Ozone effects on wheat grain quality – A summary

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ABSTRACT

We synthesized the effects of ozone on wheat quality based on 42 experiments performed in Asia, Europe and North America. Data were analysed using meta-analysis and by deriving response functions between observed effects and daytime ozone concentration. There was a strong negative effect on 1000-grain weight and weaker but significant negative effects on starch concentration and volume weight. For protein and several nutritionally important minerals (K, Mg, Ca, P, Zn, Mn, Cu) concentration was significantly increased, but yields were significantly decreased by ozone. For other minerals (Fe, S, Na) effects were not significant or results inconclusive. The concentration and yield of potentially toxic Cd were negatively affected by ozone. Some baking properties (Zeleny value, Hagberg falling number) were positively influenced by ozone. Effects were similar in different exposure systems and for spring and winter wheat. Ozone effects on quality should be considered in future assessments of food security/safety.

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1. Introduction

Ground-level ozone (O₃) is an important regional to semi-global air pollutant and as well an anthropogenic greenhouse gas (Simpson et al., 2014). Tropospheric O₃ is generated through photochemical reactions of so-called O₃ precursors, i.e. nitrogen oxides (NO_x), volatile organic compounds (VOC) including methane (CH₄), and carbon monoxide (CO), which are all emitted from anthropogenic sources. Background O₃ concentrations have risen from approximately 10 ppb before the industrial revolution (Volz and Kley, 1988) to daytime summer concentrations exceeding 40 ppb in many parts of the Northern Hemisphere (Vingarzan, 2004).

It is well established that current ozone concentrations are sufficiently high over large areas of the globe to cause significant adverse effects on crop production (Avnery et al., 2011; Van Dingenen et al., 2009). Wheat has been found to be a highly ozone sensitive crop (Feng and Kobayashi, 2009; Pleijel, 2011; Tai et al., 2014). There has been a strong focus on the effects of O₃ on

quantitative yield in crops (e.g. Feng and Kobayashi, 2009; Mills et al., 2007; Tai et al., 2014) as well as effects on photosynthesis and other physiological aspects/processes such as senescence (e.g. Feng et al., 2008; Mulholland et al., 1997; Sandelius et al., 1995) which are important for the mechanistic understanding of growth effects and for modelling. Although some studies have included quality aspects (e.g. Fuhrer et al., 1990; Pleijel, 2012; Vandermeiren et al., 1992; Zheng et al., 2013), less attention has been paid to these when it comes to research syntheses and policy related work. This paper summarises what is known about the effects of O₃ on wheat grain quality traits. The study is based on existing literature, but also includes some until now unpublished quality data for experiments already described in the literature, and covers experiments performed in Europe, North America and Asia.

Several quality traits exist for a crop like wheat. Some of these (primarily grain protein concentration, 1000-grain weight, volume weight, baking quality) may affect the market price of the yield. Others are nutritionally or otherwise important, but external to the market. These include e.g. the concentration of different nutrient mineral constituents and the content of the potentially toxic element Cd.

Certain aspects of wheat quality are linked to physical characteristics such as the average 1000-grain weight (technically: the

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mass of 1000 grains) and volume weight (technically: the density or specific mass of grain). Low values of these variables indicate a large occurrence of small and malformed grains reducing the quality for use of the grain, while higher values generally are related to larger flour yield (Manley et al., 2009; Weiss and Moreno-Sotomayer, 2006). Another group of quality properties is the concentration of various nutrients including protein, macronutrients such as Mg, K, Ca, P and S as well as micronutrients like Zn, Fe, Mn and Cu. Cd is also of interest since bread is an important pathway for human intake of this potentially highly toxic, bioaccumulating element, which may have a number of important adverse health effects, for example on kidney and bone (Satarug et al., 2010). While the concentration of various grain mineral constituents is important from a qualitative perspective, also the unit area yields of these constituents are relevant for human nutrition and for biogeochemical considerations of the balance of input/output in the agroecosystems. In case the unit area yield of protein or minerals is reduced, this may significantly and negatively affect the ability of people to access sufficient protein and minerals in areas of food scarcity in addition to the loss of food energy caused by the O₃ impairment of grain yield. Therefore we assess both O₃ effects on concentrations and on the yield of these constituents.

