THE EFFECTS OF ELEVATED CONCENTRATIONS OF CARBON DIOXIDE AND OZONE ON POTATO (*Solanum tuberosum L.)* YIELD

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CONTENTS

SUMMARY

Potatoes (*Solanum tuberosum L.* cv. Bintje) were grown in open top chambers at Teagasc, Crops Research Centre, Oak Park, Carlow and exposed to ambient and elevated concentrations of CO_2 in combination with ambient and elevated concentrations of O_3 in the 1998 and 1999 growing seasons. Exposure to elevated concentrations of O_3 caused visual damage to the leaves of the potato plants in both years of the study. In 1999, ozone damage to leaves was significantly reduced in the presence of elevated concentrations of CO_2. Stomatal conductance was reduced by elevated CO_2 and was reduced further by the elevated O_3 treatment under elevated CO_2. Exposure to elevated CO_2 increased tuber fresh weight yield by 32% in both years of the study. The yield increase was attributable to larger tuber sizes and not to an increase in the number of tubers. Tuber yield was unaffected by elevated O_3 at ambient concentrations of CO_2 in both years of the study. In 1999, the yield increase induced by elevated CO_2 was substantially reduced by the presence of elevated O_3.

Abbreviations: OTC, open top chamber; PPM, parts per million; PPB, parts per billion; CO_2, carbon dioxide; O_3, ozone; PAR, photosynthetically active radiation; UV, ultra violet; OD, outside diameter; LSD, least significant difference

INTRODUCTION

The concentration of carbon dioxide in the atmosphere has risen by approximately 40 parts per million (ppm) since 1958 and is expected to reach 550 ppm by the middle of the next century (Houghton *et al.*, 1992; Schimel *et al.*, 1996). This increase in the concentrations of CO_2, the building block for the sugars manufactured by photosynthesis, has prompted considerable interest in the potential impact of elevated CO_2 on vegetation and in particular on agricultural crops. Research has shown that a number of factors can contribute to increased biomass production as CO_2 levels rise, namely greater photosynthesis, higher water use efficiency, depression of respiration and delayed leaf senescence (Lemon, 1983; Kimball *et al*., 1990; Allen, 1990; Lawlor and Mitchell, 1991). Positive effects of elevated CO_2 on the growth and yield of a wide range of crops have been reported in the literature (Kimball, 1983). Yield responses to elevated CO_2, however, have been found to be species dependent. Species which feature good transport capacity and large sinks can best maximise the use of extra carbon fixed under elevated CO_2 (Schnyder and Baum, 1992; Korner and Miglietta, 1994).

Potato (*Solanum tuberosum* L.) is a species, which features both large sinks (Farrar and Williams, 1991) and an efficient method of sucrose transport (Riesmeier *et al* 1994), important prerequisites for a large response to elevated CO_2. Comparatively few studies have investigated the effect of elevated CO_2 concentrations on potato. Goudriann and de Ruiter (1983) reported a small negative response to CO_2 enrichment, while Wheeler and Tibbitts (1997) found that elevated levels of CO_2 stimulated above ground biomass but not tuber dry weight. In contrast, substantial stimulation of tuber yield by elevated CO_2 has been found by Wheeler *et al*. (1991). A simulation model of potato growth has predicted that a doubling of CO_2 levels to 700 ppm could stimulate tuber yield by 20-30% (Schapendonk *et al*., 1995). In the only field study done to date (Miglietta *et al*., 1998), using FACE (Free Air CO_2 enrichment), reported that a doubling of CO_2 concentration increased tuber yield by over 40%.

Levels of tropospheric ozone, a photochemical oxidant, have more then doubled in the past one hundred years and are predicted to continue rising at an even faster rate in the future (Hough and Derwent, 1990). In contrast to the stimulatory effects of CO_2, increasing concentrations of atmospheric O_3 have the potential to adversely affect crop yield (Heck *et al*., 1983). The influence of this strong oxidant on a variety of biochemical and physiological processes is well documented (Darrall, 1989). It has been established that O_3 exposure decreases leaf conductance, net photosynthesis as well as water use efficiency (Reich *et al*., 1995; Vozzo *et al*., 1995). Critical levels of O_3 for the protection of crops are known to have been exceeded in many parts of the industrialised world (Ashmore and Wilson, 1993) and the pollutant has been shown to be responsible for widespread crop loss. The deleterious effects of elevated O_3 on potatoes have been demonstrated in a number of studies (Pell *et al*., 1980, 1988; Eckardt and Pell, 1996).

