

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

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CONTENTS

SUMMARY	1
INTRODUCTION	1
MATERIALS AND METHODS	3
Experimental site	3
Open top chambers	4
Cultural practices	4
Gas generation and distribution	5
Pollutant and climate monitoring	6
Treatments	6
Stomatal conductance measurements	7
Visual scoring	7
Harvests	8
Statistics	8
RESULTS	8
DISCUSSION	14
CO ₂ effects	14
O ₃ effects	16
CO ₂ * O ₃ effects	16
CONCLUSIONS	18
ACKNOWLEDGEMENTS	18
REFERENCES	19

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 CO₂ in combination with ambient and elevated concentrations of O₃ in the 1998 and 1999 growing seasons. Exposure to elevated concentrations of O₃ 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 CO₂. Stomatal conductance was reduced by elevated CO₂ and was reduced further by the elevated O₃ treatment under elevated CO₂. Exposure to elevated CO₂ 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 O₃ at ambient concentrations of CO₂ in both years of the study. In 1999, the yield increase induced by elevated CO₂ was substantially reduced by the presence of elevated O₃.

Abbreviations: OTC, open top chamber; PPM, parts per million; PPB, parts per billion; CO₂, carbon dioxide; O₃, 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 CO₂, the building block for the sugars manufactured by photosynthesis, has prompted considerable interest in the potential impact of elevated CO₂ on vegetation and in particular on agricultural crops. Research has shown that a number of factors can contribute to increased biomass production as CO₂ 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 CO₂ on the growth and yield of a wide range of crops have been reported in the literature (Kimball, 1983). Yield responses to elevated CO₂, 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 CO₂ (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 CO₂. Comparatively few studies have investigated the effect of elevated CO₂ concentrations on potato. Goudriann and de Ruiter (1983) reported a small negative response to CO₂ enrichment, while Wheeler and Tibbitts (1997) found that elevated levels of CO₂ stimulated above ground biomass but not tuber dry weight. In contrast, substantial stimulation of tuber yield by elevated CO₂ has been found by Wheeler *et al.* (1991). A simulation model of potato growth has predicted that a doubling of CO₂ 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 CO₂ enrichment), reported that a doubling of CO₂ concentration increased tuber yield by over 40%.

Levels of tropospheric ozone, a photochemical oxidant, have more than 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 CO₂, increasing concentrations of atmospheric O₃ 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 O₃ exposure decreases leaf conductance, net photosynthesis as well as water use efficiency (Reich *et al.*, 1995; Vozzo *et al.*, 1995). Critical levels of O₃ 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 O₃ 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 CO₂ and O₃ on plants. Generally speaking, these experiments have established that elevated concentrations of CO₂ either partially or totally compensate for the negative effects of elevated O₃. 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 CO₂, together with the increased provision of

substrates for detoxification and repair processes, will afford plants with additional protection against phytotoxic air pollutants such as O₃. Some studies have reported such interactions between the effects of elevated O₃ and elevated CO₂. Fiscus *et al.*, (1997) demonstrated that suppression of soybean yield by O₃ was completely ameliorated by elevated CO₂ and that a major part of this amelioration was attributable to elevated CO₂ induced decreases in conductance that limited access of O₃ to the leaf interior. Rao *et al.*, (1995) reported that elevated CO₂ conferred increased protection to wheat plants exposed to elevated O₃ 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 O₃ by elevated CO₂ (McKee *et al.*, 1995). Barnes *et al.*, (1995) reported that the yield of wheat plants exposed to elevated O₃ was increased by elevated CO₂ but reported no interaction as proportionately similar effects of O₃ were found at elevated and ambient CO₂. 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° 51' N and longitude 6° 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 Waytograd 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 m³ 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/m², 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/m², 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 activities are given in Table 1.

