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Ozone dose–response relationships for spring oilseed rape and broccoli

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ABSTRACT

Tropospheric ozone is an important air pollutant with known detrimental effects for several crops. Ozone effects on seed yield, oil percentage, oil yield and 1000 seed weight were examined for spring oilseed rape (*Brassica napus* cv. Ability). For broccoli (*Brassica oleracea* L. cv. Italica cv. Monaco) the effects on fresh marketable weight and total dry weight were studied. Current ozone levels were compared with an increase of 20 and 40 ppb during 8 h per day, over the entire growing season. Oilseed rape seed yield was negatively correlated with ozone dose indices calculated from emergence until harvest. This resulted in an R^2 of 0.24 and 0.26 ($p < 0.001$) for the accumulated hourly O_3 exposure over a threshold of 40 ppb (AOT40) and the phytotoxic ozone dose above a threshold of 6 nmol $m^{-2}\,s^{-1}$ (POD_6) respectively. Estimated critical levels, above which 5% yield reduction is expected, were 3.7 ppm h and 4.4 mmol m^{-2} respectively. Our results also confirm that a threshold value of 6 nmol $s^{-1}\,m^{-2}$ projected leaf area, as recommended for agricultural crops (UNECE, Mills, 2004), can indeed be applied for spring oilseed rape. The reduction of oilseed rape yield showed the highest correlation with the ozone uptake during the vegetative growth stage: when only the first 47 days after emergence were used to calculate POD_6, R^2 values increased up to 0.476 or even 0.545 when the first 23 days were excluded. The highest ozone treatments, corresponding to the future ambient level by 2100 (IPCC, Meehl et al., 2007), led to a reduction of approximately 30% in oilseed rape seed yield in comparison to the current ozone concentrations. Oil percentage was also significantly reduced in response to ozone ($p < 0.001$). As a consequence oil yield was even more severely affected by elevated ozone exposure compared to seed yield: critical levels for oil yield dropped to 3.2 ppm h and 3.9 mmol m^{-2}. For broccoli the applied ozone doses had no effect on yield.

1. Introduction

Tropospheric ozone (O_3) is the third most important greenhouse gas and its concentration is still increasing. Simulations for the period 2015 through 2050 predict increases in tropospheric O_3 of up to 25% (IPCC, Meehl et al., 2007). O_3 has phytotoxic properties and represents a risk for natural vegetation and crops (Fuhrer et al., 1997; Pleijel et al., 2004). Critical exposure levels to prevent O_3 damage to vegetation were set by a European Directive (EC 2002/03). These have been exceeded all over Europe on several occasions in the last decade, with the highest concentrations appearing in the Mediterranean region (Gerosa et al., 1999; Pleijel et al., 2000). The detrimental effects of O_3 have already been confirmed for several agricultural crops (Mills et al., 2007). O_3 can cause visible leaf injury and the oxidative stress may induce defensive signal transduction responses (Bergmann et al., 1999; Faoro and Iriti, 2005). Photosynthetic activity may decrease and in general, senescence is enhanced in plants (Dawnay and Mills, 2009).

This study focuses on the impact of O_3 on the yield of two *Brassica* species: spring oilseed rape (*Brassica napus* L.) and broccoli (*Brassica oleracea* L. cv. Italica). Rapeseed meal is used as feed supplement because of its high protein content. The seeds are also an important source of vegetable oil, both for human consumption and as a biofuel. These are the main reasons for its cultivation worldwide (Stanton et al., 2010). Broccoli is part of the human diet,

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where it is an important source of vitamins. Broccoli has been attributed anticarcinogenic properties linked to the high concentration of glucosinolates, although the mechanisms are still unclear (Sarikamis, 2009; Steinbrecher et al., 2009). Both species have been considered moderately sensitive to O_3 with spring oilseed rape being more sensitive compared to broccoli (Mills et al., 2007).

Detrimental effects of O_3 on the photosynthetic capacity can influence agricultural productivity and cause economic losses (Wang et al., 2005; Mills et al., 2007). In Europe, the O_3 dose index AOT40 (the accumulated O_3 concentration over a threshold of 40 ppb) has been widely used to obtain dose–response relationships e.g. for winter oilseed rape (Wang et al., 2008), grapevine (*Vitis vinifera*) (Soja et al., 2004), sugar beet (*Beta vulgaris*), (De Temmerman et al., 2007) and soybean (*Glycine max*) (Jaoudé et al., 2008). For wheat (*Triticum aestivum*) (Pleijel et al., 2000), potato (*Solanum tuberosum* cv Bintje) (Craigon et al., 2002; Pleijel et al., 2002; Vandermeiren et al., 2005), barley (*Hordeum vulgare*) (Gerosa et al., 2004) and lettuce (*Lactuca sativa* L.) (Goumenaki et al., 2007). However, O_3 damage was more significantly correlated with the accumulated O_3 uptake which is the actual O_3 dose absorbed through the stomata (Pleijel et al., 2000; Sofiev and Tuovinen, 2001). Yield losses for wheat of up to 30% were estimated based on calculations of the O_3 flux in the Yangtze Delta (Wang et al., 2005). Ozone uptake is correlated to stomatal conductance and, consequently, it is dependent upon factors such as several environmental conditions, phenological parameters and plant species (Ashmore et al., 2004). Especially in the Mediterranean region where water availability is low and vapour pressure deficit (VPD) is higher, O_3 impact on vegetation could be overestimated by the AOT40 due to reduced stomatal uptake (Pleijel et al., 2000; Zlatev et al., 2001; Khan and Soja, 2003).