Several studies have investigated the combined effects of increasing concentrations of CO_2 and O_3 on plants. Generally speaking, these experiments have established that elevated concentrations of CO_2 either partially or totally compensate for the negative effects of elevated O_3. This has been demonstrated for soybean Reinert and Chen Ho, 1995; Mulchi *et al*., 1995), wheat (Mortensen, 1990; Mulchi *et al*., 1995; Rao *et al*., 1995; Barnes *et al*., 1995) and corn (Mulchi *et al*., 1995). It has been suggested (Allen, 1990) that decreases in stomatal conductance induced by higher CO_2, together with the increased provision of

substrates for detoxification and repair processes, will afford plants with additional protection against phytotoxic air pollutants such as O_3. Some studies have reported such interactions between the effects of elevated O_3 and elevated CO_2. Fiscus *et al*., (1997) demonstrated that suppression of soybean yield by O_3 was completely ameliorated by elevated CO_2 and that a major part of this amelioration was attributable to elevated CO_2 induced decreases in conductance that limited access of O_3 to the leaf interior. Rao *et al*., (1995) reported that elevated CO_2 conferred increased protection to wheat plants exposed to elevated O_3 by increasing the activity of antioxidant enzymes. The photosynthetic apparatus in wheat flag leaves was found to be protected against the deleterious effects of elevated O_3 by elevated CO_2 (McKee *et al*., 1995). Barnes *et al*., (1995) reported that the yield of wheat plants exposed to elevated O_3 was increased by elevated CO_2 but reported no interaction as proportionately similar effects of O_3 were found at elevated and ambient CO_2. Similarly, no interactive effect was reported by Balaguer *et al*., (1995) or by Donnelly (1998) in studies on wheat. The objective of the present study was to investigate the combined effects of increasing atmospheric concentrations of ozone and carbon dioxide on a potato crop, no such previous investigation having been carried out under field conditions.

MATERIALS AND METHODS

Experimental site

The experiment was carried out at the Crops Research Centre, Oak Park, Carlow, Ireland. Carlow lies in the centre of an intensive tillage area in the south-east of the country, and is located about 80 km south-west of Dublin and about 50 m above sea level. The Crops Research Centre, Oak Park is 4 km due north of Carlow town at latitude 52^0 $51^{'}$ N and longitude $6^0 54^{'}$ W.

The soil is a free draining medium to heavy textured grey-brown podzolic (typic Hapludalf) of about 90-100 cm depth overlying and deriving from calcareous boulder clay composed mainly of limestone with some shale and sandstone.

Open top chambers

The open top chambers used in the study were modified commercial greenhouses supplied in kit form by Waytogrow Greenhouses Ltd. (Wickford, Essex, UK). The chambers were 3 m in diameter and 2.8 m tall. Their design incorporated the various recommendations made by Buckenham *et al*. (1981), which includes a frustrum, a lip on top of the chambers, sloping inwards. These additions are designed to reduce incursions of ambient air into the chambers.

Air was blown into the chambers from fans (Thermo Air Ltd., Carlow) through a circular perforated annulus. The airflow rate was 30 m^3 per minute, which was equivalent to three air changes per minute.

Cultural practices

Subsidiary eye pieces with just one shoot were cored from tubers of certified seed (*Solanum tuberosum*, cv. Bintje) supplied from the Netherlands. In 1998, the cores were placed in pots of John Innes compost and allowed to grow before being transferred to the field. Potato plantlets, which had grown from the excised eyes, were transferred to the experimental plots on 19 and 20 May. Pots were soaked with water before the potato plantlets in their compost block were carefully taken from the pots and placed into prepared holes in the drills. Potatoes were planted at a density of 10 plants/m^2, 25 cm between drills and 40 cm between plants. In 1999, the cores were placed in moist potting compost for three days before being transferred to the experimental plots where they were planted into levelled soil at a density of 13.3 plants/m^2, 30 cm between rows and 25 cm between the plants in each row. In both years two drills were also made around each open top chamber into which tubers (cv. Bintje) were planted, the objective being to reduce the chamber edge effect.

Soil nutrient levels were determined by soil test after which fertiliser was applied at a rate of 250 kg N/ha, 250 kg P/ha and 500 kg K/ha. The potatoes were sprayed with an insecticide/fungicide mixture at approximately weekly intervals to prevent the spread of potato blight and aphid borne viral diseases. Additionally, the potatoes were sprayed with another insecticide, Dursban (active ingredient

clopyrifos), on one occasion during the 1998 growing season after damage from stem borers became evident.

