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# Chronic tropospheric ozone exposure reduces seed yield and quality in spring and winter oilseed rape

Hattie R. Roberts[a,*], Ian C. Dodd[a], Felicity Hayes[b], Kirsti Ashworth[a,*]

[a] Lancaster Environment Centre, University of Lancaster, Bailrigg, Lancaster LA1 4YQ, UK

[b] UK Centre for Ecology and Hydrology, Environment Centre Wales, Deiniol Road, Bangor, Gwynedd LL57 2UW, UK

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ABSTRACT

Oilseed rape (*Brassica napus* L.) is cultivated worldwide, producing 11.5% of global oilseeds at an economic value of 38 billion USD in 2020. It is sensitive to phytotoxic damage from exposure to tropospheric ozone ($O_3$), a major air pollutant, which disrupts plant physiological processes and thus decreases biomass accumulation. As background ozone concentrations continue to increase globally, we investigated the impact of ozone exposure on seed and oil yield of a shorter-lived spring (cv. Click) and a longer-lived winter (cv. Phoenix) oilseed rape cultivar to ozone levels (treatments with peaks of 30, 55, 80, 110 ppbv) representative of typical European conditions where these cultivars are common. Thousand Seed Weight (TSW), an important measure of final yield, decreased more in Phoenix (40%) than Click (20%) with increasing ozone exposure. Click produced more racemes and many small seeds while Phoenix produced fewer racemes and larger seeds. However, seed quality declined more substantially in Click than Phoenix. The oil content in Click's seed significantly decreased with increased ozone exposure, while less desirable components (moisture, chlorophyll, ash) increased. Scaled to field-level, our findings imply substantial economic penalties for growers, with potential losses of 175–325 USD $ha^{-1}$ in Click and 500–665 USD $ha^{-1}$ in Phoenix under ozone concentrations typical of spring and summer periods in Europe. Decreased total yield would likely outweigh the benefits of any improvement in animal oilseed cake quality (increased protein and key micronutrients for livestock feed). Neither cultivar sustained visible injury at earlier growth stages, and Phoenix sustained photosynthesis even under high exposure, thereby making ozone an invisible threat. Our findings of reduced oilseed quantity and quality threaten oilseed rape production, but differences between the cultivars may also offer an opportunity for breeders and agronomists to identify and exploit variation in ozone tolerance in oilseed rape.

## 1. Introduction

Canola or oilseed rape (hereafter OSR) is the second-most economically important oilseed crop on the planet after soya, and the most important in Europe, where over 16.8 million tonnes were produced in 2020, representing 60% of total oilseed yields (European Commission, 2020). Global production of rapeseed oil exceeded 27.7 million metric tonnes in 2020, with a market worth ~24 billion USD, while soya's market produced 60.3 million tonnes of oil worth ~55 billion USD (USDA, 2021). Moreover, the oilseed cake or protein meal, left once OSR is crushed to remove edible oil, is produced as a valuable global animal feedstock. In 2020, worldwide OSR-derived animal feed totalled 39.2 million tonnes, at a market value of ~14 billion USD, with Europe generating a third of both global OSR oil and protein meal (USDA, 2021).

Understanding the effects of changes in environmental conditions on key crops such as oilseed rape has become of significant interest for agronomists, crop breeders and policy makers to reduce crop losses and risks to food security. One important but often overlooked environmental stress is tropospheric ozone. Average global ozone concentrations have increased by ~20% since 1900, and are projected to increase by a further 18% by 2100 (Young et al., 2013; Archibald et al., 2020). Increased emissions of ozone precursors, along with rises in global temperature, have resulted in average European background concentrations exceeding 30 ppb annually (Archibald et al., 2020; Boleti et al., 2020). Daytime concentrations between 50–80 ppb in Northern Europe and >100 ppb in Central and Southern Europe have been recorded in rural areas over spring-summer periods (Pay et al., 2019; Boleti et al.,

* k.s.ashworth1@lancaster.ac.uk
*E-mail address:* h.roberts7@lancaster.ac.uk (H.R. Roberts).

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2020), which coincide with key growing dates in the agricultural calendar (Mills et al., 2018a). While episodic high- ozone events (acute exposure) have long been recognised to trigger phytotoxic damage to vegetation (e.g. Heggestad and Middleton, 1959), there is increasing awareness of the impacts of cumulative, chronic exposure to lower levels of ozone (Chen et al., 2009; Mishra and Agrawal, 2015). Under current atmospheric conditions in Europe, OSR crops are exposed to levels of ozone over days, weeks, or entire growing seasons likely to be sufficiently high to reduce yields (Lei et al., 2012; Lin et al., 2020; Mills et al., 2007; Mills et al., 2018b).

Tropospheric ozone has well-documented detrimental effects on crop physiology, due to its highly oxidising properties. Ozone enters leaves (mostly) via the stomata, resulting in cellular damage and disruption of photosynthetic pathways in ozone-sensitive species, decreasing net photosynthetic rate ($P_{net}$) (Bohler et al., 2007). Oxidation of cellular and organelle membranes also occurs, resulting in foliar chlorosis, and accelerated senescence (Tammam et al., 2019; Sharps et al., 2021). Direct damage of stomata and guard cells can also occur, leading to loss of stomatal regulation at chronic exposures of more than 40 ppbv above ambient (Mills et al., 2009), potentially exacerbating the impact. Consequently, overall productivity, and crop yields decrease in ozone-sensitive species.

Previous studies using open top chambers, free air systems, and field trials have shown OSR to be a moderately ozone-sensitive species (Mills et al., 2007), with ozone concentrations higher than 60 ppb decreasing seed yield by 15–38% and oil content by 5% (Ollerenshaw et al., 1999; Clausen et al., 2011; Namazkar et al., 2016). Experiments at both plot- and field-scale observed decreased thousand seed weight (TSW), and decreased oil content (Black et al., 2000; De Bock et al., 2011; Frenck et al., 2011; Vandermeiren et al., 2012), suggesting that ozone exposure affects crop quality as well as yield. Seed content of valuable compounds, primarily oil (for food and industrial processing) and protein (for fodder in the form of oilseed cake) may decrease by >18% in response to ozone stress as observed in OSR relatives (Singh et al., 2013). Fatty acid proportions may also be affected, with increases observed in erucic acid content (Tripathi et al., 2012), which is tightly regulated to less than 2% to avoid cardio myotoxicity in both livestock and humans (EFSA Panel on Contaminants in the Food, 2016). Furthermore, exposure to ozone may exacerbate unfavourable properties in the extracted oil, including increased moisture (>10%), chlorophyll (>20%), and glucosinolates (>3 mg/g), affecting shelf life, appearance, or palatability of edible oil (Wittkop et al., 2009). Micronutrient contents in seed cake maintain optimum livestock health, and key elements such as zinc, manganese, and iron have been observed to decrease under other abiotic stresses such as drought (Etienne et al., 2018), but have not been reported in response to ozone stress.

