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OZONE FLUX AND YIELD LOSS OF TWO WHEAT CULTIVARS UNDER ELEVATED OZONE AND DROUGHT STRESS

Thesis for doctorate to acquire the academic degree

„Doktor der Bodenkultur"

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Cuimhnich có leis a tha thu-

for my father

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1 INTRODUCTION

Current levels of ozone in the surface air are considered a major threat to humans, plants, materials and eco-systems and have been shown to cause damage to forest trees, agricultural crops and semi-natural vegetation (Kärenlampi and Skärby, 1996). A rise in ozone concentrations has occurred on a large-scale over the past decades, and as a result of the continuing rise in the emissions of precursor substances further increases must be expected in many parts of the world (Fuhrer, 2000).

Ozone is now considered to be one of the most important atmospheric pollutants in Europe, because of it's known toxicity to a wide range of plant species and it's occurrence over wide regions at concentrations sufficient to cause direct effects (UNECE, 2001, Grüters et al., 1994). Field observations and exposure to ozone in open-top chambers within the framework of the National Crop Loss Assessment Network (NCLAN) have indicated that ambient levels of ozone in the USA - similar to those measured in Europe - cause yield losses of many agronomically important crops (Heck et al., 1983, Grüters et al., 1994).

In order to address this problem and to define optimized strategies to reduce the emissions, in 1988 the United Nations Economic Commission for Europe (UN/ECE) with the Convention on Long-range Transboundary Air Pollution (LRTAP) has adopted an effect-based approach, using the critical loads/levels concept.

Critical Levels refer to the levels of ozone above which adverse effects may occur on sensitive receptors, such as plants, ecosystems or materials, according to current knowledge. They have played an important role in the development of the multi-pollutant, multi-effect protocol which was adopted in the framework of the LRTAP Convention, and are being developed to aid policy formulation to control ozone precursor emissions and reduce European ozone concentrations to acceptable levels (Bull, 1991, Emberson et. al., 2000). This protocol aims at a further control of emissions of sulphur, nitrogen oxides and volatile organic compounds (VOC) which collectively cause acidification, eutrophication, and photochemical oxidants across Europe.

Critical levels were first expressed as cumulative ozone exposures over a threshold concentration of 40 ppb (nl 1^{-1}) using the index AOT 40 during daylight hours. This corresponded to the ozone exposure which was associated with significant negative effects on the yield of crops, the biomass increment of forest trees, or the species composition of semi-natural plant communities (Fuhrer et al., 2000). From these relationships it has been possible to derive a critical level based on a given level of plant response (Emberson et al., 2000). This analysis, using single values for each of three

receptor types, were referred to as Level I assessment. It was limited by the fact that any factor which may influence a plant's response to ozone is largely ignored.

Numerous studies have concluded that stomatal resistance was the principal or sole factor controlling differential ozone uptake and foliar injury (Guderian et al., 1985, Grüters et al., 1994), provided environmental conditions restrict the influence of other resistances (Grüters et al., 1994). It has long been recognized that plant response is more closely related to the internal ozone dose, or the instantaneous flux of ozone through the stomata, than the ambient ozone exposure (Amiro et al., 1994, Fuhrer et al., 1992, Emberson et al. 2000). It is important to note that high concentrations of ozone are often associated with factors leading to reduced ozone flux, such as high vapour pressure deficits (VPD) (Grünhage and Jäger, 1994, Grünhage et al. 1997, Emberson et al. 2000). As such, the Level I critical level values, which had been used in developing European ozone control strategies both by the UN-ECE and the EU, were intended to protect the most sensitive vegetation types under the most sensitive conditions. Exceedance of these critical levels only provided an indication that some risk exists of damage to vegetation from ozone; the degree of exceedance could be used to provide a measure of the relative risk of damage to vegetation in different areas of Europe. (Emberson et al., 2000).

