



Ozone effects on yield quality of spring oilseed rape and broccoli

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ABSTRACT

The impact of elevated tropospheric ozone (O_3) on the quality of spring oilseed rape (*Brassica napus* cv Ability) and broccoli (*Brassica oleracea* L. cv Italic cv Monaco) was assessed during a three year Open – Top Chamber (OTC) experiment. Current ambient O_3 levels were compared to an increase of 20 and 40 ppb during 8 h per day over the entire growing season. The qualitative responses were expressed as a function of the accumulated hourly O_3 concentrations over a threshold of 40 ppb (AOT40) and the phytotoxic O_3 dose above a threshold of $6 \text{ nmol s}^{-1} \text{ m}^{-2}$ projected leaf area (POD₆).

Our results provide clear evidence that O_3 has an influence on the qualitative attributes of the harvested products of these *Brassica* species. The responses were comparable whether they were expressed as a function of the accumulated O_3 concentrations or of the modelled O_3 uptake. The protein concentration of oilseed rape seeds and broccoli heads was significantly increased in response to O_3 . There was also a shift in the fatty acid composition of the vegetable oil derived from seeds of oilseed rape. Oleic acid (18:1) declined significantly ($p < 0.05$) in favour of linoleic acid (18:2) ($p < 0.01$). There was no change in the relative proportion of linolenic acid (18:3). The suppression of monounsaturated fatty acids ($p < 0.05$) coincided with a positive response of the % saturated fatty acids ($p < 0.05$). In rapeseed oil the observed decrease in vitamin E content was due to a reduction of γ -tocopherol (TOC, $p < 0.001$). α -TOC, the most active form of vitamin E in humans, was not influenced by O_3 . There was no change in the glucosinolate (GSL) content of oilseed rape seeds. In broccoli an important shift occurred from indolic to aliphatic GSLs although the total GSL concentration was not changed. The increase in the aliphatic/indolic GSL ratio ($p < 0.001$) may be important in relation to the anticarcinogenic properties of these vegetables. The vitamin C (ascorbate – ASC) and α -TOC concentrations of broccoli were not influenced by O_3 ; glutathione (GSH) was slightly increased in response to a higher O_3 uptake ($p < 0.05$).

The consequences of these changes with regard to food and feed quality and human health are discussed.

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1. Introduction

O_3 has proven to decrease crop production and change its quality, with the former aspect being more extensively studied in the past than the latter (Ashmore, 2005). The focus on quantitative yield changes could result in a misleading risk assessment and unreliable economic extrapolations (Shortle et al., 1988) especially in those cases where the quality of the harvested product is crucial for industrial processing and consumer's health. O_3 induced

changes in food and feed quality have been investigated in only a limited number of crops and most studies deal with carbohydrate and crude protein content. However, crop quality may also be affected by changes in secondary metabolism including increases in antioxidant scavenging systems within the tissue such as glutathione (GSH), vitamin C and E (Iriti and Faoro, 2009). At present, only very little information exists on shifts of secondary metabolites in the marketable yield products (grains, tubers, fruits, vegetables).

The objective of the present research was to investigate the impact of an increase of tropospheric O_3 concentrations on the quality of two economically important *Brassica* species: spring oilseed rape (*Brassica napus* L.) and broccoli (*Brassica oleracea* L. cv.

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Italica). World production of oilseed rape is growing rapidly, with estimates of 58.4 million tonnes in the 2010–2011 season (USDA, 2011). It is the third most important world source of vegetable oil (Lühs and Friedt, 1994) whilst the residual seed meal is used as feed supplement because of its high protein content. The seeds are rich in linoleic and linolenic acids, essential polyunsaturated fatty acids and precursors of the Omega 6 and Omega 3 fatty acid families. They are of vital nutritional importance since mammalian metabolism does not allow the build-up of these double bonds. In rapeseed oil, vitamin E occurs as a mixture of two predominant forms, α -TOC and γ -TOC, which differ in their bioactivity and antioxidant properties: α -TOC exhibits the greatest activity in the prevention of vitamin E deficiency abnormalities whereas γ -TOC plays a role in relation to oil stability (Gliszczynska-Swiglo and Sikorska, 2004).