In Europe, OSR comprises two seasonal groupings: spring (over an area of 14,000 ha in the UK in 2020, which has tripled compared to previous four years) and winter-sown varieties (331,000 ha in the UK in 2020) (Butruille et al., 1999; DEFRA, 2020). Winter varieties are sown in mid-August to early September, harvested in July to August, and are the primary type grown in Europe. Spring varieties are sown in late March to early April, harvested in late August to September, and grown throughout Europe and Canada (AHDB, 2020). Spring varieties are faster-growing and have shorter lifespans than their winter counterparts. Previous studies on other species suggest those with shorter life cycles are more susceptible to ozone damage (Franzaring et al., 2000). It is postulated that short-lived plants that are bred for rapid growth have higher rates of leaf gas exchange over their life cycle, and therefore may be exposed to greater abiotic stress such as higher ozone uptake (Felzer et al., 2007), resulting in greater sensitivity to ozone (as in Osborne et al., 2016). Fast-growing spring OSR could therefore become economically unviable if exposure to high ozone levels substantially reduces yield or quality. In this study, we compare two modern cultivars of spring and winter OSR, to examine whether their physiological, morphological and agronomic responses to ozone exposure differ over their full life cycles, and test three specific hypotheses:

(i) Seed yield and quality will decrease in both cultivars as ozone exposure increases.
(ii) Seed yield and quality declines will reflect decreased physiology and biomass accumulation.
(iii) Decreases will be more pronounced in the spring cultivar and will occur at lower exposures.

Here we used semi-controlled environments in geodesic glasshouses and a bespoke ozone injection system to expose OSR to four different concentrations of ozone over a full growing season. This is the first study to directly compare the responses of spring and winter varieties of OSR to chronic ozone exposure over a growing season at realistic levels of ozone experienced in Europe, providing valuable information to growers on OSR yield and quality.

## 2. Materials and method

### 2.1. Plant material and care

Spring (cv. Click) and winter (cv. Phoenix) *Brassica napus* cultivars (supplied by DSV United Kingdom Ltd., Top Dawkins Barn, Wardington, Banbury, UK) were vernalised for 4 °C; for 14 days at 65%RH prior to being transplanted in bedding packs in John Innes no. 2 soil on 5th May 2019 in a glasshouse at the UK CEH Bangor experimental Henfaes Farm, Abergwyngregyn. Seedlings were transferred after three weeks into individual 6.5 L (28 cm H, 21 cm D) pots in John Innes no. 2 compost. Two weeks later, when plants had six fully unfolded leaves (growth stage 16), the middle 40 plants by size per cultivar were selected and divided between the 4 treatments using stratified randomisation. Plants were watered daily during late afternoon, and fertiliser (Phostrogen All Purpose Plant Food) and pesticide (Provanto systemic fruit and vegetable bug killer) applied as a soil drench 21, 35, 49 days after sowing to both varieties according to manufacturer's instructions, with an additional treatment at 70 days to Phoenix.

### 2.2. Experimental site and Solardome system

Ten plants per cultivar were placed in four ozone fumigation treatments conducted within separate geodesic glasshouses (dimensions 3 m D × 2.1 m H; Solardome Industries Ltd, Unit 4, Yeomans Ind Park, Nursling, UK) at Abergwyngregyn (53.23°N, -4.02°W). The computer-controlled injection system (Lab VIEW, version 8.6, National Instruments, Austin, Texas, USA) mixes a regulated flow of ozone from an ozone generator (Dryden Aqua G11, Edinburgh, UK) attached to an oxygen concentrator (Sequal 10, Pure $O_2$, Manchester, UK) with carbon-filtered air. An external fan circulated ozone-enriched air into the domes at a total flow rate of two changes per minute ($m^3$ $min^{-1}$). Ozone concentrations in each dome are recorded every 30 min using two ozone analysers with matched calibration (EnviroTech API 400A, St Albans, UK). Other environmental conditions in the domes were otherwise uncontrolled; temperature, PAR, and relative humidity were automatically measured and logged every five minutes.

### 2.3. Ozone treatments

Ozone was injected into each dome between ~9 am and 7 pm 5 days per week, to achieve a stepped diurnal profile of 20–30 ppbv elevated to the specified concentration during day (see Fig. S1 in Supplementary Information). Daytime levels of ozone in each of the Solardomes were chosen to represent realistic European ozone levels, as showxn in Table 1. Exposure commenced on 7th June, 2019 (growth stage 16) and continued until harvest: 90 days for Click, and 125 days for Phoenix.

Cumulative ozone exposure ($CEO_3$) for each treatment was calculated following Lombardozzi et al. (2013), such that:

**Table 1**
Ozone treatments used to represent spring/ summer ozone concentrations by region.

| 30 ppbv | 55 ppbv | 80 ppbv | 110 ppbv |
|---|---|---|---|
| Background; N. Europe[1] | Background; S. Europe[1] | Elevated; N. Europe[2] | Elevated; S. Europe[2] |

Background (daytime average) and elevated (daytime average) chronic tropospheric ozone concentrations used in the present study. As in
[1] Boleti et al. (2020).
[2] Pay et al. (2019). N. Europe = northern Europe; S. Europe = southern Europe.

$$CEO_3(\text{mmol mol}^{-1}h) = [O_3] \times H \times D \times 10^{-6}$$

where $[O_3]$ is ozone concentration in ppbv, $H$ is number of hours, and $D$ number of days.