Table 1: Time-table of agronomic practices

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

CO₂ was stored in liquified form in a refrigerated tank and liquid CO₂ was heated in a vapouriser after leaving the tank. The gaseous CO₂ 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. CO₂ was supplied continuously to the chambers within which concentrations were controlled by a feedback mechanism. An air sample from each open top chamber requiring CO₂ control was drawn via a diaphragm pump into an infra-red gas analyser (Model WMA-2, PP Systems, Hitchin, Herts., UK) and the CO₂ 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 CO₂
- Elevated concentrations of ozone, ambient concentrations of CO₂
- Ambient concentrations of ozone, elevated concentrations of CO₂
- Elevated concentrations of ozone, elevated concentrations of CO₂

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, CO₂ 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, CO₂ 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 O₃ treatments received ambient concentrations of O₃ plus 50 ppb. Higher concentrations of O₃, ambient O₃ plus 70 ppb, were supplied to the elevated O₃ 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, O₃ and CO₂ as well as to test for interaction between the O₃ and CO₂ 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 O₃ in 1999 (Table 3) and to the fact that

the elevated O₃ treatments were supplied with higher concentrations of O₃ 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 °C	12.4 °C	13.4 °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 CO₂, this CO₂ 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 CO₂ suppressed stomatal conductance in 1999. Significant reductions in conductance attributable to the presence of elevated CO₂ 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 CO₂ was further reduced in the presence of elevated O₃. On four occasions during which measurements were taken, the only significant difference between treatments was between the conductance of the ambient CO₂ treatments and the reduced conductance of the elevated CO₂, elevated O₃ treatment. On these occasions the conductance of the elevated CO₂, ambient ozone treatment whilst smaller, did not differ significantly from the ambient CO₂ treatment. Stomatal conductance in the elevated CO₂, elevated O₃ 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 CO₂, ambient O₃

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 CO₂ nor O₃ 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 CO₂ 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 CO₂ 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	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 O ₃						
Ambient CO ₂	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 O ₃						
Elevated CO ₂	5.6±0.4	881.9±69.3	135.3±18.8	376.6±23.5	190.0±26.5	82.1±1.3
Ambient O ₃						
Elevated CO ₂	5.9±0.9	942.4±160.7	153.0±15.6	431.5±52.8	207.9±26.9	89.3±6.1
Elevated O ₃						

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	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 O ₃						
Ambient CO ₂	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 O ₃						
Elevated CO ₂	4.1±0.6	410.3±34.5	98.6±2.6	83.0±12.3	135.7±12.9	60.1±2.1
Ambient O ₃						
Elevated CO ₂	4.7±0.9	387.1±33.2	98.6±9.6	89.4±16.8	142.1±23.3	58.7±2.8
Elevated O ₃						

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 CO₂ 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 CO₂ did not alter the number of tubers/m². In 1999, elevated CO₂ caused a significant reduction (p<0.05) in the number of tubers/m². Elevated concentrations of CO₂ 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 CO₂ 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 CO₂ 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	262.0±13.0	444.5±10.1
Ambient O ₃		
Ambient CO ₂	267.7±21.1	447.6±56.3
Elevated O ₃		
Elevated CO ₂	330.5±29.1	428.7±31.0
Ambient O ₃		
Elevated CO ₂	293.4±26.1	395.8±12.2
Elevated O ₃		

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 CO₂ and O₃ in the 50-60 mm size grade. In 1999, elevated O₃ 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 O₃ caused a significant reduction in tuber fresh weight yield under elevated CO₂ there was no significant difference between the control and the elevated O₃, ambient CO₂ treatment.

DISCUSSION

CO₂ effects

Increasing the concentration of CO₂ 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 CO₂ 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 CO₂ 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 CO₂ concentration and found that a tuber yield stimulation as large as 10% per 100 ppm increase in CO₂ could be expected.

In 1998, the number of tubers was unaffected by exposure to elevated CO₂ 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 CO₂ (Struik *et al.*, 1990). CO₂ 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 CO₂ fumigation started at emergence, exposure to elevated concentrations of CO₂ 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 CO₂ treatments. It would appear that elevated CO₂ 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 CO₂ inhibited the process of tuberization, consequently reducing the number of tubers formed.

Exposure to elevated CO₂ decreased stomatal conductance. This is a common response reported for C₃ plants exposed to elevated concentrations of CO₂ (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 CO₂. In contrast, Lawlor and Keys (1993) have proposed that a stimulation of CO₂ assimilation in the stomatal guard cells at elevated CO₂ 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.

O₃ 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 O₃ at ambient CO₂, 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 CO₂ 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 O₃ including stomatal opening, stomatal closing and no change.

