AF/02

OFFQ - Results

Impact of troposheric Ozone on Food and Feed Quality of Brassica species

DURATION OF THE PROJECT
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706.259€

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Tropospheric O3, risk assessment, Brassica napus, Brassica oleracea, oilseed rape, broccoli, yield, quality, dose response, antioxidants, glucosinolates, vitamins, food safety & security

CONTEXT

Human activities are having an unprecedented impact on the global environment and major climatic changes have been predicted (and are already being observed) as a consequence of this. Ozone (O_3) is a naturally occurring chemical present in both the stratosphere (the 'ozone layer', 10 – 40 km above the earth) and in the troposphere (0 – 10 km above the earth). Whereas stratospheric O_3 protects the Earth's surface from solar UV radiation, tropospheric O_3 is (after CO_2 and CH_4) the third most important greenhouse gas (Denman *et al*, 2007; Solomon *et al*, 2007). Besides its role as a direct greenhouse gas, O_3 has been identified as the most important rural air pollutant, affecting human health and materials, as well as vegetation (WGE, 2004).

Increased emissions associated with fossil fuel and biomass burning (Gauss *et al*, 2006; Denman *et al*, 2007), long-distance and even intercontinental transport of O_3 precursors have resulted in a steady increase in O_3 concentration in rural areas hundreds and thousands of kilometres from the original sources of pollution (Prather *et al*, 2003). Nearly one-quarter of the Earth's surface is currently at risk from mean tropospheric O_3 in excess of 60 ppb during midsummer with even higher local concentrations occurring (Fowler *et al*, 1999 a,b). This is well above the mean concentration of 40 ppb that has been determined for damage to sensitive plant species (Fuhrer *et al*, 1997; Mills *et al*, 2000; LRTAP Convention, 2007).

Several scenarios indicate that concentrations of tropospheric O_3 might further increase throughout the 21st century (Gauss *et al*, 2003); simulations for the period 2015 through 2050 project increases in tropospheric O_3 of 20 to 25% (Meehl *et al*, 2007). The global patterns of exposure of vegetation to O_3 are also changing. A prediction of the differences in annual global mean surface O_3 concentrations from 1990 to 2020 has recently been modelled by Dentener *et al* (2005), showing increases in all major agricultural areas of the northern hemisphere with large spatial variation. In North America and Western Europe reductions in peak O_3 concentrations are expected (e.g. Gardner & Dorling, 2000) but these changes are offset by the predicted increases in global background tropospheric concentrations (NEGTAP, 2001).

Furthermore, in parts of Asia, Latin America and Africa, these increases in background concentrations are combined with trends of increased emissions of O_3 precursors, suggesting that current and future impacts of O_3 on crops and forests in these areas will be very significant (Emberson *et al*, 2001).

Other important interactions may arise from the fact that O_3 as such alters the performance of herbivorous insect pests and of plant pathogens, which will themselves be influenced by climate change, e.g. as a result of greater survival under milder winter conditions.

Many studies have been conducted on the impacts of O_3 pollution on vegetation, ranging from effects at the cellular level to predicting impacts on a regional and international scale (e.g. EPA, 1996). O_3 damage to plant tissues includes visible leaf injury, decreased photosynthesis and increased senescence, which has significant repercussions for the yield and quality of major agricultural crops, biodiversity and forest health. These effects are primarily induced by an increased production of reactive oxygen species (ROS), both outside and inside the plant cell, which is a common feature of biotic (pathogens, insects) and edaphic stresses (drought, high light, UV, cold…) in plants. These stress conditions may activate the same, or at least overlapping, signal transduction pathways involving salicylic and jasmonic acid and ethylene. Consequently, O_3 itself can modify the response of plants to a range of naturally occurring environmental stresses such as drought (Bell, 1987). This explains why O_3 has been recognized as a good tool to study signalling cascades that involve apoplastic ROS formation in the regulation of gene expression and can be used to improve our understanding of the complex network of interacting signalling pathways involved in plant defence mechanisms (Rao *et al*, 2000).