For the human diet, representatives of the *Brassicaceae* are of particular importance as vegetables. In 2009 the world production of cauliflowers and broccoli reached over 19 million tonnes (FAOSTAT, 2011). The protective effect of cruciferous vegetables against cancer has been suggested to be partly due to their high content of glucosinolates (GSLs) which distinguish them from other vegetables (van Poppel et al., 1999). These GSLs are a group of sulphur-containing secondary plant metabolites, whose primary role in plants is defence, protecting plants from herbivores and stress (Sarkamis, 2009). GSLs are hydrophilic and remain in the press cake of oilseeds when these are processed and de-oiled. When rapeseed meal is used as feed supplement, they decrease digestibility and may cause goitre and haemolytic anaemia if supplemented at excessive rates (Stoewsand, 1995).

Historically, effects-related research for vegetation is based on the concentration of atmospheric O_3 , expressed as AOT40 (Accumulated O_3 concentrations over a Threshold of 40 ppb). In Belgium, the mean AOT40 accumulated in May, June and July ranged from 3.0 to 11.9 ppm h over the last 10 years (IRCEL-CELINE, 2011). Plant responses to O_3 are however more closely related to the internal O_3 dose in the leaf, or the instantaneous flux of O_3 through the stomata (e.g., Ashmore et al., 2004; Fuhrer, 2000; Mills, 2004). This flux-based approach requires mathematical modelling of the pathway of O_3 into the leaf including atmospheric, boundary layer and stomatal resistances resulting in the calculation of the so-called POD_x (Phytotoxic O_3 Dose above a threshold of $x \text{ nmol m}^{-2} \text{ s}^{-1}$ projected leaf area) (Op de Beeck et al., 2010). Such POD_x related responses also allow a more reliable extrapolation of the risk of O_3 damage to the vegetation over a wider range of climatic conditions. In this paper we explored both indices as a basis for the quantification of the O_3 effect on the quality of oilseed rape and broccoli.

2. Materials and methods

2.1. Experimental set-up

In 2007, 2008 and 2009 spring oilseed rape (*B. napus* cv Ability) and broccoli (*B. oleracea* L. cv Monaco) were exposed to different levels of ground-level O_3 in 15 OTCs located at Tervuren, approximately 12 km east of Brussels. Nine OTCs were used for oilseed rape and six for broccoli. Oilseed rape was exposed to unfiltered air (NF), NF + 20 ppb O_3 (NF+) and NF + 40 ppb O_3 (NF++). Plant spacing was 0.25 m between and 0.02 m within rows; sowing depth was 3 cm, plant density was approximately 120 plants per m^2 . Broccoli was sown and precultivated in the greenhouse. After 5–6 weeks the plants were planted in six OTCs at 50 cm spacing within and between the rows (4 plants per m^2). For broccoli only the lowest (NF) and the highest O_3 treatment (NF++) were applied. Each level of O_3 exposure was applied in three individual OTCs, each to be regarded as a true repetition, and the same experimental set-up was repeated over three growing seasons. Before sowing or planting, the plots were