Hence, a realistic estimate of the actual ozone impact, such as would be required if the benefits of pollution control are to be valued, is not feasible using the Level I approach. The need for an advanced Level II approach to assess potential ozone impacts has long been recognized. There was a general agreement that the development of a flux-based approach which addresses the ozone uptake and the toxicity of the absorbed ozone dose, would lead to the biologically most relevant estimates of ozone risks, and thus the development of a flux-based approach should be the long-term goal of a level II approach. (Fuhrer, 2000)

It is thus clear that an assessment based on ozone flux to receptor sites within the leaf, rather than ozone exposure, could provide an improved estimate of relative degree of risk of ozone damage to vegetation across Europe, and hence allow more cost-effective control strategies to be identified. Existing models of ozone flux (e.g. Baldocchi et al., 1987, Körner et al., 1995, Grünhage and Haenel, 1997) require detailed micrometeorological information, or are applicable to only a limited number of species, and cannot be readily applied at a European scale. Alternatively, models designed to model ozone deposition on a regional scale (e.g. Wesely, 1989, Erisman et al. 1994) have a limited description of the stomatal responses, which are not species-specific (Emberson et al. 2000) This approach required the development of mathematical models to estimate stomatal flux, primarily from knowledge of stomatal responses to environmental factors.

Stomatal flux based critical levels (Cle_f) for ozone take into account the varying influences of temperature, water vapour pressure deficit, light, soil water potential, ozone concentration and plant development on the stomatal flux of ozone. They therefore provide an estimate of the critical amount of ozone entering through the stomata and reaching the sites of action inside the plant. Values of Cle_f have been identified for wheat (Mapping Manual, 2004).

The objective of this study was to calculate the ozone flux into two different wheat cultivars under elevated ozone fumigation and drought stress in comparison with ambient air and well watered conditions based on the flux-model of Emberson, taking into consideration those micrometeorological parameters that most likely modify the stomatal conductivity. Consequent yield loss of the experimental plants was determined in response to interactions of species, water availability and ozone flux into the plants.

2 LITERATURE

2.1 Tropospheric ozone

2.1.1 History

The discovery of the existence of an allotrope of oxygen called ozone (from the Greek word meaning „smelly") is generally attributed to Schönbein, de la Rive, Houzeaum, and Soret, working in the mid 19th century (In: Lefohn, 1992). Subsequent investigations by Chappuis (1880), Hartley (1881a), and Huggins and Huggins (1890) established the ultraviolet and visible spectroscopy of the ozone molecule. On the basis of this spectroscopy, Hartley (1881a), and Huggins and Huggins (1890) established the ultraviolet and visible spectroscopy of the ozone molecule. On the basis of this spectroscopy, Hartley (1881b) was able to deduce that most of the atmosphere's ozone was located well above the earth's surface and that absorption by ozone molecules at these higher altitudes prevented solar radiation in the ultraviolet region from penetrating to the earth's surface. In the early part of the last century, the basic photochemical mechanism by which ozone is maintained in the stratosphere was first identified by Chapman (1930). In this reaction sequence, often referred to as the Chapman mechanism, ozone is produced by the ultraviolet photolysis of molecular oxygen at wavelengths less than 242 nm:

(R1) $O_2 + h\nu \rightarrow O + O$

Followed by the three-body reaction between atomic and molecular oxygen:

(R2) $O + O_2 (+M) \rightarrow O_3 (+M)$

M is used to refer to an atmospheric molecule, such as N_2 and O_2, which takes part in the reaction and acts to stabilize the molecule produced in the reaction.

Ozone loss in the Chapman mechanism is accomplished via

(R3) $O_3 + h\nu \rightarrow O + O_2$ and

(R4) $O + O_3 \rightarrow 2O_2$

While the Chapman mechanism was able to successfully explain many of the gross, qualitative features of stratospheric ozone, it became apparent upon closer examination that the ozone loss-reactions as described above were inadequate to remove all the O_3 generated by (R1) and (R2). Subsequent studies established the importance of additional gas-phase reactions in which hydrogen oxides, nitrogen oxides, and halogens catalyze O_3 loss (In: Lefohn, 1992).