CO₂ * O₃ effects

Exposure to approximately twice ambient concentrations of CO₂ ameliorated ozone induced leaf damage. Several studies have shown that elevated CO₂ 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 CO₂ with additional protection against O₃. McKee *et al.* (1997) and Mulholland *et al.* (1997) reported that elevated CO₂ protected against the damaging effects of O₃ on spring wheat biomass by decreasing stomatal conductance. Rao *et al.* (1995) reported that elevated CO₂ 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 CO₂ enrichment depressed various components of

the cellular antioxidant defence system suggesting that plants exposed to higher levels of CO₂ may be more sensitive to O₃ at the cellular level, a conclusion also reached by Barnes *et al.* (1995). The results of this study, however, show that elevated CO₂ 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 CO₂ consistently suppressed stomatal conductance in potato plants exposed to elevated concentrations of O₃. Lower leaf conductances in elevated CO₂ treatments would have reduced ozone flux into leaves reducing the potential for damage. It is, therefore, likely that the elevated CO₂ conferred protection against ozone damage was contributed to, if not caused by, reductions in stomatal conductance.

There was no interaction between the effects of O₃ and CO₂ 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 CO₂ was substantially reduced in the presence of elevated O₃, whereas elevated ozone alone had no effect on yield. Exposure to elevated O₃ started earlier in the 1999 growing season and higher concentrations of O₃ 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 CO₂ or a reduced CO₂ effect in the presence of elevated O₃. Previous studies on wheat and spruce have provided evidence that ozone injury may be enhanced at higher levels of CO₂ (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 CO₂. This would suggest that the observed phenomenon is the result of a reduced CO₂ effect in the presence of elevated O₃. The porometry measurements provide evidence of a greater decrease in stomatal conductance in the high CO₂ and high O₃ treatment, compared to the treatment which received just elevated CO₂. Consequently, one possible explanation for this phenomenon is reduced CO₂ uptake resulting from enhanced stomatal closure induced by the combined effect of elevated O₃ and elevated CO₂. 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 CO₂ but not at ambient CO₂ is unclear. It is also possible that the reduced effect of elevated CO₂ in the presence of elevated O₃ was caused by a reduction in the leaf area available for photosynthesis. Leaf damage resulting from exposure to elevated O₃ was substantial, even in the presence of elevated CO₂.

CONCLUSIONS

This study has shown very clearly that substantial stimulation of potato tuber yield can be expected to result from rising concentrations of atmospheric CO₂. Consequently, potato simulation models need to be modified accordingly. Elevated ozone at ambient concentrations of CO₂ 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 CO₂ reduced ozone injury most probably by limiting ozone flux into leaves. The yield increase induced by elevated CO₂ was reduced in the presence of elevated O₃.

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REFERENCES

Allen, A.H. (1990). Plant responses to rising carbon dioxide and potential interactions with air pollutants. *Journal of Environmental Quality* **19**: 15-34.

Ashmore, M.R. and Wilson, R.B. (1993). Critical Levels for Ozone; *Proceedings UN-ECE Critical Levels Workshop, Egham, UK*. Creative Press, Reading.

Balaguer, L., Barnes, J.D., Panicucci, A. and Borland, A.M. (1995). Production and utilization of assimilates in wheat (*Triticum aestivum* L.) leaves exposed to elevated O₃ and/or CO₂. *New Phytologist* **129**: 557-568.

Barnes, J.D., Ollerenshaw, J.H. and Whitfield, C.P. (1995). Effects of elevated CO₂ and/or O₃ on growth, development and physiology of wheat (*Triticum aestivum* L.). *Global Change Biology* **1**: 129-142.

Buchenham, A.H., Parry, M.A., Whittingham, C.P. and Young, A.T. (1981). An improved open-top chamber for pollution studies on crop growth. *Environmental Pollution (Series B)* **2**: 475-482.

Cavagnaro, J.B., de Lis, B.R. and Tizio, R.M. (1971). Drought hardening of the potato plant as an after-effect of soil drought conditions at planting. *Potato Research* **14**: 181-192.

Clarke, B.B., Greenhalgh-Weidman, B. and Brennan, E.G. (1990). An assessment of the impact of ambient ozone on field grown potato crops in New Jersey using the EDU method: Part 1 – white potato (*Solanum tuberosum*). *Environmental Pollution* **66**(4): 351-360.

Cochran, W.G. and Cox, G.M. (1957). *Experimental Designs*. John Wiley and Sons, Inc., New York.

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

Donnelly, A. (1998). The interactive effects of elevated CO₂, elevated O₃ and nitrogen availability on spring wheat (*Triticum aestivum* L., cv. *MINARET*). Ph.D. thesis, University of Dublin, Ireland.

