.776	0.0043
Ozone × Phen	0.000	1	0.000	0.330	0.5674
Time × Ozone × Phen	0.001	5	0.000	0.620	0.6852
Error	0.013	72	0.000		
Total	0.043	95	0.000		

Appendix 5.5.1 (Figure A5.4) Effects of ozone and phenmedipham on glutathione reductase activity, expressed on a protein basis, of sugarbeet cv Saxon, where n=4 on days 0, 1, 2, 3, 4 and 6. Results of two-way ANOVA and Duncan's Multiple Range Tests for superoxide dismutase activity, classified by ozone and/or phenmedipham. In the comparisons table, accept indicates that the two means are not significantly different at $p \leq 0.05$ and * denotes significantly different pairs.

Dependent Variable: **GLUTATHIONE REDUCTASE** d0

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.001	1	0.001	6.352	0.0453
Error	0.001	6	0.000		
Total	0.001	7	0.000		

For **GLUTATHIONE REDUCTASE** , classified by Treatment

Group	Cases	Mean	c0	o0
c0	4	0.0477		*
o0	4	0.0663	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
c0 - o0	0.0186	0.0052	3.5644	3.4523	Reject

Homogeneous Subsets:

Group 1:	c0		Group 2:	o0	
Pooled mean =	0.0477		Pooled mean =	0.0663	
95% Confidence Interval =	0.0349	0.0604	95% Confidence Interval =	0.0535	0.0790

Dependent Variable: **GLUTATHIONE REDUCTASE** d1

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.001	1	0.001	8.374	0.0135
Phen	0.003	1	0.003	30.261	0.0001
Ozone × Phen	0.000	1	0.000	0.096	0.7621
Error	0.001	12	0.000		
Total	0.004	15	0.000		

Dependent Variable: **GLUTATHIONE REDUCTASE** d2

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif

Ozone	0.001	1	0.001	6.020	0.0304
Phen	0.006	1	0.006	32.092	0.0001
Ozone × Phen	0.001	1	0.001	2.554	0.1360
Error	0.002	12	0.000		
Total	0.011	15	0.001		

Dependent Variable: **GLUTATHIONE REDUCTASE** d3

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.001	1	0.001	1.248	0.2858
Phen	0.001	1	0.001	1.978	0.1850
Ozone × Phen	0.000	1	0.000	0.005	0.9463
Error	0.006	12	0.000		
Total	0.007	15	0.000		

Dependent Variable: **GLUTATHIONE REDUCTASE** d4

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.000	1	0.000	2.376	0.1492
Phen	0.002	1	0.002	14.502	0.0025
Ozone × Phen	0.000	1	0.000	0.139	0.7158
Error	0.001	12	0.000		
Total	0.003	15	0.000		

Dependent Variable: **GLUTATHIONE REDUCTASE** d6

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.000	1	0.000	0.210	0.6550
Phen	0.001	1	0.001	14.559	0.0025
Ozone × Phen	0.000	1	0.000	0.900	0.3614
Error	0.001	12	0.000		
Total	0.003	15	0.000		

Appendix 5.6 (Figure A5.5) Effects of ozone and phenmedipham on catalase activity, expressed on a protein basis, of sugarbeet cv Saxon, where n=4 on days 0-6. Results of three-way ANOVA, classified by ozone, phenmedipham and time.

Dependent Variable: **CATALASE** d 0, 1, 2, 3, 4 and 6

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Time	117.378	5	23.476	1.842	0.1155
Ozone	18.721	1	18.721	1.469	0.2295
Phen	60.037	1	60.037	4.710	0.0333
Time × Ozone	118.232	5	23.646	1.855	0.1130
Time × Phen	149.275	5	29.855	2.342	0.0500
Ozone × Phen	2.230	1	2.230	0.175	0.6770
Time × Ozone × Phen	19.931	5	3.986	0.313	0.9038
Error	917.840	72	12.748		
Total	1403.644	95	14.775		

Appendix 5.6.1 (Figure A5.5) Effects of ozone and phenmedipham on catalase activity, expressed on a protein basis, of sugarbeet cv Saxon, where n=4 on days 0, 1, 2, 3, 4 and 6. Results of two-way ANOVA and Duncan's Multiple Range Tests for superoxide dismutase activity, classified by ozone and/or phenmedipham. In the comparisons table, accept indicates that the two means are not significantly different at $p \leq 0.05$ and * denotes significantly different pairs.