As plants grew, their canopies became less erect and it became necessary to stake the stems of each plant. The dates of important activites are given in Table 1.

Table 1: Time-table of agronomic practises

Year	Date	
1998	14 & 15 April	Eyes excised and planted in glasshouse
	19 & 20 May	Plants transferred to open top chambers
	15 May	Simazine @ 1 l/ha applied
	27 May	250 kg N; 250 kg P; 500 kg K applied
	5 August	Intermediate harvest
	6 October	Final harvest
1999	10–12 May	Eyes excised
	20–21 May	Cores planted in open top chambers
	28 May	250 kg N; 250 kg P; 500 kg K applied
	20 July	Intermediate harvest
	6 September	Final harvest

Gas generation and distribution

CO_2 was stored in liquified form in a refrigerated tank and liquid CO_2 was heated in a vapouriser after leaving the tank. The gaseous CO_2 was divided into two streams, each going to a bank of three mass flow controllers from which the gas was conducted through tubing to the air circulation system of each chamber. CO_2 was supplied continuously to the chambers within which concentrations were controlled by a feedback mechanism. An air sample from each open top chamber requiring CO_2 control was drawn via a diaphragm pump into an infra-red gas analyser (Model WMA-2, PP Systems, Hitchin, Herts., UK) and the CO_2 concentration regulated to the required concentration by adjusting the flow rate to the chamber by means of its associated mass flow controller.

Ozone was supplied for approximately seven hours a day, five days a week during the growing seasons. This gas was generated from industrial grade oxygen by silent electrical discharge (ABB Ozone generator Type LN 103, Asea Brown Boveri Ltd., Baden, Switzerland). Ozone enriched air from the ozone generator was divided between two stainless steel manifolds before being distributed to the

chambers. Ozone concentrations in the chambers was monitored by passing chamber air through an ozone analyser (Model No. 8810, Monitor Labs Inc., San Diego, CA., USA). Flow rate adjustment by rotameters allowed ozone concentrations in individual chambers to be controlled.

Pollutant and climate monitoring

Ambient ozone concentrations were monitored using a UV absorption analyser (Model No. 8810, Monitor Labs. Inc., San Diego, CA., USA). This analyser and the analyser referred to above, which was used for monitoring ozone concentrations within the chambers, were both calibrated at the start of the season using a secondary transfer ozone standard which itself had been calibrated from a primary standard. Thereafter the ozone analysers were calibrated at monthly intervals using a Monitor Labs. 8500 calibrator. Ambient air was sampled at a point 6 m above ground level and drawn into the analyser through 6.4 mm OD Teflon tubing by means of a teflon membrane pump (Model No. N726, KNF Neuberger, Oxford, UK). An index of pollutant exposure was calculated for each treatment. The index used for this calculation (AOT40 index) calculates the censored sum of all hourly concentrations above a threshold of 40 ppb.

Treatments

In this study potatoes were exposed to four treatments as follows:-

- Ambient concentrations of ozone, ambient concentrations of CO_2
- Elevated concentrations of ozone, ambient concentrations of CO_2
- Ambient concentrations of ozone, elevated concentrations of CO_2
- Elevated concentrations of ozone, elevated concentrations of CO_2

Each treatment was replicated three times. The concentration of carbon dioxide in the elevated treatments was 680 ppm which was supplied throughout day and night. In 1998, CO_2 was supplied from 25 June, 37 days after the plants had been transferred to the open top chambers, until the final harvest (6 October). In 1999, CO_2 was supplied from 9 June when the plants were beginning to emerge until the final harvest (6 September) Elevated ozone was typically supplied to the treatments 7 hours a day, five days a week. In 1998, the elevated O_3 treatments received ambient concentrations of O_3 plus 50 ppb. Higher concentrations of O_3, ambient O_3 plus 70 ppb, were supplied to the elevated O_3 treatments in 1999.

Ozone fumigation started on 23 June in the 1998 growing season and on 10 June in 1999.

Stomatal conductance measurements

Stomatal conductance was measured on several dates towards the end of the 1999 growing season. A Delta T type AP4 porometer (Delta T devices, 128 Low Road, Burwell, Cambridge) was used for these measurements which were taken on sunny days with minimal cloud cover during those hours of the day when PAR levels were highest. Measurements were made on the terminal leaflet of the first fully expanded mainstem leaf of five plants in each chamber.