### 2.4. Physiological and environmental sampling

Physiological and environmental measurements were carried out three times over the growing season for Click and four times for Phoenix using four randomly selected plants (with the same plants used for seed quality analyses). Each time, net photosynthesis rate ($P_{net}$), stomatal conductance ($g_s$), and chlorophyll content of the youngest fully expanded leaves were measured between 10am – 4pm daily (with sampling randomised over treatments), from three replicates per treatment. A handheld Soil Plant Analysis Development (SPAD) meter (CCM 200; Opti-sciences, Hudson, New Hampshire, USA) provided a relative measure of chlorophyll content. In addition to $P_{net}$ and $g_s$, leaf temperature, relative humidity, and Vapour Pressure Deficit (VPD) were logged and trace gas samples were collected over a 20 min period using a LI-COR 6400XT (LI-COR Biosciences, Lincoln, Nebraska, USA) using a 2 × 3 cm LED chamber head. Experimental conditions within the chamber head were set to 400 ppm $CO_2$, 1000 μmol $m^{-2}$ $s^{-1}$ PAR, and 20 °C leaf temperature at a 500 mmol $sec^{-1}$ flow rate. A hand-held ThetaProbe (Delta-T Devices Ltd., Cambridge, UK) was used to measure soil moisture of the surface soil to 6.5 cm depth, to determine that plants were well-watered prior to measurement.

### 2.5. Yield parameters (seed quantity)

Plants were harvested when siliques had completely ripened and dried, and leaves had senesced and abscised (90 days after the start of exposure in Click and 125 days in Phoenix). This maximised the number of plants that reached seed yield for subsequent analysis. Dried siliques were picked and placed into paper envelopes (one raceme per envelope), and number of racemes per plant, number of siliques per raceme, number of seed per silique, thousand seed weight, and total seed mass per plant were recorded.

### 2.6. Seed quality analysis

Oil, protein, chlorophyll, ash, moisture, and glucosinolate content, and fatty acid composition of the harvested seed were determined by Near Infrared (NIR) spectroscopic analysis (DA 7250, Perten Instruments AB, SE-126 09 Hägersten, Sweden) at John Innes Centre, East Anglia, UK. Micronutrient and macronutrient contents (nitrogen, phosphorus, potassium, sulphur, magnesium, N:S Ratio, copper, manganese, zinc, boron, and iron) was determined by grain suite analyses by Natural Resource Management Centre (Cawood Scientific Limited, Bracknell, Berkshire, UK).

### 2.7. Statistical analysis

Data were compiled in Microsoft Excel (Microsoft Corporation, 2018. Microsoft Excel), and interrogated in R Studio (Version 1.2.5033, RStudio Team (2019); RStudio: Integrated Development for R. RStudio, Inc., Boston, MA, USA). Morphological, physiological and seed quality parameters were tested against fixed factors of cultivar and cumulative ozone exposure. After testing for normal distribution and homogeneity of variances, curvilinear and linear models with lowest Akaike information criterion (AIC) values were used to determine effects of ozone exposure on physiology, morphology and seed yield/ quality within cultivars. Analyses of covariance (ANCOVA) were used to explain the effects of cumulative ozone exposure and cultivar. Two-sample T-tests on quality parameters were conducted for the highest and lowest ozone treatments.

### 2.8. Economic assessment

Ozone-induced economic loss was estimated using the four-year UK average (2017–2020) yield of spring and winter OSR (2.9 and 3.3 t $ha^{-1}$, respectively), and a yield loss derived from our TSW measurements for 80 ppbv and 110 ppbv treatments taking 30 ppbv as the zero-loss baseline. The 4-year (2017–2020) AHDB average OSR price per tonne (466.26 USD) was converted into a value per hectare. In line with industry practice, a premium of 1.5% increment above baseline selling price was assumed for every 1% oil content above 40% (Federation of Oils, Seeds and Fats Associations Ltd (FOSFA) document 26A), as presented in Table 2.

## 3. Results

### 3.1. Pre-harvest data

Net photosynthesis ($P_{net}$) significantly decreased with increasing ozone exposure in both varieties. However, it decreased to a greater extent (by 53%) in Click, between 30 and 110 ppbv, than in Phoenix (18% - Fig. 1a). $P_{net}$ dropped more substantially, by 67% in Click and 47% in Phoenix, from the commencement of flowering (Day 21 for Click corresponding to $CEO_3 = 0.025$ mmol $mol^{-1}$ $h$ and Day 56 for Phoenix at $CEO_3 = 0.049$ mmol $mol^{-1}$ $h$) in the 110 ppbv treatment. Initial stomatal conductance ($g_s$) in Click was twice that of Phoenix at (0.66 and 0.32 mol $m^{-2}$ $s^{-1}$, respectively) as shown in Fig. 1b. Similarly, $g_s$ significantly decreased with increasing ozone exposure in Click, but only weakly in Phoenix. In Click, $g_s$ decreased by 77% (from 0.66 to 0.29 mol $m^{-2}$ $s^{-1}$ between 30 and 110 ppbv). Again, $g_s$ decreased more once flowering commenced under 110 ppbv, by 46% in Phoenix and 70% in Click. $P_{net}$ and $g_s$ decreased more significantly at a lower cumulative exposure in Click than Phoenix. Taken together, leaf gas exchange of the spring cultivar Click was more sensitive to ozone exposure than the winter cultivar Phoenix.

Decreased leaf gas exchange ($P_{net}$ and $g_s$) appeared to follow decreases in leaf chlorophyll content. Both varieties presented similar linear relationships between $P_{net}$ and chlorophyll content, and $g_s$ and chlorophyll content with lower values in Click than Phoenix at 110 ppbv (Fig. 2a,b). Hence, decreased chlorophyll content (indicative of increased senescence) was associated with both $P_{net}$ and $g_s$.