While many uncertainties remain in our understanding of the cycle of tropospheric ozone, it has become apparent that tropospheric ozone is influenced by a highly complex set of photochemical reactions that can be profoundly perturbed by human activities (Jenkin, 2000).

2.1.2 Ozone Photochemistry

While many uncertainties remain in our understanding of atmospheric photochemical processes, it is now well established that significant production and destruction of tropospheric ozone can occur as a result of photochemical reactions (Lefohn, 1992, Jenkin, 2000).

In regions directly influenced by anthropogenic emissions, the relatively high levels of nitrogen oxides catalyze ozone photochemical generation from the oxidation of anthropogenic and natural hydrocarbons. During the summer months this mechanism can result in frequent air pollution episodes characterized by ozone levels sufficiently high to threaten human health and harm agricultural crops and forests. The control of ozone during these air pollution episodes is a complex problem; recent research concerning the role of natural hydrocarbons suggests that an ozone control strategy that includes nitrogen oxide emissions controls may prove to be effective.

In the remote troposphere ozone photochemistry can be globally significant and can result in either ozone production or destruction, depending upon the local levels of NO. As a result, ozone photochemical production in remote areas appears to be greatest in the upper troposphere where NO levels are high, while ozone photochemical destruction appears most intensive in the remote marine boundary layer where NO levels are typically below 10 pptv. A small shift in global NO levels can trigger a major change in the tropospheric ozone balance in favour of significantly enhanced rates of ozone photochemical production (Lefohn, 1992, Jenkin, 2000).

The possibility cannot be discounted that rising anthropogenic emission rates of nitrogen oxides have already perturbed global NO levels and are, at least in part, responsible for the apparent increase in tropospheric ozone concentrations (Lefohn, 1992)

Like much of the photochemistry of the troposphere, the production of tropospheric ozone requires the presence of OH radicals. In the remote troposphere, primary production of OH radicals occurs from the near-UV photolysis of ozone itself via:

(R5) $O_3 + h\nu \rightarrow O(^1D) + O_2(\lambda \leq 320nm)$

and

(R6) $O(^1D) + H_2O \rightarrow 2OH$

(Levy, 1971).

In polluted atmosphere, primary production of radicals can also occur from the direct emissions of aldehydes (RCHO, where R=H, CH_3, etc.) and nitrous acid (HONO), followed by their photolysis (Lefohn, 1992, Chatfield, 1996, Jenkin, 2000, Stedman, 2004).

Once OH radicals have been generated, photochemical production of ozone is possible in the troposphere by converting these OH radicals into peroxy radicals in the presence of nitrogen oxides. In the remote, marine troposphere where there are relatively few non-methane hydrocarbons, ozone production is often triggered by the oxidation of carbon monoxide (CO):

(R7) $CO + OH \rightarrow CO_2 + H$

(R8) $H + O_2 (+M) \rightarrow HO_2 (+M)$

(R9) $HO_2 + NO \rightarrow OH + NO_2$

(R10) $NO_2 + h\nu \rightarrow NO + O$ ($\lambda \leq 420$ nm)

(R2) $O + O_2 (+M) \rightarrow O_3 (+M)$

Net: $CO + 2O_2 + h\nu \rightarrow CO_2 + O_3$

The oxidation of methane (CH_4) can also be an important source of tropospheric ozone:

(R11) $CH_4 + OH \rightarrow CH_3 + H_2O$

(R12) $CH_3 + O_2 (+M) \rightarrow CH_3O_2 (+M)$

(R13) $CH_3O_2 + NO \rightarrow CH_3O + NO_2$

(R14) $CH_3O + O_2 \rightarrow HCHO + HO_2$

(R9) $HO_2 + NO \rightarrow OH + NO_2$

2x (R10) $NO2 + h\nu \rightarrow NO + O$

2x (R2) $O + O_2 (+M) \rightarrow O_3 (+M)$

Net: $CH_4 + 4O_2 + 2h\nu \rightarrow HCHO + H_2O + 2O_3$

The generation of Formaldehyde (HCHO) from the above oxidation sequence can also result in the production of O_3 via:

(R14) $HCHO + h\nu \rightarrow H + HCO$ ($\lambda \leq 330$ nm)

and

(R15) $HCO + h\nu \rightarrow H + CO$ ($\lambda \leq 360$ nm)

Followed by reactions (R8), (R9), (R10) and (R2).