Visual scoring

Leaves were assessed for ozone damage during the 1999 growing season as follows: 0, no damage; 1, 0-25% of the leaf surface damaged; 2, 25-50% of the leaf surface damaged; 3, >50% of the leaf surface damaged. An index for each assessment was expressed as a percentage of the maximum possible damage as follows:

$$\text{Index} = \frac{100(x+2y+3z)}{3(w+x+y+z)}$$

where w = leaves in class 0, x = leaves in class 1, y = leaves in class 2, z = leaves in class 3. Eighteen leaves per plant were scored in the intermediate harvest where leaf 1 was the newest leaf to emerge, the injury observed on these eighteen leaves was combined and expressed as total plant injury. On 29 July the fifth leaf down the stem from the first fully expanded leaf on ten randomly selected plants in each chamber was marked with a tag. All subsequent visual scoring was done on these leaves which were approximately equal in age, the assessment being done on either the whole leaf, the terminal leaflet or both.

Harvests

An intermediate harvest was taken in both years of the study in which five plants were harvested at random from each chamber. The following parameters were determined, main stem height, number of main stem leaves, green leaf area index, total number of tubers, in addition to dry weights of green leaves, stems and tubers. A tuber was defined as a swelling at least twice the diameter of the subtending stolon.

Fifteen plants were sampled at random from each experimental plot in the final harvest of 1998 while ten plants were sampled from each plot in 1999, (dates are given in Table 1). The following parameters were determined, dry weight of above ground biomass, number of tubers in grades <35 mm, 35-50 mm, 50-60 mm, 60-70 mm, 70-80 mm, >80 mm, fresh and dry weights of tubers in the above grades.

Statistics

The experiment was laid out as a randomised complete block design. Analysis of variance was carried out to test the effect of block, O_3 and CO_2 as well as to test for interaction between the O_3 and CO_2 treatments. The statistical package Minitab was used (Minitab Inc., State College, PA, USA). Missing data was calculated using a missing value equation (Cochran and Cox, 1957). Data from the visual scoring of ozone damage was in the form of percentages. The percentage data was transformed using an ArcSine transformation (Snedecor, 1962) as percentage data is not necessarily normally distributed and, consequently, does not necessarily meet the requirements for analysis of variance.

RESULTS

A summary of the meteorological conditions during two growing seasons is presented in Table 2. Both growing seasons were cold and wet when compared to the ten-year average. The 1998 growing season was cold and wet with below average levels of irradiance. In contrast, the 1999 growing season was better with higher levels of irradiance and better temperatures, even though rainfall was high. The AOT40 index value is higher for 1999 than for 1998. The higher value is attributable to higher ambient levels of O_3 in 1999 (Table 3) and to the fact that

the elevated O_3 treatments were supplied with higher concentrations of O_3 in 1999 in comparison to 1998.

Table 2: Meteorological information for each growing season

Parameter	10 year average	1998	1999
Average daily air temperature	15.0 ^{0}C	12.4 ^{0}C	13.4 ^{0}C
Average daily irradiance	184.4 W/m^2	164.4 W/m^2	183.7 W/m^2
Total growing season rainfall	196.4mm	385.1mm	451.4 mm

Table 3: Pollutant exposure – AOT40 value calculated from the first day of ozone fumigation until final harvest. The AOT40 index is calculated as the censored sum of all hourly concentrations above a threshold of 40 ppb

Treatment	1998	1999
Chamber with ambient air	429.7 ppbh	2724.1 ppbh
Elevated ozone treatment	11105.9 ppbh	14066.5 ppbh

Whole leaves and terminal leaflets were scored for ozone damage in 1999 and results are presented in Tables 4 and 5. Ozone damage was evident in the elevated ozone treatments, there being a significant ozone effect ($p<0.01$) each time scoring was carried out. Leaf damage increased during the measurement period so that over 70% of the leaf surface was affected by ozone damage in the elevated ozone treatment on 26 August. Damage was reduced in the presence of elevated concentrations of CO_2, this CO_2 effect was statistically significant when whole leaves were scored at the intermediate harvest and when terminal leaflets were scored on 5 August, 20 August and on 27 August.