Starch is the main source of energy in wheat grain, consisting often in the range of 60–70% of grain mass, and wheat is the second most important energy source for the human population. Thus, starch concentration was relevant to include in the study.

Finally, since wheat is used to a large extent for baking, some quality traits are related to baking quality. Here the Zeleny value, the Hagberg falling number and gluten are frequently used indicators. The Zeleny value is determined by a sedimentation test that measures the rate of sedimentation of wheat flour suspended in an acid solution. Sedimentation occurs due to swelling of glutenin, which is a major protein in wheat flour. A high Zeleny value (sedimentation volume) is associated with a high protein content and good baking quality (Eckert et al., 1993; Reeves et al., 1978). Hagberg falling number is a measure of α -amylase activity in wheat grains and its resistance to enzymatic degradation. Low falling numbers represent high α -amylase activity and thus more germinated grains, resulting in poor baking qualities, such as sticky dough and poorly structured loaves (Kindred et al., 2005). Additionally, a higher falling number enables longer storage time for flour and grains (Hruskova et al., 2004).

The aim of this study is to provide a comprehensive overview of all known effects of O₃ on wheat grain quality based on all available ecologically realistic experiments, using open-top chambers (OTC) or free air concentration enrichment with ozone (FACE) and presented as meta-analysis (to check the statistical significance of the effects) and as response regressions (to assess the magnitude of effect in relation to exposure).

2. Materials and methods

2.1. Database

Relevant papers were searched using Web of Science and selected to be included if the following criteria were fulfilled:

1. Daytime ozone concentration, [O₃]_{day}, was reported (7-h, 8-h, 12-h, 6-h or 4-h).
2. At least one of the following yield quality responses was reported: 1000-grain weight, volume weight, grain protein concentration, mineral nutrient concentration (P, K, Ca, Zn, Fe, Mn, Mg, Cu, S, Na), Cd concentration, or baking quality properties (Hagberg falling number, Zeleny value, wet and dry gluten concentrations). Grain yield data were also extracted from the

data sources to calculate yields of protein and minerals but grain yield as such is not the main focus of the present paper.

3. Ozone exposure was at least 14 days.
4. [O₃]_{day} for elevated ozone treatments was at least 30 ppb during exposure.
5. Plants were rooted in field soil, i.e. pot experiments were excluded.

The total number of wheat cultivars included in the study was 19.

After selection, a total of 36 publications were used for this meta-analysis (Appendix 1). Analyses were performed only for variables for which data existed from at least three experiments. Data used, but not available in the open literature, were mineral concentrations (ICP-Mass Spectrometry) for the Gelang et al. (2000) experiment, starch for the Pleijel et al. (1998) experiment and minerals and protein for the Chinese experiment described in Zhu et al. (2011) (minerals, protein 2007–2009 and all quality data for 2010). Data from figures were digitized using data extraction software (Get Data Graph Digitizer 2.26; <http://getdata-graph-digitizer.com/>).

2.2. Meta-analysis

Two parallel meta-analyses were performed for the yield quality variables, one with charcoal filtered air (CF) as control and the other with non-filtered air (NF) as control. Since FACE experiments never included a reduced O₃ treatment corresponding to the CF treatment used in most OTC experiments, FACE experiments could not be included in meta-analyses using CF as the control. In order to explain variation in yield quality responses, the following categories were included: (1) wheat type (spring vs. winter wheat); (2) fumigation facility (OTC vs. FACE). Parameter values were considered independent if they were made on different cultivars or O₃ concentrations, following the approach of previous meta-analyses (Curtis and Wang, 1998; Feng et al., 2008).

The meta-analysis was conducted using a meta-analytical software package (MetaWin2.1.3.4, Sinauer Associates, Inc. Sunderland, MA, USA) (Rosenberg et al., 2000). To estimate the treatment effect, the natural log of the response ratio (r = variable in elevated [O₃]/variable in control) was used as the metric for analysis (Hedges et al., 1999; Rosenberg et al., 2000) and reported as the percentage changes from control as $(r - 1) \times 100\%$ (Curtis and Wang, 1998; Feng et al., 2008). Negative percentage changes indicate a decrease in the variable in response to elevated [O₃] treatment, while positive values indicate an increase.