The antioxidant defence response influences the production of secondary metabolites such as vitamins and natural toxins e.g. glucosinolates (GSLs). The production and breakdown of GSLs is an important inducible defence system that is found exclusively in plants of the family *Brassicaceae*. Their breakdown products have been shown to possess a range of antifungal, antibacterial and antimicrobial activities (Fenwich *et al*, 1989). Most importantly, these biochemicals have been attributed anticarcinogenic properties and therefore considerable effort is being put in trying to understand and manipulate metabolic pathways leading to an increase of these anti-carcinogenic compounds in the human diet. On the other hand, GSLs exert anti-nutritional and even toxic effects, especially in animal feedstuffs, such as rapeseed meal, decreasing the digestibility and causing e.g. goitre and haemolytic anaemia. From a nutritional point of view, vitamin C (ascorbic acid, ASC) and E (α-tocopherol, TOC) are antioxidants with mainly beneficial health effects.

AGRI-FOOD

The evidence is accumulating that diets rich in plant antioxidants derived from fruits and vegetables are associated with lower risks of coronary heart disease and cancer.
The close phylogenetic relationship of *Brassica* crops with the model plant species *Arabidopsis thaliana*, for which the entire genome sequence has been available since 2000, provides another important argument for adopting *Brassica* as the paradigm for transfer and testing of fundamental knowledge to crop plants.
In conclusion, there is no doubt that predicted increases in tropospheric O_3 will impact on future agro-ecosystems and their management. This study aims to contribute to the risk assessment of the impact of these predicted increases in tropospheric O_3 on the yield, quality and safety of *Brassica* species as primary source for human nutrition and animal feed. The influence of elevated O_3 on secondary metabolism such as antioxidants and GSLs will also improve our understanding of plant defence responses and signaling pathways in general. Increasing knowledge of the plant-environment interactions will surely provide novel strategies to stabilize agricultural yield and quality in a fluctuating environment. This knowledge is imperative to be able to detect, monitor and understand the full impact of our changing environment, in order to identify the risks and justify the appropriate actions (EPSO, 2005).

OBJECTIVES

- To contribute to the risk assessment of increasing tropospheric O_3 pollution by the establishment of reliable O_3 dose/exposure response relationships for oilseed rape and broccoli crops. Therefore not only the economic yield is considered, but also quality traits of the marketable end products are taken into account. This will be achieved by exposure of oilseed rape and broccoli to different O_3 levels under “near-field” conditions and analysis of the consumable end product.
- To develop a flux model for the estimation of the O_3 uptake by oilseed rape and broccoli as influenced by climatic parameters such as air humidity, solar radiation, temperature and crop phenology. This will be achieved by relating changes in the plants’ stomatal conductance, obtained through field measurements, to hourly means of these climatic parameters, evolution of crop growth and O_3 concentrations.
- To determine the impact of increasing tropospheric O_3 concentrations on changes in secondary metabolism of *Brassica* species, especially on the antioxidant (vit C & E, glutathione (GSH)) and GSL composition because these compounds are highly important in relation to the health and safety aspects of human food and animal feed.
- It will be further investigated whether the O_3 induced changes in glucosinolate content and composition of the marketable end products may have consequences on the human diet and animal feed intake.
- To identify physiological and biochemical biomarkers for O_3 stress by investigating the interaction between stress induction, changes in secondary metabolites and yield effects. Therefore the physiological assessments of plant stress responses (photosynthesis and chlorophyll fluorescence) will be linked to biochemical analyses of antioxidants and GSLs at the leaf level.
- Elucidation of the impact of a long term (not acute) increase in tropospheric O_3 levels on leaf/plant metabolism and defence pathways by means of transcriptome analysis.

CONCLUSIONS

The impact of increasing tropospheric O_3 concentrations on the food and feed safety and security of *Brassica* species proved to be dependent on the nature of the marketable end product. This was clearly illustrated by the species presented in this study. For oilseed rape (*Brassica napus*) the seed production represents the economic value of the crop, whereas for broccoli (*Brassica napus* cv italic), it is the fresh vegetable, harvested before seed set, that will appear on the market.
Our primary aim was to develop quantitative O_3 dose-response relationships for risk assessment of present and future O_3 damage to these *Brassica* species. The functions were not only based on ground level O_3 concentrations but also on the biologically more relevant O_3 uptake. Therefore the variation of the stomatal O_3 uptake was modelled as a function of climatic conditions (soil moisture, air humidity, temperature, global radiation) and phenology. For the subsequent flux modelling two approaches were explored. The first model is an empirical multiplicative model which is currently used for flux-based O_3 risk assessment for vegetation over Europe. The second model, referred to as the coupled model, is a more mechanistic model. As the comparison of the modelled and measured stomatal conductance did not indicate a clear distinction between the predictive performance of both models, only the empirical model was used for further calculation of the absorbed O_3 dose and comparison of concentration versus dose based O_3-yield responses.