Dependent Variable: **CATALASE** d0

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.064	1	0.064	0.018	0.8967
Error	21.022	6	3.504		
Total	21.087	7	3.012		

Dependent Variable: **CATALASE** d1

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.735	1	0.735	1.738	0.2120
Phen	20.539	1	20.539	48.573	0.0000
Ozone × Phen	0.194	1	0.194	0.459	0.5108
Error	5.074	12	0.423		
Total	26.542	15	1.769		

Dependent Variable: **CATALASE** d2

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	117.450	1	117.450	3.417	0.0893
Phen	96.530	1	96.530	2.808	0.1196
Ozone × Phen	11.093	1	11.093	0.323	0.5804
Error	412.477	12	34.373		
Total	637.550	15	42.503		

Dependent Variable: **CATALASE** d3

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	3.159	1	3.159	0.260	0.6195
Phen	77.198	1	77.198	6.348	0.0269
Ozone × Phen	5.954	1	5.954	0.490	0.4975
Error	145.943	12	12.162		

Total	232.254	15	15.484		

Dependent Variable: **CATALASE** d4

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	3.678	1	3.678	0.147	0.7083
Phen	1.482	1	1.482	0.059	0.8119
Ozone × Phen	0.133	1	0.133	0.005	0.9430
Error	300.595	12	25.050		
Total	305.889	15	20.393		

Dependent Variable: **CATALASE** d6

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	11.802	1	11.802	12.098	0.0046
Phen	13.564	1	13.564	13.904	0.0029
Ozone × Phen	4.787	1	4.787	4.907	0.0468
Error	11.706	12	0.975		
Total	41.859	15	2.791		

For **CATALASE**, classified by Treatment

Group	Cases	Mean	o6	c6	op6	p6
o6	4	10.2080				*
c6	4	10.8318				*
op6	4	10.9555				*
p6	4	13.7672	*	*	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
o6 - p6	3.5591	0.4938	7.2071	3.3193	Reject
o6 - op6	0.7475	0.4938	1.5136	3.2216	Accept
o6 - c6	0.6237	0.4938	1.2630	3.0734	Accept
c6 - p6	2.9354	0.4938	5.9441	3.2216	Reject
c6 - op6	0.1237	0.4938	0.2506	3.0734	Accept
op6 - p6	2.8117	0.4938	5.6936	3.0734	Reject

Homogeneous Subsets:

Group 1:	o6 c6 op6		Group 2:	p6	
Pooled mean =	10.6651		Pooled mean =	13.7672	
95% Confidence Interval =	10.0439	11.2863	95% Confidence Interval =	12.6912	14.8431

Appendix 5.7 (Figure A5.6) Effects of ozone and phenmedipham on general peroxidase activity, expressed on a protein basis, of sugarbeet cv Saxon, where n=4 on days 0-6. Results of three-way ANOVA, classified by ozone, phenmedipham and time.

