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IMPACT OF TROPOSHERIC OZONE ON FOOD AND FEED QUALITY OF BRASSICA SPECIES

"OFFQ"

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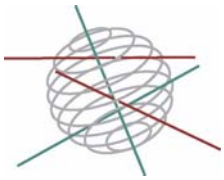

Agro-Food

FINAL REPORT PHASE 1
SUMMARY

IMPACT OF TROPOSHERIC OZONE ON FOOD AND FEED QUALITY OF BRASSICA SPECIES "OFFQ"

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Summary

The preliminary screening of O_3 sensitivity of four cultivars of *Brassica napus* L. and *Brassica oleracea* L. cv Italica during two short term fumigation experiments, was mainly destined to determine the cultivar to be used in the main OTC experiment. At the same time the sampling protocol for the biochemical and molecular analyses and measuring techniques for photosynthesis and fluorescence assessments were tested. Despite the limited number of replications the following conclusions could be drawn:

- Acute O_3 exposure caused a reduction of the ASC concentration in broccoli leaves, but without significant differences between cultivars. The GSH concentration was not affected in either plant species.
- Oilseed rape leaves showed a significant increase in gluconapin and glucobrssicanapin in response to O_3. There was however no significant change in total GSL content for either species.
- For the physiological and biochemical assessments we observed a large variation within each population, despite the highly controlled climatic conditions under which the plants were grown. This was an indication that a high number of replications would be needed to obtain reliable results, especially under less stable conditions such as the OTCs.
- The acute O_3 exposure caused a decrease of photosynthetic activity and chlorophyll concentration, but this effect was only significant for a limited number of cultivars.
- Cultivars Ability (spring oilseed rape) and Monaco (broccoli) were selected for the main OTC experiment because they showed the most significant physiological O_3 response

The short term acute O_3 fumigation has been repeated in 2008 with only one cultivar per species (Ability and Monaco) and the plants were exposed to more elevated O_3 concentrations to obtain significant data on their physiological, biochemical & molecular responses to an acute O_3 exposure. Contrary to previous experiment, visible leaf injury was now indeed observed on *Brassica napus* leaves. The number of physiological assessments were limited, but the chlorophyll fluorescence measurements clearly indicated a comparable decrease in photosynthetic efficiency for both species. The number of active photosynthetic reaction centers per leaf cross section, performance index and potential photosynthetic activity of photosystem II was reduced (significantly for broccoli), whereas energy dissipation showed a tendency to increase. The biochemical analyses from this experiment have not been completely finished yet. This acute O_3 exposure caused a significant increase in total glucosinolate concentration of broccoli leaves, an effect that was not observed during the previous closed chamber experiment.

The Open-Top Chamber experiment was destined to test the effect of a long term, chronic increase in O_3 concentrations on the yield quantity and quality of *Brassica napus* and *Brassica oleracea* cv Italic under near field conditions. The main interest was to investigate whether a further increase in ambient O_3 pollution may induce changes in glucosinalate and vitamin content of the consumable end products which is important for the health and safety of the food and feed chain.
There was an important difference in growth conditions and O_3 exposure levels between 2007 and 2008, especially for *Brassica napus*. In 2007 emergence of oilseed

rape was not optimal due to a serious drought that lasted for three weeks. In the OTCs plant density was more seriously reduced in comparison to the OPs (60 vs. 90 plants/m²) mainly because the constant airflow caused a higher water evaporation of the soil. Especially the NF treatment yielded some conflicting results due to the irregular plant emergence in 2007. For broccoli the results of both growing seasons were much more comparable and most of the data could be pooled for statistical analysis. In 2008 the O_3 exposure level was much more elevated because the the ambient concentrations were higher in comparison to 2007. As a consequence, in 2008 visible leaf injury was observed on both species in the most elevated O_3 treatments, which was not the case in 2007. Whereas the bad weather conditions of 2007 limited the number of field measurements, in 2008 crop growth and its physiological evolution could be perfectly monitored throughout the growing season. It was also decided, as recommended by the follow-up committee, to put more effort into the OTC assessment and reduce some of the OP measurements in 2008.

Exposure to an increased level of O_3 pollution caused a number of physiological changes in oilseed rape crops, that were mainly related to increased senescence. This was apparent after flowering and resulted in a more rapid loss of leaf area, decrease of photosynthetic activity (A_{sat}) and of photochemical efficiency assessed by several chlorophyll fluorescence techniques (potential and effective photochemical efficiency, performance index, photochemical reaction centers per leaf cross section, energy fluxes through photosystem II). This was accompagnied by an increased loss of energy at the level of the antenna chlorophylls. At the leaf level the appearance of these effects depend on the leaf's phenological age. Chlorophyll measurements on younger, upper canopy and older, lower canopy leaves show that the same process takes place in all the leaves, but the effects become more significant as the leaves grow older.