### 3.2. Chlorophyll content

Chlorophyll content responded differently to ozone exposure between seeds and foliage, and between cultivars (Fig. 3). As outlined above, leaf chlorophyll content in the youngest, fully expanded leaf significantly declined with increasing ozone exposure in both varieties, but to a greater extent in Click (83.4% between 30 and 110 ppbv) than Phoenix (40.8%). By contrast, seed chlorophyll content significantly increased with ozone exposure in Click, and was 3 times higher under 110 ppbv than 30 ppbv. Although Phoenix received the highest cumulative exposure, nearly double that of Click's ($CEO_3$ = 0.032 mmol $mol^{-1}$ $h$ vs 0.017 mmol $mol^{-1}$ $h$ under the 110 ppbv treatment), seed

**Table 2**
Economic assessment outputs based on thousand seed weight (TSW), 4-year UK yields and delivered prices (2017–2020).

| Variety | Ozone treatment (ppbv) | Average UK yield (2017–2020) | TSW | TSW change (%) | Yield (pots to field scale) (t ha$^{-1}$) | Delivered prices (USD t$^{-1}$ 2017–2020) | Oil content (%) | FOFSA oil premium (%) | Price increase (%) | USD ha$^{-1}$ increase | Price + oil premium (USD t$^{-1}$) | Total price (USD ha$^{-1}$) | Price change from 30ppbv (USD ha$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Click | 30 | 2.1 | 2.9 | | 2.1 | 466.3 | 48.1 | 8.1 | 12.1 | 1.1 | 522.9 | 1092.9 | |
| (Spring) | 55 | | 3.5 | +17.7 | 2.5 | | 48.2 | 8.2 | 12.3 | 1.1 | 523.6 | 1287.9 | +195.0 |
| | 80 | | 2.6 | -12.2 | 1.8 | | 44.9 | 4.9 | 7.3 | 1.1 | 500.5 | 918.0 | -174.9 |
| | 110 | | 2.3 | -21.4 | 1.6 | | 40.9 | 0.0 | 0.0 | 1.0 | 466.3 | 765.7 | -327.2 |
| Phoenix | 30 | 3.3 | 7.2 | | 3.3 | | 44.0 | 4.0 | 6.0 | 1.1 | 494.2 | 1643.3 | |
| (Winter) | 55 | | 5.6 | -21.9 | 2.6 | | 42.8 | 2.8 | 4.2 | 1.0 | 485.8 | 1262.2 | -381.1 |
| | 80 | | 5.1 | -29.5 | 2.3 | | 43.0 | 3.0 | 4.5 | 1.0 | 487.2 | 1141.7 | -501.6 |
| | 110 | | 4.3 | -40.4 | 2.0 | | 43.9 | 3.9 | 5.8 | 1.1 | 493.5 | 978.2 | -665.1 |

UK average yield and delivered prices derived between 2017–2020 (AHDB, 2020). Oil premium prices calculated from industry practice in line with international guidelines (Federation of Oils, Seeds and Fats Associations Ltd. document 26A).

chlorophyll content did not significantly differ, fluctuating between 6.7–7.9 ppm across all treatments. Taken together, seed and foliar chlorophyll content of Click was more responsive to ozone exposure than Phoenix.

### 3.3. Seed yield and quality

Thousand seed weight (TSW) was significantly lower in Click, the faster-growing spring cultivar, than Phoenix for all treatments (Fig. 4a). At 30 ppbv of ozone, TSW differed by a factor of 2.5 (2.9 g (1000 seeds)$^{-1}$ vs 7.2 g (1000 seeds)$^{-1}$) whereas the smallest difference (~2.0 g (1000 seeds)$^{-1}$) between cultivars occurred under exposure to 55 ppbv of ozone. TSW significantly decreased with increasing ozone concentration in both varieties between 30 and 110 ppbv, by 40% in Phoenix and 20% in Click. TSW decreased at the same rate in both varieties between cumulative exposures of ~0.07 mmol mol$^{-1}$ *h* and ~0.11 mmol mol$^{-1}$ *h*. Although TSW of Phoenix was more sensitive to ozone exposure than Click, TSW remained higher for the winter cultivar under all treatments.

Total seed mass per plant did not significantly differ between varieties (Fig. 4b), as the significantly greater number of racemes per plant in Click (Fig. 4c) compensated for the lower TSW. Total seed mass decreased similarly in both varieties with increasing ozone exposure, although the greater cumulative ozone exposure of Phoenix decreased seed yield by 44% from 30 to 80 ppbv. Increased raceme number between 55 and 110 ppbv in Phoenix to some extent ameliorated the impact of greater ozone exposure on total seed mass. Although the individual yield components (raceme number and TSW) showed differing sensitivity to ozone exposure between the two cultivars, total seed mass was similarly sensitive to ozone exposure.

Seed quality was much more affected by exposure to ozone in Click than Phoenix. The average proportion of oil per seed decreased from 48% to 41% as cumulative exposure increased above 0.07 mmol mol$^{-1}$ *h* (corresponding to 55 ppbv treatment) (Fig. 5a). Total protein content was inversely proportional to oil content, rising from ~18% under 30 and 55 ppbv to 24% at 110 ppbv (Fig. 5b). Total ash and moisture content significantly increased by 24% and 15% with increasing ozone exposure in Click (Fig. 6). Greater ozone exposure increased concentrations of four nutrients (Fig. 6): sulphur increased 46%, with more modest increases in manganese (17%), iron and zinc (both 15%). Fatty acid composition, erucic acid, and glucosinolate proportions, did not significantly change with increased ozone exposure in Click (Table S2). Although small changes were measured between treatments in Phoenix, proportions of key seed quality parameters (oil, protein, ash, moisture, saturated fatty acid composition, erucic acid, glucosinolates, micronutrients) did not significantly differ with increased ozone exposure. Total oil content fell to a minimum of 43% at 55 ppbv in Phoenix, with little difference between other treatments (Fig. 5a). In contrast, average total protein content initially rose from 18% with a peak of 22% at 80 ppbv (Fig. 5b). Overall, Click's quality parameters largely decreased, while Phoenix's remained unchanged with increasing ozone exposure.