Even though flux-based indices may be more biologically relevant than concentration-based indices, they do not directly account for the fact that plants have the potential to neutralise part of the O_3 that is absorbed through the stomata (Massman et al., 2000). Hypothetically, the use of a threshold flux under which the O_3 uptake is not added to the total dose, might serve as a surrogate for a plant's biochemical resistance to O_3 stress. This often leads to an improvement of the dose–response relationship e.g. for potato yield losses, the best correlation was found at a threshold of 7 nmol m^{-2} s^{-1} PLA (projected leaf area) (Pleijel et al., 2002), for wheat also good correlations were found when no threshold was used (Pleijel et al., 2000). A threshold of 6 nmol m^{-2} s^{-1} has been suggested for all agricultural crops (UNECE, Mills, 2004) and the O_3 dose index is currently defined as the phytotoxic O_3 dose above a threshold of 6 nmol m^{-2} s^{-1} PLA (POD_6). Only for wheat and potato sufficient data were available to define POD_6-based critical levels of 1 and 5 mmol m^{-2} PLA respectively, above which 5% yield loss may be expected (UNECE, Mills, 2004; Paoletti and Manning, 2007). Although flux-based approaches have proven to be more accurate in predicting O_3 effects, a good correlation exists between these O_3 dose indices and AOT40, as O_3 concentration is the most determinant factor in both calculations (Cieslik, 2004; Paoletti et al., 2007).

The objective of this paper is to correlate the yield response of spring oilseed rape and broccoli to different O_3 dose and exposure indices: Phytotoxic O_3 dose (POD_x) at different threshold levels is compared to AOT40. The timing of the O_3 doses in relation to the susceptibility of the plant is also taken into account by testing the dose–response correlation over different time frames from plant emergence until harvest. Several studies have indeed established empirically that there exist phenological variations in sensitivity, which may be related to changes in O_3 uptake, capacity for detoxification or assimilate partitioning during plant growth (Younglove et al., 1994; Pleijel et al., 1998).

2. Materials and methods

2.1. Experimental set-up

During three consecutive growing seasons (2007–2009) plants were exposed to O_3 in open-top chambers (OTCs) located at Tervuren, approximately 12 km east of Brussels (50°50′0″ N and 4°31′22″ E, 80 m above sea level). The soil was a well-drained heavy loam, rich in nutrients (De Temmerman et al., 2007).

Nine OTCs were used to cultivate spring oilseed rape (*B. napus* L. cv. Ability) and six OTCs were used for broccoli (*B. oleracea* L. cv. Italica cv. Monaco). The hexagonal OTCs were 3 m in diameter, resulting in a soil surface of 7.35 m^2; the height was 2.8 m. Oilseed rape was exposed to three different levels of O_3: non-filtered ambient air (NF) and non-filtered air with addition of 20 and 40 ppb O_3 (NF+, NF++). For broccoli only the lowest (NF) and the highest O_3 treatment (NF++) were applied. Ozone 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 O_3 41 M), air temperature (Pt 1000), soil temperature (thermocouple Pt 1000), VPD (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 chambers.

Average air temperature, soil temperature, VPD, global radiation, O_3 concentration (8 h average), AOT40 and POD_6 for the different growing seasons, species and treatments are shown in Table 1. For spring oilseed rape O_3 concentrations were considerably higher in 2008 and 2009 compared to 2007, as was global radiation. Nevertheless the highest mean air and soil temperature were measured in this first growing season; between 2008 and 2009 only small differences in environmental conditions were observed. For broccoli O_3 concentrations were most elevated in 2009, as were global radiation, VPD, soil and air temperature. In 2008 global radiation and O_3 concentrations were higher compared to 2007.

Fertiliser was applied according to the recommendations based on the soil analysis by the Soil Service of Belgium. Spring oilseed rape was sown in full soil on 18 April 2007, 10 April 2008 and 1 April

Table 1
Climatic variables in the open top chambers for the growing seasons of 2007, 2008 and 2009 for oilseed rape and broccoli. Mean values are given for air temperature, soil temperature, vapour pressure deficit and global radiation. Total values are given for 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
VPD (kPa)	0.41	0.47	0.56	0.42	0.45	0.64
GR (MJ m^{-2} 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; VPD: Vapour pressure deficit; GR: Global radiation; avg: average; AOT40 accumulated ozone exposure over a threshold of 40 ppb; POD_6: Phytotoxic Ozone Dose over a threshold of 6 nmol m^{-2} s^{-1}.