Table 4: Visual scoring of whole leaves. Leaves were assessed for ozone damage as follows: 0, no damage; 1, 0-25% of the leaf surface damaged; 2, 25-50% of the leaf surface damaged; 3, >50% of the leaf surface damaged. An index for each assessment was expressed as a percentage of the maximum possible damage. Transformed data presented. Original percentage data in italics

Date	Intermediate harvest 20/7/99	13/8/99	20/8/99	27/8/99
Ambient CO_2	0.0±0.0	0.0±0.0	8.8±8.8	11.7±11.7
Ambient O_3	(*0%*)	(*0%*)	(*6.7%*)	(*11.7%*)
Ambient CO_2	13.9 ± 3.0	49.6±6.2	50.4±4.5	57.7±3.7
Elevated O_3	(*6.3%*)	(*57.8%*)	(*59.3%*)	(*71.1%*)
Elevated CO_2	0.0±0.0	0.0±0.0	7.2±3.6	3.5±3.5
Ambient O_3	(*0%*)	(*0%*)	(*2.3%*)	(*1.1%*)
Elevated CO_2	5.4 ± 0.4	37.4±13.6	34.4±9.7	44.6±9.7
Elevated O_3	(*0.9%*)	(*37.8%*)	(*33.7%*)	(*48.9%*)
CO_2 effect	P<0.05	n.s.	n.s.	n.s.
O_3 effect	P<0.01	P<0.01	P<0.01	P<0.01
O_3*CO_2 effect	P<0.05	n.s.	n.s.	n.s.
LSD (0.05)	5.3	15.0	14.5	15.9
LSD (0.01)	8.0	22.8	21.9	24.1

Table 5: Visual scoring of terminal leaflets. Leaves were assessed for ozone damage as follows: 0, no damage; 1, 0-25% of the leaf surface damaged; 2, 25-50% of the leaf surface damaged; 3, >50% of the leaf surface damaged. An index for each assessment was expressed as a percentage of the maximum possible damage. Transformed data presented. Original percentage data in italics

Date	5/8/99	13/8/99	20/8/99	27/8/99
Ambient CO_2	0.0±0.0	0.0±0.0	6.1±6.1	10.4±10.4
Ambient O_3	(*0%*)	(*0%*)	(*3.33%*)	(*8.9%*)
Ambient CO_2	23.4±4.7	50.9±5.9	52.7±3.1	55.5±3.0
Elevated O_3	(*16.7%*)	(*60.0%*)	(*63.1%*)	(*67.8%*)
Elevated CO_2	0.0±0.0	0.0±0.0	4.9±4.9	3.5±3.5
Ambient O_3	(*0%*)	(*0%*)	(*2.2%*)	(*1.1%*)
Elevated CO_2	3.5±3.5	35.4±7.7	30.8±9.1	39.7±7.6
Elevated O_3	(*1.11%*)	(*34.4%*)	(*28.1%*)	(*41.1%*)
CO_2 effect	P<0.05	n.s.	P<0.01	P<0.05
O_3 effect	P<0.01	P<0.01	P<0.01	P<0.01
O_3*CO_2 effect	P<0.05	n.s.	P<0.01	n.s.
LSD (0.05)	11.5	14.8	8.9	12.6
LSD (0.01)	17.3	22.4	13.6	19.0

Elevated CO_2 suppressed stomatal conductance in 1999. Significant reductions in conductance attributable to the presence of elevated CO_2 were recorded on July 28 ($p<0.05$), at the three times on August 20 when measurements were made ($p<0.05$) ($p<0.01$) and ($p<0.05$) as well as on September 1 ($p<0.01$). Elevated ozone did not have a significant effect on stomatal conductance. In general, however, stomatal conductance in treatments exposed to elevated CO_2 was further reduced in the presence of elevated O_3. On four occasions during which measurements were taken, the only significant difference between treatments was between the conductance of the ambient CO_2 treatments and the reduced conductance of the elevated CO_2, elevated O_3 treatment. On these occasions the conductance of the elevated CO_2, ambient ozone treatment whilst smaller, did not differ significantly from the ambient CO_2 treatment. Stomatal conductance in the elevated CO_2, elevated O_3 treatment was significantly lower than the control in all three measurements conducted on August 20 ($p<0.05$) ($p<0.01$) ($p<0.05$) and on September 1 ($p<0.01$). Stomatal conductance in the elevated CO_2, ambient O_3

treatment differed significantly from the control on the second measurement of August 20 ($p<0.05$) as well as on September 1 ($p<0.01$).