### 3.4. Economic Assessment

When the observed changes in TSW are scaled to field-level, Click's final yield decreased from 2.09 t ha$^{-1}$ at 30 ppbv to 1.84 t ha$^{-1}$ and 1.64 t ha$^{-1}$ under 80 and 110 ppbv, respectively (Table 2). Increased TSW and oil content (between 30 and 55 ppb) are not statistically significant, but represent an instability of gross profits with increased ozone exposure. More substantial final yield losses occurred in Phoenix: from 3.33 t ha$^{-1}$ at 30 ppbv to 2.34 t ha$^{-1}$ and 1.98 t ha$^{-1}$ under 80 and 110 ppbv, respectively. The total oil content in both varieties was >40% across all treatments and would, therefore, still attract price premiums. However, premiums would fall under increasing exposure in both cultivars. Our findings suggest the premium would decrease from 12.5% at 30 ppbv to 7.35% under chronic exposure to 80 ppbv of ozone for Click. The

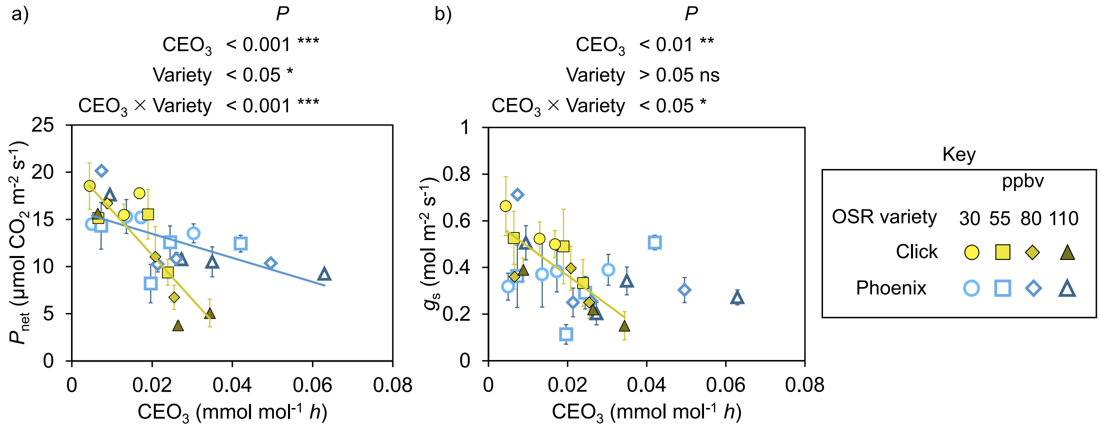


**Fig. 1.** Net photosynthetic rate (a) and stomatal conductance (b) plotted against cumulative ozone exposure ($CEO_3$) for Click (yellow) and Phoenix (blue). P-values represent ANCOVA outputs. Asterisks indicate $P < 0.05$ *, $P < 0.01$ **, $P < 0.001$ ***. Error bars indicate ± SEM, some of which are smaller than the symbols denoting ozone treatment. Regression lines are only shown for statistically significant $P < 0.05$) relationships; outputs in Table S1 (Supplementary Information). Each data point represents an average of measurements logged over 20 min taken from youngest, fully expanded leaves across 3 replicates (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

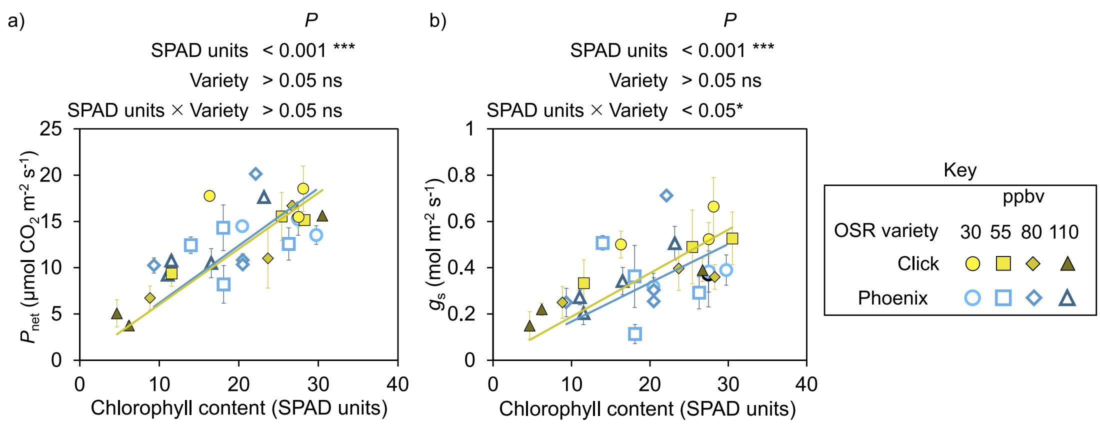


**Fig. 2.** Net photosynthetic rate (a) and stomatal conductance (b) plotted against leaf chlorophyll content (SPAD units) for Click (solid yellow) and Phoenix (unfilled blue). P-values represent ANCOVA outputs. Asterisks indicate $P < 0.05$ *, $P < 0.01$ **, $P < 0.001$ ***. Error bars indicate ± SEM, some of which are smaller than the symbols denoting ozone treatment. Regression lines are only shown for statistically significant $P < 0.05$) relationships; outputs in Table S1 (Supplementary Information). Each data point represents an average of measurements logged over 20 min taken from youngest, fully expanded leaves across 3 replicates (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

premium would drop from 6% at 30 ppbv to ~4% at 55 and 80 ppbv for Phoenix. No premium would have been paid for Click seed under 110 ppbv, but Phoenix's recovered slightly to 5.8%. Overall, the combined losses in seed yield and oil content would have led to economic losses of up to 30% in Click and 40% in Phoenix between 30 and 110 ppb, with growers' profits narrowing under increasing ozone exposure.

## 4. Discussion

This is the first study to directly compare the physiological, morphological and seed quality responses of spring and winter oilseed rape (OSR) cultivars to chronic ozone exposure and explore the findings within the context of industry practice. Most importantly, greater ozone exposure decreased seed yield and quality in both cultivars (Figs. 3–5), despite some evidence of increased raceme number compensating for smaller seed in Click (Fig. 4c). Therefore, our first hypothesis was accepted. However, while oil content significantly decreased, and ash, moisture, protein, and micronutrients increased in the spring cultivar Click, seed quality of the winter cultivar Phoenix was largely unchanged. Furthermore, Click was more physiologically sensitive to ozone exposure than Phoenix, with net photosynthetic rate ($P_{net}$), stomatal conductance ($g_s$), relative chlorophyll content and biomass accumulation decreasing under lower cumulative exposure (Figs. 1,3); thus, our second hypothesis was also partially accepted. Overall, our results support our third hypothesis and provide further evidence that shorter-lived cultivars (spring OSR) are more sensitive to chronic ozone exposure than longer-lived cultivars (winter OSR) regarding quality and physiology.