This explains why the light saturated CO_2 assimilation and Fv/Fm of upper canopy leaves in broccoli are not significantly affected by O_3. These leaves remain green and healthy during the entire growing season. At harvest the young green broccoli florets are harvested before plants are allowed to senescence and produce seeds. However, the limited number of measurements on lower canopy leaves do reveal a decrease of photoysynthesis in response to higher O_3 concentrations. Fluorescence measurements after dark adaptation also seem slightly more sensitive to detect decreases in photosynthetic efficiency.

At the moment, biochemical and molecular analyses at the leaf level have only been partly completed. Data from 2007 give some indications of an increased antioxidant capacity in response to O_3. In both years the α-tocopherol concentration of broccoli leaves, sampled during the generative phase, was reduced by O_3. In oilseed rape leaves there is a tendency for total and reduced ascorbate to be increased by O_3 during the generative phase whereas the opposite tendency appears for broccoli. The latter was also observed after the acute O_3 fumigation.

As indicated, the irregular emergence of oilseed rape in 2007 poses some problems, especially for the interpretation of the yield effects, both on quantity and quality. 2008 offers a much higher degree of reliability. The highest level of O_3 exposure induced a 29 % decrease of seed yield in 2008. Though not significantly, the same degree of reduction was achieved in 2007, when comparing NF^{++} to the intermediate O_3 exposure level (NF^{+}). This was correlated to a decrease of the 1000 seed weight (-14 and -9%, not significant), but there were no changes in seed density (weight/volume). Lipids and proteins are quantitatively the two most important fractions of oilseed rape seeds and account for more than 60% of the seed weight.

The protein content of the seeds was not affected by O_3. In 2008, O_3 induced a clear decrease of oleic acid and of the oil %, accompagnied with an increase in linoleic acid and % saturated fatty acids. The same tendency appears to be present for the NF^{++} compared to NF^{+} treatments in 2007. The increase of the linoleic acid content is important with regard to human nutrition because it is an omega 6 fatty acid that we cannot synthesize ourselves. In animal feed, glucosinolates may decrease digestibility and cause goitre and haemolytic anaemia if supplemented at excessive rates. In this respect it is important that the glucosinolate concentration of rapeseed meal does not exceed certain safety limits. The seeds from our experiments mainly contained progoitrine, gluconapin and 4OH-glucobrassicin. O_3 did not cause a significant change in total glucosinolate concentration. Neither did we detect a significant shift in the relative GSL composition.
Fresh marketable yield of broccoli vegetables was significantly reduced by elevated O_3 in 2007, but not in 2008, despite the higher O_3 exposure level. This was also reflected in the dry weight of the individual plant parts and total aboveground plant weight. The reason for this discrepancy is not clear yet. Perhaps the earlier harvest in 2008 had an influence, or the differences in O_3 uptake (flux) may provide an explanation. A preliminary parameterisation of the stomatal conductance for O_3 allowed a first estimate of the actual O_3 uptake in comparison to the O_3 concentrations. This already indicates a large variation in O_3 uptake depending on climatic conditions. Moreover, in comparison to the AOT40, broccoli shows a relatively higher O_3 uptake than oilseed rape. These data surely need further investigation to determine O_3 uptake-response relationships for application within O_3 risk assessments.
The quality of broccoli vegetables in relation to human nutrition is determined by their protein, vitamin and glucosinolate content. The protein content of broccoli plant parts was not significantly affected by an increase in O_3 exposure in 2007 and 2008. The data from 2007 did not indicate a change in vitamin content either, but the data of 2008 are not yet available. The long term increase in O_3 concentration caused a significant increase of the alipahtic GSLs glucoiberin and glucoraphanin, the latter being the most abundant GSL in broccoli vegetables. This did not, however, result in a significant increase of the total GSL content as the effect seemed to be counteracted by a (not significant) decrease of the indole GSLs glucobrassicin and neo-glucobrassicin. This is an important shift since it is mainly the ratio aliphatic/indol GSLs that is important with regard to human health. The anticarcinogenic properties of broccoli are attributed to the aliphatic compounds such as glucoraphanin, the indol GSLs on the contrary are often considered carcinogenic.

In summary we may state that the first phase of the OFFQ project has been executed according to the time schedule of the proposal, except for the molecular analyses that have experienced some delay. Thanks to the input of the follow-up committee and additional staff, this problem is now solved and this information will become available in 2009. The results obtained until now clearly confirm that increased ambient O_3 pollution will induce physiological and biochemical changes for the investigated *Brassica* crops. Whether the effects on yield quality will have a significant impact on the health and safety aspects of the food and feed chain still needs to be further investigated. The challenge during the second phase will also be to relate these physiological and biochemical effect to the changes at the molecular level.