Dependent Variable: **GENERAL PEROXIDASE** d 0, 1, 2, 3, 4 and 6

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Time	0.017	5	0.003	3.826	0.0040
Ozone	0.016	1	0.016	17.165	0.0001
Phen	0.022	1	0.022	24.102	0.0000
Time × Ozone	0.009	5	0.002	2.037	0.0835
Time × Phen	0.012	5	0.002	2.563	0.0343
Ozone × Phen	0.000	1	0.000	0.024	0.8777
Time × Ozone × Phen	0.002	5	0.000	0.482	0.7885
Error	0.065	72	0.001		
Total	0.143	95	0.002		

Appendix 5.7.1 (Figure A5.6) Effects of ozone and phenmedipham on general peroxidase activity, expressed on a protein basis, of sugarbeet cv Saxon, where n=4 on days 0, 1, 2, 3, 4 and 6. Results of two-way ANOVA and Duncan's Multiple Range Tests for superoxide dismutase activity, classified by ozone and/or phenmedipham. In the comparisons table, accept indicates that the two means are not significantly different at $p \leq 0.05$ and * denotes significantly different pairs.

Dependent Variable: **GENERAL PEROXIDASE** d0

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.009	1	0.009	3.073	0.1302
Error	0.017	6	0.003		
Total	0.025	7	0.004		

Dependent Variable: **GENERAL PEROXIDASE** d1

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.001	1	0.001	5.147	0.0425
Phen	0.001	1	0.001	9.094	0.0108
Ozone × Phen	0.000	1	0.000	4.355	0.0589
Error	0.001	12	0.000		
Total	0.003	15	0.000		

For **GENERAL PEROXIDASE**, classified by Treatment

Group	Cases	Mean	c1	o1	p1	op1
c1	4	0.0413				*
o1	4	0.0422				*
p1	4	0.0461				*
op1	4	0.0687	*	*	*	

Comparison	Difference	Std Error	q Stat	Table q	Result
c1 - op1	0.0275	0.0052	5.2842	3.3193	Reject
c1 - p1	0.0048	0.0052	0.9288	3.2216	Accept
c1 - o1	0.0009	0.0052	0.1819	3.0734	Accept
o1 - op1	0.0265	0.0052	5.1024	3.2216	Reject
o1 - p1	0.0039	0.0052	0.7469	3.0734	Accept
p1 - op1	0.0226	0.0052	4.3554	3.0734	Reject

Homogeneous Subsets:

Group 1: c1 o1 p1 Group 2: op1

Pooled mean =	0.0432		Pooled mean =	0.0687	
95% Confidence Interval =	0.0366	0.0497	95% Confidence Interval =	0.0574	0.0800

Dependent Variable: **GENERAL PEROXIDASE** d2

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.002	1	0.002	2.649	0.1296
Phen	0.009	1	0.009	9.690	0.0090
Ozone × Phen	0.000	1	0.000	0.130	0.7249
Error	0.011	12	0.001		
Total	0.023	15	0.002		

Dependent Variable: **GENERAL PEROXIDASE** d3

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.004	1	0.004	5.704	0.0342
Phen	0.018	1	0.018	26.613	0.0002
Ozone × Phen	0.000	1	0.000	0.197	0.6648
Error	0.008	12	0.001		
Total	0.030	15	0.002		

Dependent Variable: **GENERAL PEROXIDASE** d4

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.000	1	0.000	0.887	0.3649
Phen	0.002	1	0.002	3.888	0.0721
Ozone × Phen	0.000	1	0.000	0.060	0.8101
Error	0.005	12	0.000		
Total	0.007	15	0.000		

Dependent Variable: **GENERAL PEROXIDASE** d6

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.000	1	0.000	0.756	0.4016
Phen	0.004	1	0.004	7.183	0.0200
Ozone × Phen	0.001	1	0.001	2.948	0.1117
Error	0.006	12	0.000		
Total	0.011	15	0.001		

Appendix 5.8 (Figure A5.7) Effects of ozone and phenmedipham on glutathione s-transferase activity, expressed on a protein basis, of sugarbeet cv Saxon, where n=4 on days 0-6. Results of three-way ANOVA, classified by ozone, phenmedipham and time.