Results from the intermediate destructive harvest are presented in Tables 6 and 7. Neither CO_2 nor O_3 had any significant effect on leaf area index or plant height in either year. Similarly, above ground biomass was not affected in 1998 and stem dry weight was not affected in 1999. Elevated CO_2 significantly increased tuber dry weight ($p<0.05$) and leaf dry weight ($p<0.05$) in 1998 but not in 1999. In contrast, tuber number ($p<0.05$) was significantly depressed by the presence of elevated CO_2 in 1999.

Table 6: Parameters measured at the intermediate harvest taken on 5 August 1998

	Leaf area index	Tuber dry weight (g m^{-2})	Tuber number /m^2	Above ground biomass (g m^{-2})	Leaf dry weight (g m^{-2})	Plant height (cm)
Ambient CO_2 Ambient O_3	5.8±0.7	656.6±65.2	173.3±18.7	352.6±61.7	156.9±19.9	94.8±7.6
Ambient CO_2 Elevated O_3	6.1±0.5	631.0±52.3	135.3±15.7	327.1±18.1	158.3±16.9	91.3±0.7
Elevated CO_2 Ambient O_3	5.6±0.4	881.9±69.3	135.3±18.8	376.6±23.5	190.0±26.5	82.1±1.3
Elevated CO_2 Elevated O_3	5.9±0.9	942.4±160.7	153.0±15.6	431.5±52.8	207.9±26.9	89.3±6.1
CO_2 effect	n.s.	P<0.05	n.s.	n.s.	P<0.05	n.s.
O_3 effect	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
O_3*CO_2 effect	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
LSD (0.05)	2.1	354.5	66.1	141.9	61.4	15.0
LSD (0.01)	3.3	537.0	100.1	214.9	92.9	22.78

Table 7: Parameters measured at the intermediate harvest taken on 20 July 1999

	Leaf area index	Tuber dry weight (g m^{-2})	Tuber number /m^2	Stem dry Weight (g m^{-2})	Leaf dry Weight (g m^{-2})	Plant height (cm)
Ambient CO_2 Ambient O_3	4.0±0.6	347.2±24.9	110.2±6.2	83.7±10.8	131.5±18.2	63.1±1.0
Ambient CO_2 Elevated O_3	4.5±0.5	322.1±27.7	128.9±7.3	80.2±1.6	132.0±4.2	61.3±3.7
Elevated CO_2 Ambient O_3	4.1±0.6	410.3±34.5	98.6±2.6	83.0±12.3	135.7±12.9	60.1±2.1
Elevated CO_2 Elevated O_3	4.7±0.9	387.1±33.2	98.6±9.6	89.4±16.8	142.1±23.3	58.7±2.8
CO_2 effect	n.s.	n.s.	P<0.05	n.s.	n.s.	n.s.
O_3 effect	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
O_3*CO_2 effect	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
L.S.D.(0.05)	1.4	115.9	26.6	30.6	43.9	6.1
L.S.D.(0.01)	2.1	175.7	98.4	46.4	66.5	9.2

Elevated CO_2 significantly increased the dry weight of potato tubers in 1998 ($p<0.05$) and in 1999 ($p<0.01$). In 1998, the increase in dry weight was caused by an increase in the weight of individual tubers in the separate size grades and elevated CO_2 did not alter the number of tubers/m^2. In 1999, elevated CO_2 caused a significant reduction ($p<0.05$) in the number of tubers/m^2. Elevated concentrations of CO_2 significantly depressed ($p<0.05$) the number of tubers in the <35 mm and 35-50 mm grades whilst significantly increasing ($p<0.05$) the number of tubers in the 50-60 mm and 60-70 mm grades. The yield increase at elevated CO_2 was attributable to the greater number of tubers in these larger grades, as the fresh weight and dry weight increases in these larger size grades were considerably greater than the weight reductions which were recorded in the lower grades. Above ground biomass was significantly ($p<0.05$) increased by elevated CO_2 in 1998 but not in 1999 (Table 8).

Table 8: Dry weight of above ground biomass (g m^{-2}) measured at final harvest

	1998	1999
Ambient CO_2 Ambient O_3	262.0±13.0	444.5±10.1
Ambient CO_2 Elevated O_3	267.7±21.1	447.6±56.3
Elevated CO_2 Ambient O_3	330.5±29.1	428.7±31.0
Elevated CO_2 Elevated O_3	293.4±26.1	395.8±12.2
CO_2 effect	P<0.05	n.s.
O_3 effect	n.s.	n.s.
CO_2 * O_3 effect	n.s.	n.s.
LSD (0.05)	65.5	130.23
LSD (0.01)	99.2	197.28

In 1998, elevated concentrations of ozone did not have a significant effect on the final harvest tuber number, tuber fresh weight or tuber dry weight. There was a significant interaction between the effect of CO_2 and O_3 in the 50-60 mm size grade. In 1999, elevated O_3 had no significant effect on tuber dry weight or tuber number but caused a statistically significant reduction in tuber fresh weight ($p<0.05$). Additionally, in the case of total tuber fresh weight, there was a significant interaction ($p<0.05$) between the effects of the two gases. Although elevated O_3 caused a significant reduction in tuber fresh weight yield under elevated CO_2 there was no significant difference between the control and the elevated O_3, ambient CO_2 treatment.