Only a limited part of the papers reported data that would allow computation of sample variance (standard deviations or standard errors with replication). Therefore, all variables were analysed using an un-weighted approach in which the variance of the effect size was calculated using resampling techniques after 59,999 iterations (Adams et al., 1997; Feng et al., 2008; Rosenberg et al., 2000). Confidence limits around the effect size were calculated using a bootstrap method (Rosenberg et al., 2000). Estimates of the effect size were assumed to be significant if the 95% confidence intervals (CI) did not overlap zero (Curtis and Wang, 1998). The difference between categorical variables was considered significant if the 95% CIs did not overlap (Curtis and Wang, 1998; Feng et al., 2008).

2.3. Response functions

Response functions were derived between the relative effects of O₃ in the experiments vs. [O₃]_{day}. Effects were related to the effect estimated at zero [O₃]_{day} for each experiment. At zero [O₃]_{day} exposure, the biological variables were set to take the value of 1 on

a relative scale, i.e. it was assumed that there was no O₃ effect associated with zero [O₃]_{day} in each experiment. Although other O₃ indices, especially the POD (Phytotoxic Ozone Dose, Grunhage et al., 2011) and AOT40 (Accumulated Exposure over Threshold of 40 ppb hourly O₃, Fuhrer et al., 1997), may result in stronger relationships, the [O₃]_{day} was preferred in the present study since it is reported for almost all experiments and in this review the priority was to include as much as possible of the data reported for O₃ effects on wheat quality. The time-resolved data for calculating AOT40 (ozone concentrations) and POD (ozone concentrations and several meteorological variables) are only available for a very limited number of experiments.

The following limitations were defined for inclusion of data in the response functions:

1. Experimental treatments were excluded if [O₃]_{day} was larger than 100 ppb, in order to avoid the influence on the regressions from a few very high O₃ treatments.
2. Data were also excluded from the response function if they contained only two data points (treatments) in combination with NF being the control treatment and [O₃]_{day} for NF was larger than 30 ppb, to avoid the uncertainty in the determination of the intercept associated with large extrapolation in combination with only two observations.
3. Experiments with an [O₃]_{day} range (difference between highest and lowest O₃ treatment) smaller than 15 ppb were excluded since random effects become large in relation to the difference in exposure for such experiments.

In order to correct for different time intervals (the number of daytime hours included in the average) in [O₃]_{day} measurements, a daily average for 40 European monitoring stations was calculated for the time period May to July for each time interval (4-h, 6-h, 7-h, 8-h, and 12-h). O₃ data were retrieved from The European Monitoring and Evaluation Programme (EMEP, www.emep.int). [O₃]_{7-h} was the most frequently occurring measurement interval for the selected experiments and chosen as a standard. The average [O₃]_{12-h} for EMEP measurements was about 4.1% lower than the 7-h standard and therefore all [O₃]_{12-h} were adjusted to [O₃]_{7-h} with a conversion factor of 1.041. However, the average [O₃]_{4-h}, [O₃]_{6-h} and [O₃]_{8-h} were estimated to differ less than 1% from the 7-h standard and were consequently not corrected.

All regression response functions, including that for GY, are presented with respect to number of experiments, number of experimental treatments, countries and continents contributing data to the analysis, coefficient of determination (R^2), statistical significance, intercept and slope in Table 1. A small selection of the response regression is shown as figures in the Results section.

3. Results

3.1. Grain yield, 1000-grain weight and volume weight

In Figs. 1 and 2, the effects of O₃ on grain yield (GY), 1000-grain weight and volume weight are presented using the CF treatment and the NF treatment as the reference, respectively. Except for volume weight using CF as the reference (Fig. 1) all variables were significantly negatively affected by O₃. For GY and 1000-grain

Table 1

Overview over the response regressions of the study including the number of experimental treatments, experiments, countries and continents providing data for each response variable included. Further, the linear regression response functions for all variables are presented by coefficient of determination (R^2), statistical significance (P), slope (represented by the % effect per 10 ppb O₃) and the intercept.