In comparison to the current situation, seed yield losses of spring oilseed rape may be reduced by 30% within 100 years if future ambient 7 or 12 hr average O_3 concentrations increase to a range of 51 – 75 ppb, as predicted by Assessment Report Four (Meehl *et al*, 2007). Oil yield will be even more affected due to an additional decrease of the oil percentage. Since oilseed rape is the third most important world source of vegetable oil, this implicates a considerable additional economic loss for farmers. Such an effect should also be taken into account for the estimation of biofuel production under future scenarios of increasing tropospheric O_3 levels. There was also a shift in the fatty acid composition of the vegetable oil derived from seeds of oilseed rape. Oleic acid (18:1), which constitutes about 60% of the total fatty acid content, declined significantly in favour of linoleic acid (18:2)..

The suppression of monounsaturated fatty acids coincided with a positive response of the % saturated fatty acids. After removal of the oil, the residual rapeseed meal contains proteins that are used as a feed supplement. In general, there is an inverse relationship between seed oil and protein content and also in our study as the seed protein content was significantly increased by O_3 exposure. In rapeseed oil, vitamin E occurs as a mixture of α- and γ- tocopherol (TOC). The observed decrease in vitamin E content was due to a reduction of γ-TOC which may have an effect on the oxidative stability and storage life of rapeseed oil. α-TOC, the most active form of vitamin E in humans, was not influenced by O_3. Ozone exposure did not result in any significant changes in the GSL content or composition of *Brassica napus* seeds so no consequences are to be expected with regard to feed safety. However, it must be mentioned that the GSL level of the investigated spring cultivar (Ability), was very low and consequently statistically significant changes are more difficult to detect. Therefore it would be interesting to investigate this in e.g. winter cultivars of *Brassica napus* with a higher endogenous GSL content.

Despite the fact that elevated O_3 exposure did not have an effect on the fresh weight of broccoli vegetables, the quality was undoubtedly influenced. Aliphatic GSLs (glucoiberin and glucoraphanin) showed a strong tendency to increase as a consequence of higher O_3 uptake during crop growth although this did not result in a general increase of the total content since the indol fraction (mainly glucobrassicin and neoglucobrassicin) was significantly decreased.

With a season long increase of the ground-level O_3 concentration by 40 ppb during daylight hours, the ratio of aliphatic/indol GSLs in broccoli vegetables was significantly raised from 0.97 to 1.72 which may be considered beneficial with regard to their anticarcinogenic properties. Other positive consequences of more elevated O_3 exposure on broccoli quality were an increase of the protein concentration and of the antioxidant GSH.

The effects of elevated O_3 exposure on yield quantity were in accordance changes in canopy development and physiological performance of the upper canopy leaves of both plant species. The reduction of the seed yield *of Brassica napus* cv Ability can be caused by a more rapid decrease in the photosynthetic performance of the leaves due to earlier senescence. The absence of any biomass or yield effects for broccoli may be due to the fact that the CO_2 assimilation in the upper canopy leaves was not yet affected by O_3 at the time of harvest. This may be due to the short growth period and limited O_3 exposure but possibly the detrimental O_3 effects on marketable yield are not yet measurable because broccoli harvest occurs before senescence sets in.