OSR is grown to provide oil for human consumption and oilcake for animal fodder. Oilseed composition is closely monitored and controlled to ensure that the oil and derived products are fit for consumption. International guidelines from the Federation of Oils, Seeds and Fats Association (FOSFA) stipulates that seeds require a minimum of 40% total oil content and 6–10% moisture when received by a crusher (FOSFA, 2016). Seeds that fail to meet these FOSFA quality standards may be rejected. If loads are accepted, growers then receive a payment premium of 1.5% for every 1% increase in oil content above this minimum, with similar penalties as oil content falls below 40%. All seed analysed in this study passed the minimum FOSFA standards. However, the reduction in oil content in seeds from plants exposed to higher levels of ozone, particularly in Click, would result in growers forfeiting the premium payments they currently rely on to improve profit margins. For example, the decrease in oil content in Click from 48% under European background ozone concentrations of 30 ppbv to 41% following chronic

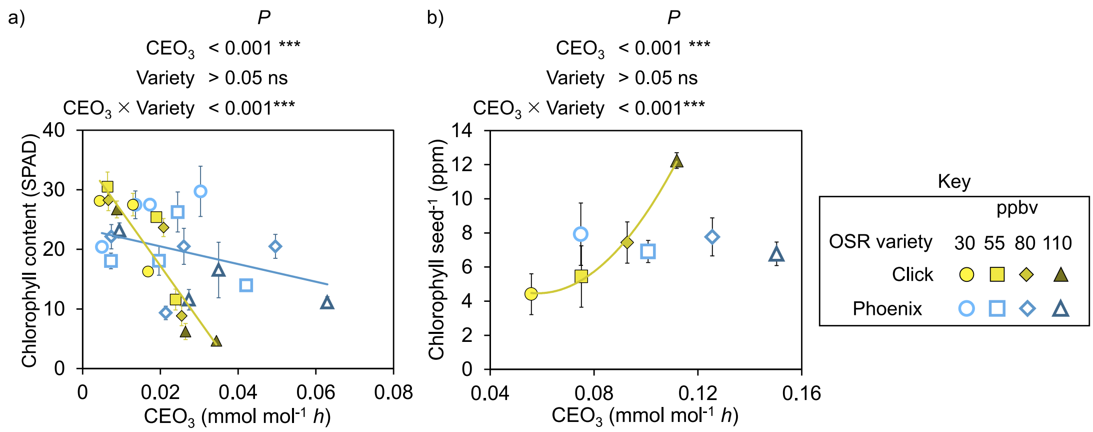


**Fig. 3.** (a) Leaf chlorophyll content (SPAD units) plotted against cumulative ozone exposure ($CEO_3$). Each data point represents an average of measurements logged over 20 min taken from youngest, fully expanded leaves across 3 replicates. (b) Seed chlorophyll content ($n = 4$) (NIR analysis) plotted against $CEO_3$ for Click (solid yellow symbols) and Phoenix (unfilled blue symbols). P-values represent ANCOVA outputs. Asterisks indicate $P < 0.05$ *, $P < 0.01$ **, $P < 0.001$ ***. Error bars indicate ± SEM, some of which are smaller than the symbols denoting ozone treatment. Regression lines are only shown for statistically significant $P < 0.05$) relationships; outputs in Table S1 (Supplementary Information) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

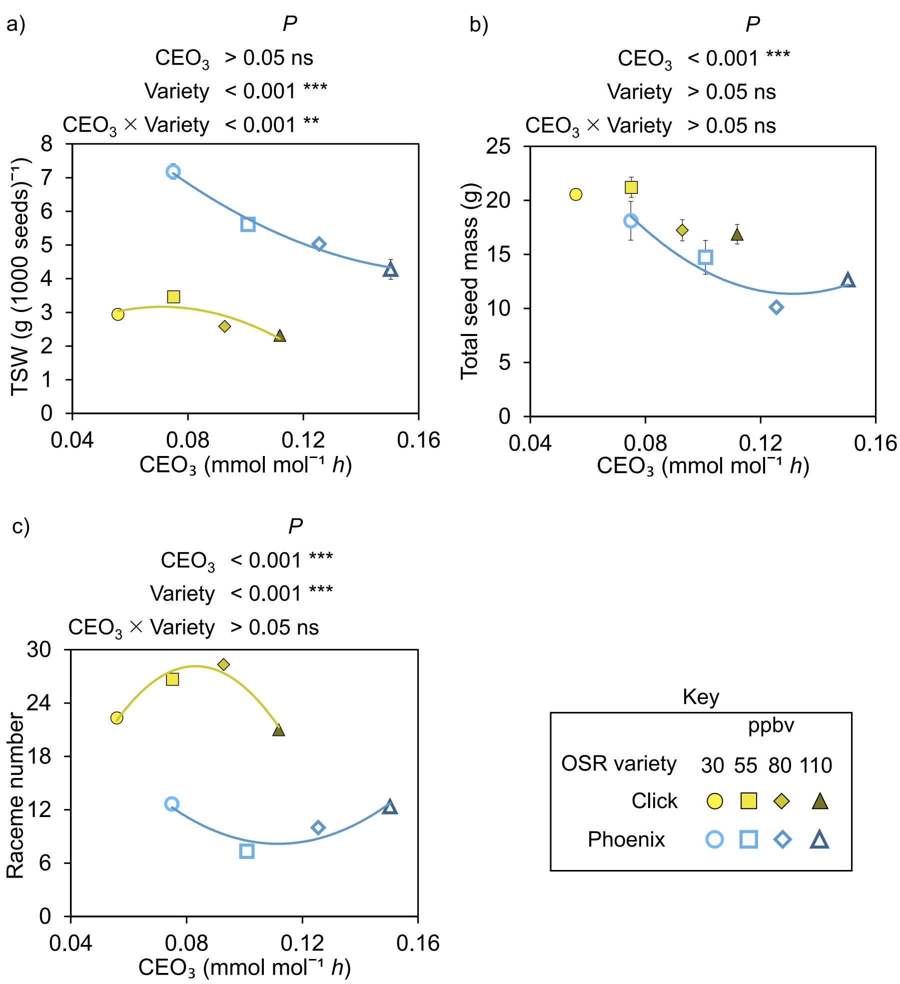


**Fig. 4.** Thousand seed weight (TSW) (a), total seed mass (b), and raceme number (c) of Click (solid yellow) harvested at 90 days, and Phoenix (unfilled blue) harvested at 125 days against cumulative ozone exposure ($CEO_3$). P-values represent ANCOVA outputs. Asterisks indicate $P < 0.05$ *, $P < 0.01$ **, $P < 0.001$ ***. Error bars indicate ± SEM, some of which are smaller than the symbols denoting ozone treatments. Regression lines are only shown for statistically significant $P < 0.05$) relationships (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