Eckardt, N.A. and Pell, E.J. (1996). Effects of ethylenediurea on ozone-induced acceleration of foliar senescence in potato (*Solanum tuberosum* L.). *Environmental Pollution* **92**(3): 299-306.

Farrar, J.F. and Williams, M.L. (1991). The effects of increased atmospheric carbon dioxide and temperature on carbon partitioning, source sink relations and respiration. *Plant Cell and Environment* **14**: 819-830.

Fiscus, E.L., Reid, C.D., Miller, J.E., Heagle, A.S. (1997). Elevated CO₂ reduces O₃-induced yield losses in soybeans: possible implications for elevated CO₂ studies. *Journal of Experimental Botany* **48**: 307-313.

Goudrian, J. and de Ruiter, H.E. (1983). Plant growth in response to CO₂ enrichment, at two levels of nitrogen and phosphorus supply. 1. Dry matter, leaf area and development. *Netherlands Journal of Agricultural Science* **31**: 157-169.

Heck, W.W., Adams, R.M., Cure, W.W., Heagle, A.E., Heggstad, H.E., Kohut, R., Kress, L.W., Rawlings, J.O. and Taylor, O.C. (1983). A reassessment of crop loss from ozone. *Environmental Science and Technology* **17**: 573a-581a.

Hough, A.M. and Derwent, R.G. (1990). Changes in the global concentration of tropospheric ozone due to human activities. *Nature* **344**: 645-648.

Houghton, J.T., Callander, B.A. and Varney, S.K. (1992). International Panel on Climate change; the supplementary report to the IPCC Scientific assessment. Cambridge University Press, Cambridge, 200pp.

Kimball, B.A. (1983). Carbon dioxide and agricultural yield: an assemblage and analysis of 430 prior observations. *Agronomy Journal* **75**: 779-782.

Kimball, B.A., Rosenberg, N.J., Allen, L.H., Heichel, G.H., Stuber, C.W., Kissel, D.E. and Ernst, S. (Eds) (1990). Impact of Carbon Dioxide, Trace Gases

and Climate Change on Global Agriculture. *American Society of Agronomy, Madison, USA.*

Korner, C.H. and Miglietta, F. (1994). Long term effects of naturally elevated CO₂ on Mediterranean grassland and forest trees. *Oecologia*, **99**: 343-351.

Lawlor, D.W. and Mitchell, R.A.C. (1991). The effects of ozone in ambient air on ribulose biphosphate carboxylase/oxygenase activity decreases photosynthesis and grain yield in wheat. *Journal of Plant Physiology* **130**: 189-200.

Lawlor, D.W. and Keys, A.J. (1993). Understanding photosynthetic adaption to changing climate. In: *Plant adaptation to environmental stress*, (Eds. L. Fowden, T. Mansfield and J. Stoddart), pp85-106, Chapman and Hall, London.

Lemon, E.R. (1983). CO₂ and Plants: The Response of Plants to Rising Levels of Atmospheric Carbon Dioxide. Westview Press, Boulder.

McKee, I.F., Farage, P.K. and Long, S.P. (1995). The interactive effects of elevated CO₂ and O₃ concentration on photosynthesis in spring wheat. *Photosynthesis Research* **45**: 111-119.

Miglietta, F., Magliulo, V., Bindi, M., Cerio, L., Vaccari, F.P., Loduca, V. and Peressotti, A. (1998). Free air CO₂ enrichment of potato (*Solanum tuberosum* L.): development, growth and yield. *Global Change Biology* **4**: 163-172.

McKee, I.F., Bullimore, J.F., and Long, S.P. (1997). Will elevated CO₂ concentrations protect the yield of wheat from O₃ damage? *Plant, Cell and Environment* **20**: 77-84.

Morison, J.I.L. (1989). Plant growth in increased CO₂. In: *Carbon Dioxide and Other Greenhouse Gases: Climatic and Associated Impacts* (Eds Fantechi R, Ghazi A), pp. 228-244. Kluwer Academic Publishers, Dordrecht.

Mortensen, L.M. (1990). Effects of ozone on growth of *Triticum aestivum* L. at different light, air humidity and CO₂ levels. *Norwegian Journal of Agricultural Sciences* **4**(4): 343-348.

Mott, K.A. (1988). Do stomata respond to CO₂ concentrations other than intercellular? *Plant Physiology* **86**: 200-203.