Dependent Variable: **GLUTATHIONE S-TRANSFERASE** days 0, 1, 2, 3, 4 and 6

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Time	0.001	5	0.000	3.657	0.0053
Ozone	0.001	1	0.001	18.704	0.0000
Phen	0.000	1	0.000	8.890	0.0039
Time × Ozone	0.000	5	0.000	1.532	0.1906
Time × Phen	0.000	5	0.000	1.294	0.2760
Ozone × Phen	0.000	1	0.000	0.002	0.9644
Time × Ozone × Phen	0.000	5	0.000	0.627	0.6796
Error	0.003	72	0.000		
Total	0.005	95	0.000		

Appendix 5.8.1 (Figure A5.7) Effects of ozone and phenmedipham on glutathione s-transferase activity, expressed on a protein basis, of sugarbeet cv Saxon, where n=4 on days 0, 1, 2, 3, 4 and 6. Results of two-way ANOVA and Duncan's Multiple Range Tests for superoxide dismutase activity, classified by ozone and/or phenmedipham. In the comparisons table, accept indicates that the two means are not significantly different at $p \leq 0.05$ and * denotes significantly different pairs.

Dependent Variable: **GLUTATHIONE S-TRANSFERASE** d0

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.000	1	0.000	3.082	0.1297
Error	0.001	6	0.000		
Total	0.001	7	0.000		

Dependent Variable: **GLUTATHIONE S-TRANSFERASE** d1

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.000	1	0.000	0.974	0.3431
Phen	0.000	1	0.000	2.802	0.1200
Ozone × Phen	0.000	1	0.000	5.782	0.0332
Error	0.000	12	0.000		
Total	0.000	15	0.000		

For **GLUTATHIONE S-TRANSFERASE**, classified by Treatment

Group	Cases	Mean	c1	op1	o1	p1
c1	4	0.0209			*	*
op1	4	0.0250				
o1	4	0.0262	*			
p1	4	0.0272	*			

Comparison	Difference	Std Error	q Stat	Table q	Result
c1 - p1	0.0064	0.0016	4.0785	3.3193	Reject
c1 - o1	0.0053	0.0016	3.3917	3.2216	Reject
c1 - op1	0.0042	0.0016	2.6610	3.0734	Accept
op1 - p1	0.0022	0.0016	1.4175	3.2216	Accept
op1 - o1	0.0011	0.0016	0.7307	3.0734	Accept
o1 - p1	0.0011	0.0016	0.6868	3.0734	Accept

Homogeneous Subsets:

Group 1:	c1 op1		Group 2:	op1 o1 p1	
Pooled mean =	0.0229		Pooled mean =	0.0261	
95% Confidence Interval =	0.0205	0.0254	95% Confidence Interval =	0.0242	0.0281

Dependent Variable: **GLUTATHIONE S-TRANSFERASE** d2

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.000	1	0.000	2.495	0.1402
Phen	0.000	1	0.000	10.191	0.0077
Ozone × Phen	0.000	1	0.000	0.194	0.6675
Error	0.000	12	0.000		
Total	0.001	15	0.000		

Dependent Variable: **GLUTATHIONE S-TRANSFERASE** d3

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.000	1	0.000	2.599	0.1329
Phen	0.000	1	0.000	4.803	0.0489
Ozone × Phen	0.000	1	0.000	0.305	0.5907
Error	0.000	12	0.000		
Total	0.001	15	0.000		

Dependent Variable: **GLUTATHIONE S-TRANSFERASE** d4

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.000	1	0.000	8.953	0.0112
Phen	0.000	1	0.000	7.651	0.0171
Ozone × Phen	0.000	1	0.000	3.677	0.0793
Error	0.000	12	0.000		
Total	0.000	15	0.000		

Dependent Variable: **GLUTATHIONE S-TRANSFERASE** d6

Due To	Sum of Squares	DF	Mean Square	F-Stat	Signif
Ozone	0.000	1	0.000	1.855	0.1982
Phen	0.000	1	0.000	0.001	0.9713
Ozone × Phen	0.000	1	0.000	0.013	0.9119
Error	0.000	12	0.000		
Total	0.001	15	0.000		