exposure to 110 ppbv of ozone, typical of hot Southern European summers, represents a loss of 12% in premiums. Exposure to 80 ppbv, typical of hot Northern European summers, decreased premiums by over a third. For a crop such as OSR with very tight profit margins, this represents a high risk for growers. Although seed oil content was not affected in the Phoenix, profit from this winter cultivar would be substantially lower due to reductions in total seed mass.

Based on average UK yields and prices for OSR in 2020 (DEFRA, 2020), our results suggest that high ozone concentrations (80 and 110 ppbv) could result in a loss of between 174.87 and 327.22 USD $ha^{-1}$ for

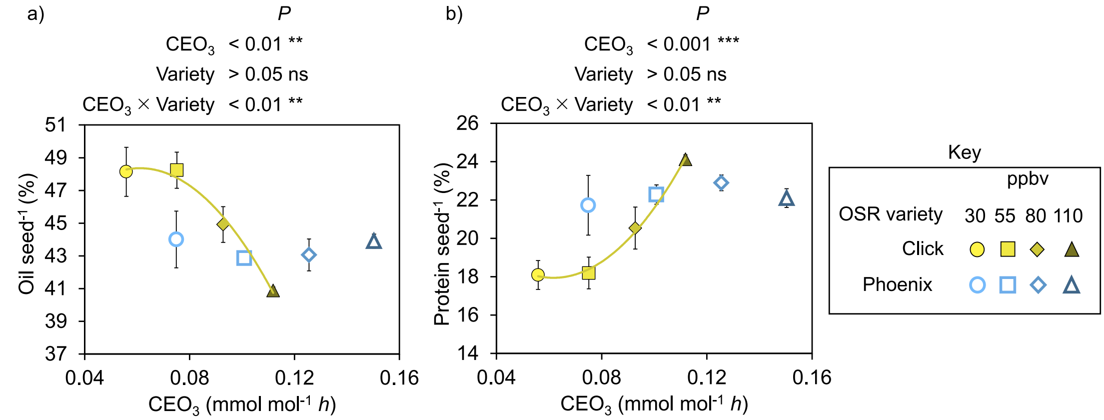


**Fig. 5.** Changes in (a) seed oil content, and (b) seed protein content in Click (solid yellow) harvested at 90 days, and Phoenix (unfilled blue) harvested at 125 days against cumulative ozone exposure (CEO$_3$). Changes derived from NIR spectroscopy (John Innes Centre). P-values represent ANCOVA outputs. Asterisks indicate $P < 0.05$ *, $P < 0.01$ **, $P < 0.001$ ***. Error bars indicate ± SEM, some of which are smaller than the symbols denoting ozone treatment. Regression lines are only shown for statistically significant $P < 0.05$) relationships; outputs in Table S1 (Supplementary Information) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

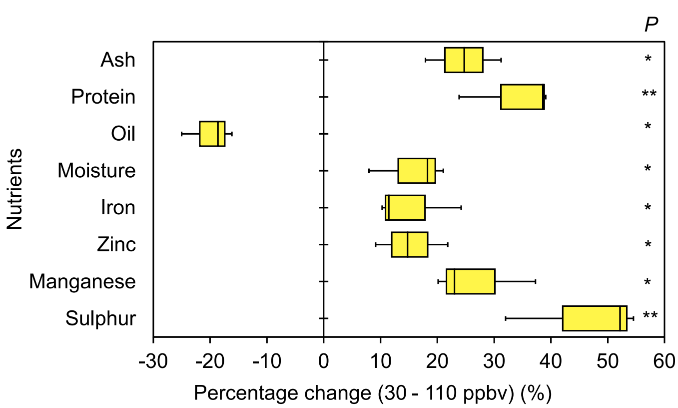


**Fig. 6.** Key macro- and micronutrient changes in spring oilseed rape (cv. Click) between 30 and 110 ppb chronic ozone exposure, with the *t*-test significance output shown on the right. Ash, protein, oil and moisture changes derived from NIR spectroscopy (John Innes Centre), while iron, zinc, manganese, sulphur were derived from a grain suite analysis (NRM). Asterisks indicate $P < 0.05$ *, $P < 0.01$ **, $P < 0.001$ ***. Absolute values for quality parameters discussed in both varieties are reported in Table S1 and S2 (Supplementary Information).

Click and 501.61 to 665.13 USD ha$^{-1}$ for Phoenix (Table 2), which may deter growers from planting this crop. The ozone-induced yield changes observed in this study are, therefore, sufficient to cause concern for growers in current and projected future climates. Moreover, yield instability of Click with increased ozone exposure presents a further risk to OSR growers. OSR yields in optimised field trials in UK averaged 3.3 t ha$^{-1}$ (spring) and 5.6 t ha$^{-1}$ (winter) between 2017 and 2020 (AHDB, 2021). However, on-farm yields were substantially lower, averaging 2.1 t ha$^{-1}$ (spring) and 3.3 t ha$^{-1}$ (winter) over four years (as in Table 2). UK OSR farm yield (2017–2020) has fluctuated between 1.8- 2.2 (spring) and 2.7–3.5 t ha$^{-1}$ (winter) (Bayer Crop Science, 2020). The ozone-induced yield losses of between 0.3 and 0.5 t ha$^{-1}$ (Click) and 1.0 and 1.3 t ha$^{-1}$ (Phoenix) projected by this study are therefore of real concern. In particular, the losses projected by this study surpass previously reported pest- and disease-induced yield and oil losses. For example, Turnip yellows virus and cabbage stem flea beetle decreased yields by 10–40% (Stevens et al., 2008) and 9% (Wynn et al., 2017), respectively. Furthermore, stress-induced yield losses may be additive as stresses frequently co-occur (Pullens et al., 2019).