Most consequences of long term chronic O_3 exposure in plants appear at a late growth stage, and this was also the case for changes in leaf antioxidants. However, here as well both species responded differently. In general, in oilseed rape there was an increase of the antioxidant level, especially of the water soluble antioxidants, whereas in broccoli leaves TOC levels were reduced in response to O_3. As in the vegetables, the ratio aliphatic/indol GSLs in broccoli leaves showed a tendency to increase under elevated O_3 (not significant). Microarray results showed a high variability in gene expression of field grown oilseed rape and broccoli. In conjunction with the low O_3 exposure, this may explain why no clear shifts in metabolic pathways were detetected. An up regulation of PS I genes in the microarray could not be confimed through RT-PCR analysis. In contrast, effects on enzymatic activities and metabolites involved in antioxidative defense was clearly detected and were most pronounced during generative growth in oilseed rape. In general, enzymes in direct contact with ROS showed an increased activity, especially APX, which is activated post transcriptionally. An increased pool of ASC and GSH is available in ozone exposed oilseed rape plants during generative growth. A higher redox potential for ASC is maintained by an increase in MDHAR activity, by an upregulation of the corresponding gene.
Our findings clearly illustrate that O_3 not only has an influence on primary (e.g. CO_2 assimilation) but also on secondary metabolism and antioxidative defence pathway in these plants which has repercussions for both quantity and quality of crop yield. For *Brassica napus* the yield losses associated with increasing tropospheric O_3 concentrations are of economic importance, but a distinction must be made between the loss in oil production and increase in protein concentrations.

Changes in fatty acid composition may have and influence on the nutritional quality of rapeseed oil, whereas the reduction in γ-TOC may have consequences for its oxidative stability and storage life. Although no yield reduction was observed for fresh broccoli vegetables, O_3 did have a significant effect on their quality. Especially the increase in the ratio of aliphatic/indol GSLs may be important with regard to the anticarcinogenic properties for which broccoli consumption is highly recommended.

CONTRIBUTION OF THE TO A SUSTAINABLE DEVELOPMENT POLICY

Ozone has long been recognised as causing losses in crop productivity and changes in the quality of agricultural products. There is now a strong demand from policy makers for the quantification of O_3 damages to be fed into cost-benefit analysis of emission control strategies (Holland *et al*, 2006). This project supplied such information for some major Brassica crops: *Brassica napus* (oilseed rape) and *Brassica oleracea* (broccoli).

Economic losses are expected for oil seed rape if O_3 concentrations continue to rise. In comparison to the current situation, seed yield losses of spring oilseed rape may be reduced by 30% within 100 years if future ambient 7 or 12 hr average O_3 concentrations increase to a range of 51 – 75 ppb, as predicted by Assessment Report Four (IPCC, Meehl *et al*, 2007). Oil yield is even more affected due to an additional decrease of the oil percentage which will need to be taken into account for the estimation of biofuel production under future scenarios of increasing tropospheric O_3 levels. Based on our data the critical AOT40 (Accumulated Ozone exposure over a threshold of 40 ppb) to prevent 5% seed or oil yield are respectively 3.7 and 3.2 ppm h from emergence until harvest, which implies that the presently accepted critical level of 3 ppm h for agricultural crops (UNECE) will also protect spring oilseed rape.

For the quantification of O_3 responses the flux-based method is preferred on the grounds that it estimates yield losses and quality effects against received dose of O_3, rather than against simple exposure to ambient levels. However, so far the flux-based method could only be applied to wheat and potato. We now developed such a model for spring oilseed rape and broccoli so that a wider range of crops may be included in the O_3 risk assessment. The flux-based critical level above which 5% yield reduction for oilseed rape may be expected, is estimated at a POD_6 (Phytotoxic Ozone dose above a threshold of 6 nmol s^{-1} m^{-2} projected leaf area) of 4.4 mmol m^{-2} (for seed production) and 3.9 mmol m^{-2}. PLA (for oil production).
These concentration- and flux-based critical levels can be compared to modelled O_3 concentrations and fluxes for 50 km x 50 km grid squares across Europe as supplied by EMEP (European Monitoring and Evaluation Programme*)* to identify those areas that are most at risk for O_3 damage to oilseed rape, at present but also as predicted for the future.

This project also illustrates that the focus on yield changes could however result in a misleading risk assessment and economic extrapolations since also qualitative attributes of the harvested products may be affected by O_3. Depending on nature of these quality traits for industrial processing and consumer's health, the consequences of increasing tropospheric O_3 concentrations may have beneficial or detrimental consequences on the food and feed chain.

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