DISCUSSION

CO_2 effects

Increasing the concentration of CO_2 from 355 ppm to 680 ppm increased tuber fresh weight by 32% in both years of the study. Substantial stimulation in tuber fresh weight yield has been reported in a number of CO_2 enrichment studies (Wheeler *et al*., 1991; Miglietta *et al*., 1998). The percentage yield increase recorded in this study is consistent with that predicted by Schapendonk *et al*.

(1995) who predicted that a doubling of present CO_2 concentration to 700 ppm would stimulate the tuber yield of early sown potato varieties such as cv. Bintje by 29%. Similarly, Miglietta *et al*. (1998) regressed tuber yield against levels of CO_2 concentration and found that a tuber yield stimulation as large as 10% per 100 ppm increase in CO_2 could be expected.

In 1998, the number of tubers was unaffected by exposure to elevated CO_2 and the increase in tuber yield was attributable to increases in the size of tubers within individual grades. The absence of an effect on tuber number is not surprising given that the number of tubers would have been determined before the plants were exposed to elevated CO_2 (Struik *et al*., 1990). CO_2 fumigation commenced late in the 1998 growing season, 37 days after the potato plantlets had been transferred to the field site. In 1999, when elevated CO_2 fumigation started at emergence, exposure to elevated concentrations of CO_2 reduced tuber numbers. The 1999 yield increase was attributable to the fact that the remaining tubers, though smaller in number, grew larger and were classified into larger size grades in comparison to tubers from the ambient CO_2 treatments. It would appear that elevated CO_2 altered the hierarchy of tubers causing some tubers to be favoured as sinks for assimilate and others to be resorbed. It has been demonstrated that tuber hierarchy can alter in response to environmental change (Plodowska *et al*., 1989) which can also result in the resorption of tubers (Cavagnaro *et al*., 1971). Another possibility is that exposure to elevated CO_2 inhibited the process of tuberization, consequently reducing the number of tubers formed.

Exposure to elevated CO_2 decreased stomatal conductance. This is a common response reported for C_3 plants exposed to elevated concentrations of CO_2 (Allen, 1990; Morison, 1989). The explanation of the exact mechanism remains inconclusive. Mott (1988) suggested that the stomata are induced to close because of high concentrations of intracellular CO_2. In contrast, Lawlor and Keys (1993) have proposed that a stimulation of CO_2 assimilation in the stomatal guard cells at elevated CO_2 concentrations competes for ATP with the ion transport process which transfers ions into the guard cells maintaining the turgor required to keep these cells open.

O_3 effects

High concentrations of ozone caused visible leaf damage in both years of the study, the first symptoms appearing on August 20 of the 1998 growing season (91

days after emergence) and on 5 July in 1999 (26 days after emergence). Visual symptoms of ozone damage were most severe in 1999, more than 70% of the area of the leaves used for scoring was damaged. Exposure to elevated O_3 at ambient CO_2, however, had no significant effect on tuber yield or above ground biomass in both 1998 and 1999, despite the severity of the leaf damage. The results suggest the potato crop had sufficient compensatory mechanisms to avoid yield reduction from serious leaf damage as it approached maturity. Similarly, Wille and Kleinkopf (1992) reported that severe defoliation due to late season hail damage had little effect on tuber yield. This result does, however, contrast with the work of Clarke *et al*., (1990) who showed that tuber yield was reduced by 25% and by 31% for two different varieties when 75% of the foliage exhibited ozone toxicity symptoms. It would appear that, in the case of the potato variety used in this study, there are quite different thresholds for visible injury and yield reduction.