Mulchi, C., Rudorff, B., Lee, E., Rowland, R. and Pausch, R. (1995). Morphological responses among crop species to full-season exposures to enhanced concentrations of atmospheric CO₂ and O₃. *Water, Air and Soil Pollution* **85**: 1379-1386.

Mulholland, B.J., Craigon, J., Black, C.R., Colls, C.R., Atherton, J. and Landon, G. (1997). Effects of elevated carbon dioxide and ozone on the growth and yield of spring wheat (*Triticum aestivum* L.). *Journal of Experimental Botany*, **48**(306): 113-122.

Pell, E.J., Weissberger, W.C. and Speroni, J. (1980). Impact of ozone on quantity and quality of greenhouse grown potato plants. *Environmental Science and Technology* **14** (5): 568-571.

Pell, E.J., Pearson, N.S. and Vinten-Johansen, C. (1988). Qualitative and quantitative effects of ozone and/or sulphur dioxide on field grown potato plants. *Environmental Pollution* **53**: 171-186.

Plodowska, J.W., Jongebloed, P.H.J., Van De Sanden, P.A.C.M. and Struik, P.C. (1989). Effects of a short period of drought on changes in tuber volume and specific leaf weight. I. Long-term changes. *Potato Research* **32**: 245-253.

Polle, A., Pfirmann, T., Chakrabarti, S. and Rennenberg, H. (1993). The effects of enhanced ozone and enhanced carbon dioxide concentrations on biomass, pigments and antioxidative enzymes in spruce needles (*Picea abies* L.). *Plant, Cell and Environment* **16**: 311-316.

Rao, M.V., Hale, B. and Ormrod, D.P. (1995). Amelioration of ozone-induced oxidative damage in wheat plants grown under high carbon dioxide. *Plant Physiology* **109**: 421-432.

Reich, P.B., Schoettle, A.W. and Amundson, R.G. (1985). Effects of low concentrations of O₃, leaf age and water stress on leaf diffusive conductance and water use efficiency in soybean. *Physiologia Plantarum* **63**: 58-64.

Reinert, R.A. and Chen Ho, M. (1995). Vegetative growth of soybean as affected by elevated carbon dioxide and ozone. *Environmental Pollution* **89**: 89-96.

Riesmeier, J.W., Willmitzer, L. and Frommer, W.B. (1994). Evidence for an essential role of the sucrose transporter in phloem loading and assimilate partitioning. *Embo Journal* **13**: 1-17.

Schapendonk, A.H.C.M., Pot, C.S. and Goudriaan, J. (1995). Simulated effects of elevated carbon dioxide concentration and temperature on the productivity of potato. In: *Potato Ecology and Modelling of Crops under Conditions Limiting Growth* (Eds. Haverkort, A.J. and D.K.L. MacKerron), pp101-113. Kluwer, Amsterdam.

Schimel, D.S., Braswell, B.H., McKeown, R., Ojima, D.S., Parton, W. and Pulliam, W. (1996). Climate and nitrogen controls on the geography and timescales of terrestrial biogeochemical cycling. *Global Biogeochemical cycles* **10**: 677-692.

Schnyder, H. and Baum, U. (1992). Growth of the grain of wheat (*Triticum aestivum* L.). The relationship between water content and dry matter accumulation. *European Journal of Agronomy* **1**: 51-57.

Snedecor, G.W. (1962). Statistical Methods. Iowa State Library Press, Ames, Iowa.

Struik, P.C., Haverkort, A.J., Vreugdenhil, D., Bus, C.B. and Dankert, R. (1990). Manipulation of tuber size distribution of a potato crop. *Potato Research* **33**: 417-432.

Vozzo, S.F., Miller, J.E., Pursley, W.A. and Heagle, A.S. (1995). Effects of ozone and water deficit on field grown soybean. I. Leaf gas exchange. *Journal of Environmental Quality* **24**: 663-70.

Wheeler, R.M. and Tibbitts, T.W. (1997). Influence of changes in daylength and carbon dioxide on the growth of potato. *Annals of Botany* **79**: 529-533.

Wheeler, R.M., Tibbitts, T.W. and Fitzpatrick, A.H. (1991). Carbon dioxide effects on potato growth under different photoperiods and irradiance. *Crop Science* **31**: 1209-1213.

Wille, M.J. and Kleinkopf, G.E. (1992). Effect of simulated hail damage on yield and quality of russet burbank potatoes. *American Potato Journal* **69**: 705-714.