Variable	Treatments	Experiments	Countries	Continents	R ²	P		% Effect per 10 ppb	Intercept
Grain yield	115	35	9	3	0.513	<0.001	***	−4.7	1.00
1000-Grain weight	107	33	9	3	0.532	<0.001	***	−3.9	1.01
Grain number	77	21	6	3	0.062	<0.05	*	−1.0	0.97
Volume weight	23	9	3	2	0.354	<0.01	**	−1.1	1.01
Protein	Concentration	69	22	6	0.399	<0.001	***	2.6	1.00
	Yield	69	22	6	0.503	<0.001	***	−4.1	1.02
Zn	Concentration	17	5	2	0.803	<0.001	***	3.3	1.01
	Yield	17	5	2	0.203	0.070	ns	−2.4	0.97
Mn	Concentration	19	6	3	0.204	0.052	ns	8.0	0.93
	Yield	19	6	3	0.000	0.985	ns	0.0	0.94
Cd	Concentration	17	5	1	0.107	0.200	ns	−1.5	1.00
	Yield	17	5	1	0.487	<0.01	**	−5.5	0.97
K	Concentration	33	10	4	0.440	<0.001	***	3.6	0.94
	Yield	33	10	4	0.647	<0.001	***	−4.9	0.98
Ca	Concentration	25	8	4	0.497	<0.001	***	4.4	0.92
	Yield	25	8	4	0.680	<0.001	***	−5.2	0.98
Mg	Concentration	25	8	4	0.633	<0.001	***	5.3	0.93
	Yield	25	8	4	0.574	<0.001	***	−4.9	0.99
Na	Concentration	11	3	3	0.005	0.837	ns	−0.9	0.86
	Yield	11	3	3	0.199	0.170	ns	−5.4	0.89
P	Concentration	25	8	4	0.620	<0.001	***	4.4	0.94
	Yield	25	8	4	0.619	<0.001	***	−5.1	0.99
Fe	Concentration	9	2	2	0.565	<0.05	*	2.9	0.97
	Yield	9	2	2	0.230	0.191	ns	−3.3	0.91
Cu	Concentration	9	2	2	0.299	0.128	ns	4.8	0.92
	Yield	9	2	2	0.042	0.599	ns	−1.8	0.90
S	Concentration	11	3	3	0.179	0.195	ns	1.3	0.97
	Yield	11	3	3	0.397	<0.05	*	−4.3	0.96
Starch concentration	29	9	3	1	0.410	<0.001	***	−1.6	1.02
Hagberg falling number	14	6	2	1	0.309	<0.05	*	2.9	1.03
Zeleny value	16	6	2	1	0.717	<0.001	***	3.8	1.00
Dry gluten	8	4	1	1	0.465	0.062	ns	3.0	1.00
Wet gluten	8	4	1	1	0.439	0.073	ns	2.8	1.01

Fig. 1. Meta-analysis for grain yield, 1000-grain weight and volume weight using CF as the reference. For grain yield and 1000-grain weight comparison is made also with data separated for spring wheat and winter. Numbers within brackets represent the number of comparisons included in the meta-analysis for each variable.

Fig. 2. Meta-analysis for grain yield, 1000-grain weight, volume weight using NF as the reference. For grain yield and 1000-grain weight comparison is made also with data separated for spring wheat and winter wheat and with respect to the exposure system used, open-top chamber (OTC) or free air ozone enrichment (FACE). Numbers within brackets represent the number of comparisons included in the meta-analysis for each variable.

weight there were strongly significant negative effects, which differed relatively little between spring and winter wheat. The comparison of effects in OTC experiments and FACE experiments (Fig. 2) suggested no statistically significant differences. For 1000-grain weight the difference was very small, whereas a larger but non-significant difference was noted for GY with larger effects in OTCs than FACE.

The regression response function for volume weight is displayed as Fig. 3. The negative effect by O_3 on this quality trait was comparatively small, and reported in a limited number of experiments, but resulted in a statistically significant regression response function. The response function for 1000-grain weight had a stronger negative slope (Table 1). In fact, as obvious from Fig. 4, which exhibits the effects of O_3 on GY plotted vs. the corresponding effects on 1000-grain weight, the negative effects of O_3 on GY in wheat is dominated by effects on 1000-grain weight, leaving the

negative effect of O_3 on grain number (Table 1) a minor factor.

3.2. Protein and starch

Grain protein concentration (GPC) was significantly positively affected by O_3 , while grain protein yield (GPY) was significantly negatively affected (Figs. 5 and 6). Results for spring and winter wheat, as well as for OTC compared to FACE experiments, did not suggest significant differences between groups. Starch concentration was negatively affected with the exception of spring wheat (non-significant with small margin) using NF as the reference treatment (Fig. 6).