Exposure to elevated ozone at ambient CO_2 had no significant effect on stomatal conductance. This result contrasts with recent work on a range of species where similar concentrations of ozone have been shown to induce stomatal closure (Fiscus *et al.*, 1997; Balaguer *et al*., 1995; Barnes *et al*., 1995). Evidence has been produced to show that such ozone induced stomatal closure is the result rather than the cause of reduced photosynthesis (Fiscus *et al*., 1997; McKee *et al*., 1995). Darrall (1989), however, reviewing stomatal responses to ozone reported a diversity of stomatal responses below 200 ppb O_3 including stomatal opening, stomatal closing and no change.

CO_2 * O_3 effects

Exposure to approximately twice ambient concentrations of CO_2 ameliorated ozone induced leaf damage. Several studies have shown that elevated CO_2 can reduce ozone injury (Rao *et al*., 1995; Mortensen, 1990; Mulchi *et al*., 1995; Reinert and Chen Ho, 1995; Donnelly, 1998). It was Allen (1990) who postulated that decreases in stomatal density and aperture combined with increased availability of substrates for detoxification would afford plants grown in elevated CO_2 with additional protection against O_3. McKee *et al*. (1997) and Mulholland *et al*. (1997) reported that elevated CO_2 protected against the damaging effects of O_3 on spring wheat biomass by decreasing stomatal conductance. Rao *et al*. (1995) reported that elevated CO_2 conferred increased protection to wheat plants exposed to elevated ozone by increasing the activity of antioxidant enzymes. In contrast, Polle *et al*. (1993) reported that CO_2 enrichment depressed various components of

the cellular antioxidant defence system suggesting that plants exposed to higher levels of CO_2 may be more sensitive to O_3 at the cellular level, a conclusion also reached by Barnes *et al*. (1995). The results of this study, however, show that elevated CO_2 alleviates the damaging effects of ozone on potato leaves. Without further evidence the precise mechanism of this protective effect is open to speculation. The 1999 porometry measurements showed that exposure to elevated CO_2 consistently suppressed stomatal conductance in potato plants exposed to elevated concentrations of O_3. Lower leaf conductances in elevated CO_2 treatments would have reduced ozone flux into leaves reducing the potential for damage. It is, therefore, likely that the elevated CO_2 conferred protection against ozone damage was contributed to, if not caused by, reductions in stomatal conductance.

There was no interaction between the effects of O_3 and CO_2 on yield in 1998 when ozone had no effect on either biomass or tuber yield. In 1999, however, there was an interaction between the two treatments in the final harvest, as the yield increase induced by elevated CO_2 was substantially reduced in the presence of elevated O_3, whereas elevated ozone alone had no effect on yield. Exposure to elevated O_3 started earlier in the 1999 growing season and higher concentrations of O_3 were used in comparison to 1998. The question arises as to whether this result is the manifestation of increased susceptibility to ozone injury at higher levels of CO_2 or a reduced CO_2 effect in the presence of elevated O_3. Previous studies on wheat and spruce have provided evidence that ozone injury may be enhanced at higher levels of CO_2 (Polle *et al*., 1993; Barnes *et al*., 1995). However, visual scoring of leaves for ozone damage in this study has clearly shown that ozone injury was substantially reduced in the presence of elevated CO_2. This would suggest that the observed phenomenon is the result of a reduced CO_2 effect in the presence of elevated O_3. The porometry measurements provide evidence of a greater decrease in stomatal conductance in the high CO_2 and high O_3 treatment, compared to the treatment which received just elevated CO_2. Consequently, one possible explanation for this phenomenon is reduced CO_2 uptake resulting from enhanced stomatal closure induced by the combined effect of elevated O_3 and elevated CO_2. There are many reports in the literature of ozone induced stomatal closure (Fiscus *et al*., 1997; Barnes *et al*., 1995, Darrall, 1989). Why ozone should induce stomatal closure at elevated CO_2 but not at ambient CO_2 is unclear. It is also possible that the reduced effect of elevated CO_2 in the presence of elevated O_3 was caused by a reduction in the leaf area available for photosynthesis. Leaf damage resulting from exposure to elevated O_3 was substantial, even in the presence of elevated CO_2.

CONCLUSIONS

This study has shown very clearly that substantial stimulation of potato tuber yield can be expected to result from rising concentrations of atmospheric CO_2. Consequently, potato simulation models need to be modified accordingly. Elevated ozone at ambient concentrations of CO_2 had no effect on tuber yield despite substantial injury to foliage, suggesting that there are different thresholds for visible injury and reductions in yield. Exposure to elevated CO_2 reduced ozone injury most probably by limiting ozone flux into leaves. The yield increase induced by elevated CO_2 was reduced in the presence of elevated O_3.

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