High seed chlorophyll content is undesirable in food products. Chlorophyll oxidises oils and accelerates rancidity thereby reducing shelf life (Onyilagha et al., 2011), creates a colour that makes the product visually unappealing (Bommarco et al., 2012), and necessitates additional resources to refine (HGCA, 2006; HGCA, 2003). Oil prices are reduced by up to 0.2% t$^{-1}$ once seed chlorophyll content increases above 20 ppm (Bommarco et al., 2012). Moreover, Click and Phoenix are both hybrid cultivars, which have half the chlorophyll content of conventionally bred varieties (HGCA, 2003). Therefore, while chlorophyll content of all seeds harvested in this study were below the 20 ppm quality threshold, should the three-fold increases between lowest and highest exposures seen in this study be replicated in older hybrids, seed chlorophyll content would cause problems for the refining chain and therefore final market with chronic ozone exposures >55 ppbv.

While ozone stress decreased yield and/or oil content, and therefore income from the human food product market, other changes may offer growers increased quality in oilseed cake. Protein and micronutrient (specifically iron, manganese, sulphur and zinc) content all rose (Figs. 4 and 5), which may be favourable for animal fodder, particularly seed cake (Arrutia et al., 2020). As global demand for animal protein is projected to double by 2050 (Westhoek et al., 2011), this may provide an unexpected bonus for growers of OSR already supplying the feedstock market or a new opportunity for others. OSR protein content currently ranges between 20–35%, and an increase of 33.4%, as in our study, would make OSR directly competitive to other high protein feedstock. For example, soya averages 45–49% and fava bean 30–36% protein (Mattila et al., 2018; Heuzé et al., 2020). However, the concomitant increase in less favourable components (moisture, ash and chlorophyll) and substantial decreases in total seed mass may negate any benefit, as in other crops such as soya (Broberg et al., 2020).

The two cultivars differed considerably in their ozone sensitivity, which adds to a body of evidence of intraspecific differences in ozone sensitivity, such as soya (Bailey et al., 2019) and wheat (Pandey et al., 2019). Although selective breeding has favoured crops with higher rates of $g_s$ (Lu et al., 1998; Roche, 2015) and $P_{net}$ (Long et al., 2006; Koester et al., 2016), which is correlated with higher yields, such crops risk higher cumulative ozone exposure and ozone uptake via stomata. Both $g_s$ and $P_{net}$ of the fast-growing spring cultivar (Click) decreased substantially as cumulative exposure increased. Click's photosynthetic declines were correlated with significantly lower TSW and seed quality in plants grown under higher ozone concentrations. In contrast, the slower-maturing winter cultivar (Phoenix) maintained stomatal conductance and photosynthesis under increasing exposure. Hence, increased cumulative exposure over a longer growing season decreased carbon assimilation, which affected Phoenix's yields, but did not affect quality. Phoenix's 40% TSW decrease indicates ozone is an invisible threat to OSR, as leaf-level physiological measurements were not a reliable guide to seed filling. Despite increased ozone tolerance being attributed to low relative growth rates (Franzaring et al., 2000), intraspecific mechanisms are not widely discussed. Plants with longer growth cycles may divert more photosynthetic products to protective mechanisms than shorter-lived plants, which instead decrease biomass accumulation and seed filling (Zhu, 2002; Felzer et al., 2007; Kant et al., 2015). Thus, while this study presents differential ozone sensitivity between two OSR varieties, further study is warranted to identify varieties that may exhibit heritable ozone tolerance in OSR. Moreover, the effects of other environmental and phenological variables need further investigation, as this study grew plants in pots in a single soil type under glasshouse conditions for a shorter duration than in the field. Despite such uncertainties, the economic penalties presented here highlight the importance of further investigation of the effects ozone alongside other abiotic stresses, nutrient application, and different soil types.

Ozone is well-documented to accelerate leaf senescence (Miller et al., 1999; Franzaring et al., 2000; Yendrek et al., 2017). Ozone induces elicitor signalling to plant cell nuclei, which upregulates senescence-associated genes and antioxidants, and downregulates $P_{net}$-associated genes, which decreases Rubisco and chlorophyll synthesis (Pell et al., 1997; Yendrek et al., 2015; Grulke and Heath, 2020). This contributes to re-mobilisation and re-assimilation of nutrients from leaves to seeds, hence decreasing foliar (Calatayud et al., 2004) and increasing seed chlorophyll content (Masclaux-Daubresse et al., 2010). Such nutrient remobilisation is particularly concerning, as OSR typically has a low nitrogen use efficiency, with only half of absorbed nitrogen being present in harvested seeds (Schjoerring et al., 1995). Therefore, exploiting the genotypic variation in nutrient remobilisation and delayed senescence may provide an opportunity to improve yields and selectively breed ozone-tolerant OSR cultivars (Avice and Etienne, 2014; Girondé et al., 2015).

## 5. Conclusion

Our study compares the responses of two European modern OSR cultivars (one spring and one winter) to chronic exposure to realistic ozone levels over a growing season and adds to mounting evidence of intraspecific differences in yield, seed quality, and physiology. Moreover, indications of final yield differences did not manifest in classic ozone injury symptoms at earlier growth stages, indicating chronic ozone stress poses a hidden threat to the cultivation of OSR. Chronic ozone exposure reduced seed quantity and quality at relatively moderate levels of ozone (>55 ppbv), resulting in potentially large reductions (of up to 665.13 USD $ha^{-1}$) in selling price, threatening the commercial viability of OSR. With increased background and peak concentrations of ozone projected for the near future, our findings provide a timely warning for growers and agronomists, and a call to identify and exploit traits linked to ozone tolerance in oilseed rape.

## Data

The data from these experiments will be available from the Natural Environment Research Council (NERC) Centre for Environmental Data Analysis (CEDA) archive; a DOI will be made available when the manuscript is accepted for publication.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.agrformet.2022.108859.

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