Fig. 7a shows the response regression for GPC, exhibiting a consistent positive response. In Fig. 7b the significant negative response regression for grain starch concentration is presented.

Fig. 3. Regression response functions between volume weight with average daytime 7-h ozone concentration.

3.3. Concentration and yield of minerals

Generally, fewer experiments have reported data on grain mineral content as compared to 1000-grain weight and grain protein. However, there is substantial evidence to conclude that for the important nutrients Mg, Ca, K, P, Zn, Mn there was a significant positive effect by O_3 on the concentration but a significant negative one on the yield when CF (Fig. 8) was used as the reference. For Zn and Mn the effect on concentration was not statistically significant when using NF as the reference (Fig. 9). For P, K, Mg, Ca and Cu both the effects on concentration and yield were statistically significant when using NF as the reference (Fig. 9). In the case of especially Na

and Fe the variability in the response was large and no firm conclusions can be drawn with the respect to the O_3 effects on these elements. For the potentially toxic, non-essential element Cd, and unlike all the nutrient elements, there was a significant negative effect of O_3 both on concentration and yield, regardless if CF or NF was used as the reference for the meta-analysis (Figs. 8 and 9). It should be noted that the highest levels of grain Cd in the experiments included were in the range of 55–60 $\mu\text{g kg}^{-1}$ (data not shown), which is below e.g. the limit value for Cd concentrations of wheat grain of the EU (EC Regulation No 1881/2006): 200 $\mu\text{g kg}^{-1}$. However, both concentration and total intake are important for the dose and cereal products are indeed a major contributor to dietary cadmium exposure (EFSA, 2009). For the element S the effect on concentration was not statistically significant, neither using CF, nor using NF as the reference.

As a representative example, the P grain concentration and yield responses to O_3 are presented as response functions in Fig. 10. All four macronutrients, and with somewhat smaller effects also the micronutrient Zn, had similar responses with substantial and statistically significant positive O_3 response regression for concentration and approximately equally strong and significant negative effects for yields (Table 1). These effects were of the same magnitude for the four macronutrient minerals.

3.4. Baking properties

Baking properties were reported in rather few experiments, some of which were limited to CF-NF comparison. Thus, meta-analysis was only made with CF as reference. All four studied variables, Zeleny value, Hagberg falling number and dry and wet gluten content, were positively affected by O_3 (Fig. 11). The response regression for Zeleny value shown in Fig. 12 is positive and statistically significant. It is stronger and based on a slightly larger number of data points than the similar positive relationship for Hagberg falling number (Table 1).

4. Discussion

The qualitative review by Wang and Frei (2011) clearly showed that O_3 has a strong potential to affect a wide range of quality traits in crops and that both negative and positive effects are likely to occur. Indeed, the comprehensive quantitative assessment of the present study shows that O_3 has a wide range of significant effects on wheat quality and that some of the effects can be regarded as negative, while other are positive. Among the unequivocally adverse effects are the strong negative influence on 1000-grain weight and the smaller but significant effect on volume weight, both indicating a larger number of very small and/or malformed kernels are formed under O_3 exposure. The significant negative effect of O_3 on starch concentration is consistent with this, since for small/malformed kernels the fraction of starch is likely to be reduced. Wang and Frei (2011) found a negative effect of O_3 on starch concentrations in some crops, but not in cereals. However, they suggest that environmental stresses, such as O_3 , could act to inhibit key enzymes for starch synthesis, causing hampered conversion of sugar to starch. Here, we show a strong negative relationship of starch concentrations with O_3 based on larger database for wheat.

It is obvious that the effect of O_3 on wheat grain is to reduce the average 1000-grain weight and only to a much smaller extent the grain number, as was also observed in some earlier studies (e.g. Pleijel and Uddling, 2012). This observation is in line with the general post-anthesis senescence-promoting effect of O_3 (Grandjean and Fuhrer, 1989), which shortens the period of grain growth (Gelang et al., 2000). The reduction in 1000-grain weight is

Fig. 4. Ozone effect on grain yield vs. the corresponding effect on 1000-grain weight obtained in the regression analysis. The broken line represents the theoretical situation where the effects of O_3 on grain yield are entirely explained by effects on 1000-grain weight.