fertilized with ammonium sulphate, triple phosphate, patent potassium, lime, sodium and borax, based on preliminary soil analysis and recommendations by the 'Soil Service of Belgium'. Depending upon the incidence of insects, snails and risks for fungal diseases, several plant protection products were applied: Ortiva (azoxystrobin), Geyser (difenoconazole), Dursban (chlorpyrifos-ethyl), Karate (lambda-cyhalothrin), Patriot (deltamethrin) and Mesurol Pro (methiocarb). The application always occurred at the same dose in all treatments and with respect to the prescribed frequency and waiting times before sampling and harvest. Underground irrigation was activated when tensiometers indicated a soil water suction of more than 0.5 bar. O_3 addition was performed daily between 11:00 h and 19:00 h (local time). O_3 was produced from pure oxygen with an O_3 generator (CMG 5–4, Innovatec, Rheinbach, Germany) and the supply was adjusted with mass flow controllers (models 5850 TR, Brooks Instrument B.V., Fisher-Rosemount, The Netherlands). The O_3 concentration (Environment S.A. model O3 41M), air temperature (Pt 1000), soil temperature (thermocouple Pt 1000), vapour pressure deficit (dry and wet bulb temperature difference, Pt 1000) and global radiation (pyranometer sensor LI-2000SA, LiCor, USA) were continuously monitored both in and outside the OTCs. Details on climatic conditions and O_3 concentrations for the different growing seasons, species and treatments are given in Table 1 (see also De Bock et al., 2011). Between the chambers, the standard deviation for the 8 h average O_3 exposure varied between 0.5 and 4.2 ppb, and between 0.04 and 1.99 ppm h for the AOT40. Fig. 1 represents the 8 h O_3 average in the NF treatment for each growing season.

At the end of the growing season, in each chamber 6 to 9 subplots of oilseed rape of 1 m row length were harvested. The seeds were threshed at the APPO (Association pour la Promotion des Protégéagineux et des Oléagineux) in Gembloux (Belgium). Seed samples were purified and stored at 6 °C for further quality analysis. For broccoli 12 plants per chamber were harvested. Fresh samples of the broccoli florets were immediately frozen in liquid nitrogen and stored at –80 °C.

2.2. Quality analyses

2.2.1. Glucosinolates

Sample preparation and analysis were performed according to the (slightly modified) International Standard ISO 9167-1 (1992). For oilseed samples, 200 mg of air dried seeds were used; for broccoli florets 1 g fresh material was used. The samples were ground in

Table 1

Climatic variables and O_3 data in the OTCs for growing seasons 2007, 2008 and 2009. Mean values are given for air temperature, soil temperature, vapour pressure deficit and global radiation. Total accumulated O_3 exposure and uptake from emergence (oilseed) or planting (broccoli) until harvest are expressed as AOT40 and POD_6 .

Variable	Oilseed rape			Broccoli		
	2007	2008	2009	2007	2008	2009
Air temperature (°C)	16.6	16.5	16.0	17.3	17.4	18.5
Soil temperature (°C)	16.3	15.6	15.1	16.6	16.4	17.4
Vapour pressure deficit (kPa)	0.41	0.47	0.56	0.42	0.45	0.64
Global radiation ($\text{MJ m}^{-2} \text{ d}^{-1}$)	11.8	14.3	14.4	12.8	14.1	15.6
8 h avg [O_3] NF (ppb)	30	33	32	29	29	32
8 h avg [O_3] NF+ (ppb)	46	53	48			
8 h avg [O_3] NF++ (ppb)	63	73	63	58	63	66
AOT40 NF (ppm h)	0.71	2.11	1.51	0.38	0.74	1.28
AOT40 NF+ (ppm h)	5.66	10.74	10.95			
AOT40 NF++ (ppm h)	14.97	24.11	23.51	8.94	12.85	15.21
POD_6 NF (mmol m^{-2})	8.22	13.59	17.10	7.27	8.22	16.94
POD_6 NF+ (mmol m^{-2})	20.06	23.53	29.13			
POD_6 NF++ (mmol m^{-2})	31.29	38.08	43.72	24.37	28.89	40.14

NF: Non Filtered air; NF+, NF++: Non Filtered air with additional O_3 ; avg: average; AOT40 accumulated ozone exposure over a threshold of 40 ppb; POD_6 : Phytotoxic Ozone Dose over a threshold of $6 \text{ nmol m}^{-2} \text{ s}^{-1}$.

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