Fig. 5. Meta-analysis for starch concentration, starch yield, grain protein concentration, grain protein yield using CF as the reference. Comparison is made also with data separated for spring wheat and winter wheat. Numbers within brackets represent the number of comparisons included in the meta-analysis for each variable.

Fig. 6. Meta-analysis for starch concentration, starch yield, grain protein concentration, grain protein yield using NF as the reference. Comparison is made also with data separated for spring wheat and winter wheat and for protein with respect to the exposure system used, open-top chamber (OTC) or free air ozone enrichment (FACE). Numbers within brackets represent the number of comparisons included in the meta-analysis for each variable.

a logical consequence of the shortened grain filling period in combination with a lower O_3 sensitivity during earlier stages of plant growth (Soja et al., 2000) when the number of grain initials is largely determined.

Among O_3 effects that were positive can be mentioned the negative effect on both Cd concentration and Cd yield, as well as the positive effects on baking properties. The concentration and quality (Zeleny value) of gluten protein were positively influenced. The same was true for the Hagberg falling number reflecting lower α -amylase activity. Several factors may contribute to this pattern, including the general increase in grain protein concentration, but also the early or premature ripening of the grain under elevated O_3 . For example, a late ripening often leads to a high α -amylase activity in the grain, lowering the quality and in worst case (under humid conditions) promoting germination of the grains before harvest (Lunn et al., 2001). In the case of the non-essential element Cd, the uptake and transport is known to depend on transpiration (Salt

et al., 1995). O_3 is known to reduce both the stomatal conductance and the leaf life span (Mulholland et al., 1997), which can explain the observed pattern for Cd. Fig. 8, using CF as the reference, indicates that the two non-essential elements included, Cd and Na, had a similar response with reduced concentrations under elevated O_3 , in contrast to all the essential elements for which the concentrations enhanced. This points towards a general pattern with respect to the different physiological mechanisms governing the uptake of essential and non-essential elements, where the passive transport with the transpiration stream through the plant is a strong feature for non-essential elements, while specific uptake mechanisms play the important role for the essential elements. However, in the case of Na, but not Cd, the pattern was different when using NF as the basis for comparison (Fig. 9). It should be noted that both in the case of Cd and the different aspects of baking quality, the amount of data available was relatively small and only represented by experiments performed in Europe.

Fig. 7. Response functions for a) grain protein concentration (GPC), b) grain starch concentration, with average daytime ozone concentration.

In the case of grain protein and most of the important minerals (especially Mg, P, Ca, K, Mn and Zn) it can be noted that a consistent pattern was observed with a significant positive effect on concentration and an approximately equally strong negative effect on the yield using CF as the basis for comparison. This means that the quality of the grain is improved with respect to concentration, but the amount of protein and minerals accumulated per unit area is reduced, which may have serious effects on human nutrition. Further, this shows that a smaller amount of nitrogen and the mentioned minerals was taken up from the soil. Our findings are consistent with what was observed in some individual studies, e.g. of Ca, Mg, K, and P by [Führer et al. \(1990\)](#), K and P by [Vandermeiren et al. \(1992\)](#), K and Ca by [Feng et al. \(2008\)](#); and Mg, P, and K by [Pleijel et al. \(2006\)](#), but the consistency of the combined results from many studies is striking and has not been shown before.

Several factors may contribute to the observed pattern of reduced yield and increased concentration of nitrogen and several essential minerals under ozone exposure. The duration of plant growth in annual crops is reduced by ozone ([Grandjean and Führer, 1989](#)). Thus, the period of nutrient uptake from the soil is shortened. Additionally, there is evidence that the root-to-shoot ratio is negatively affected by ozone ([Cooley and Manning, 1987](#)). As photosynthesis is reduced by ozone stress ([Mulholland et al., 1997](#)), so are photosynthates available for root growth, disproportionately

Fig. 8. Meta-analysis for mineral concentration and yield: plant macronutrients P, K, Mg, Ca, S, plant micronutrients Zn, Mn, Fe, Cu, elements non-essential to plants Cd, Na, using CF as the reference. Numbers within brackets represent the number of comparisons included in the meta-analysis for each variable.

affecting the roots. Further, plant growth rate is smaller under elevated ozone. This will tend to reduce nutrient uptake also in the absence of partitioning effects. All these factors promote a reduction of the uptake and yield of nitrogen and mineral nutrients. At the same time it is well known from general crop physiology ([Kibite and Evans, 1984](#)) that there is a general anti-correlation between crop yield and nitrogen (protein) concentration, since the uptake of

Fig. 9. Meta-analysis for mineral concentration and yield: plant macronutrients P, K, Mg, Ca, S, plant micronutrients Zn, Mn, Fe, Cu, elements non-essential to plants Cd, Na, using NF as the reference. Numbers within brackets represent the number of comparisons included in the meta-analysis for each variable.

Fig. 10. Response functions for phosphorus a) concentration, and b) yield with average daytime ozone concentration.

nitrogen of crops is in most cases strongly source limited. Thus, crops under stress, e.g. by ozone, will maintain nitrogen uptake, although reduced, to a larger extent than biomass accumulation (Wang and Frei, 2011). The net result is an enhanced protein concentration. From the result of the present study, a similar principle

Fig. 12. Response functions for Zeleny value with average daytime ozone concentration.

seems to apply also for other essential elements.

For almost all variables for which a comparison was possible, the effects of O₃ were similar for spring and winter wheat. This suggests that the main two groups of wheat varieties behave in a similar way with respect to the sensitivity to O₃. Similarly, for the variables permitting comparison, the effects were of the same magnitude and did not differ significantly when comparing OTC and FACE exposure systems. OTC exposed plants tended to exhibit stronger responses to O₃, although it was not statistically significant in any single case. It has to be kept in mind also that the OTC experiments included some treatments with rather large elevation O₃, while the O₃ enhancement in the FACE experiments was modest (Zhu et al., 2011).

In the present analysis we did not attempt to separate the O₃ sensitivity of different wheat cultivars other than the comparison of spring and winter wheat. Since each cultivar was mostly used in one or just a few experiments, and there are important differences in agro-environmental conditions in different experimental sites, the comparison of cultivars does not lend itself to the kind of meta-analysis performed here. However, there are examples of experiments where different cultivars were compared in the same experiments, excluding influence of environmental conditions, showing differences in yield magnitude and quality when comparing old and modern cultivars (Pleijel et al., 2006) and when

Fig. 11. Meta-analysis for baking properties Zeleny value, Hagberg falling number, wet gluten, dry gluten using CF as the reference. Numbers within brackets represent the number of comparisons included in the meta-analysis for each variable.

comparing different modern cultivars (Sarkar and Agrawal, 2010). The fact that correlations of response functions were in any cases high, and confidence intervals of meta-analyses small, in the present investigation indicates that the response of many wheat quality variables to O₃ is relatively consistent over the range of 19 cultivars included in the present study. Nevertheless the study of cultivar differences in O₃ sensitivity is an important matter (Avnery et al., 2013), which deserves further study as part of the strategy to avoid crop loss from O₃. These analyses should include both yield magnitude and quality.

Recently Tai et al. (2014) quantified the threat to global food security from O₃ pollution in a climate change context, where their modelling suggested significant effects for yields on four major food crops including wheat. In order to obtain a comprehensive picture of the threats by O₃ to food security/safety a number of important quality effects have to be added, such as reduced 1000-grain weight and volume weight as well as the reduced yields of protein and important minerals. Further assessments of the O₃ pressure on global agriculture should include quality and nutritional aspects with direct consideration of the potential for adverse health effects and malnutrition associated with reduced yields of protein and a range of important minerals. As far as possible, they should also cover interactions with other aspects of global change that might have strong effects on agriculture such as elevated CO₂, rising temperatures and changes in water availability (Challinor et al., 2009; Lobell and Gourdji, 2012).

5. Conclusions

The main conclusions for this study were:

- Ozone significantly reduces 1000-grain weight (strongly), volume weight and starch concentration of wheat.
- Ozone enhances the concentrations but strongly reduces the yield of important wheat grain constituents such as protein, phosphorus, magnesium, potassium, calcium, zinc and manganese.
- Ozone negatively affected the concentration and yield of the potentially toxic element cadmium.
- Ozone positively affects the baking properties of wheat, as a result of enhanced protein concentration and possibly also the ozone promoted premature senescence.
- Future assessments of O₃ effects on global agronomy should include quality and nutritional aspects as well as interactions with CO₂ and climate change.

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