Similar to the methane oxidation sequence, oxidation of non-methane hydrocarbons can also result in ozone generation via reaction sequences such as

(R16) $RH + OH \rightarrow R + H_2O$

(R17) $R + O_2 (+M) \rightarrow RO_2$

(R18) $RO_2 + NO \rightarrow RO + NO_2$

(R19) $RO + O_2 \rightarrow HO_2 + R'CHO$

(R9) $HO_2 + NO \rightarrow OH + NO_2$

2x (R10) $NO_2 + h\nu \rightarrow NO + O$

2x (R2) $O + O_2 (+M) \rightarrow O_3 (+M)$

Net: $RH + 4O_2 + 2h\nu \rightarrow RCHO + H_2O + 2O_3$

RH is used to denote a hydrocarbon with R representing a hydrocarbon chain, such as CH_3CH_2 in the case of ethane, and R' is used to denote a chain with one fewer C atom. Similar to the photolysis of HCHO, additional O_3 molecules can be generated from the photolysis of R'CHO produced from (R19).

Peroxy radicals are a key intermediate leading to O_3 generation in all above reaction sequences. However, the production of peroxy radicals in the atmosphere does not guarantee that O_3 will be produced; in order to produce O_3, the peroxy radicals must first react with NO. When the NO_x ($NO+NO_2$) levels are relatively small, the peroxy radicals can react with other constituents besides NO, and as a result, O_3 is not produced. Under these conditions, photochemistry can actually result in a net loss of O_3 from (R5) and (R6), reactions of O_3 with olefinic hydrocarbons such as propylene and isoprene, and reaction with HO_2 radicals via:

(R20) $HO_2 + O_3 \rightarrow OH + 2O_2$

As a result of these complex reactions the relationship between ozone photochemistry and the concentrations of NO_x and hydrocarbons can be quite nonlinear (In: Lefohn, 1992).

In "low NO_x" environments (NO_x<400pptv), NO_x plays the central role in controlling ozone photochemistry. As NO_x concentrations vary from 10 to 400 pptv, the photochemistry rapidly increases and shifts from representing a net sink to representing a net source of ozone. Increasing propylene under low "NO_x" conditions tends to increase the rate of ozone destruction due to the reaction between ozone and propylene.

In contrast to "low NO_x" environments, hydrocarbons can significantly enhance ozone production in more polluted regions where NO_x>400pptv.

In areas with extremely high NO_x concentrations, characteristic of some urban areas, the rate of ozone production can actually become depressed by additional NO_x and, essentially, solely limited by the availability of hydrocarbons.

An additional complication in ozone photochemistry arises from the possible effects of halogenated compounds, which may catalyze ozone destruction in the troposphere (Lefohn, 1992, Chatfield, 1996, Jenkin, 2000, Stedman, 2004).

2.1.3 Uptake of Ozone by Vegetation

Surface deposition may lead to chemical changes in the cuticle and cuticular waxes on the leaf surface, but movement into the interior tissues is the essential prerequisite for most of the biochemical and physiological effects of exposure that have been observed.

Without access to the internal sites of reaction, a gaseous pollutant such as ozone may be relatively harmless to vegetation. Thus, uptake plays a key role in determining the effects on metabolism and physiology, and in the description and quantification of dose response (Lefohn, 1992, WHO Regional Office for Europe, Kohut, 2007).

2.1.3.1 The nature of the Atmosphere-Plant-Soil System

Since the plant is part of the larger system involving the atmosphere, vegetation, and soil, it is appropriate to review the features of this overall system as they relate to ozone pollution.

As depicted in the simple diagram of the overall system in Fig. 1 vegetation provides only one of the major „sinks" for any ozone presented in the ambient air. The pollutant may also be adsorbed by soil and other materials and be deposited in snow and bodies of water.

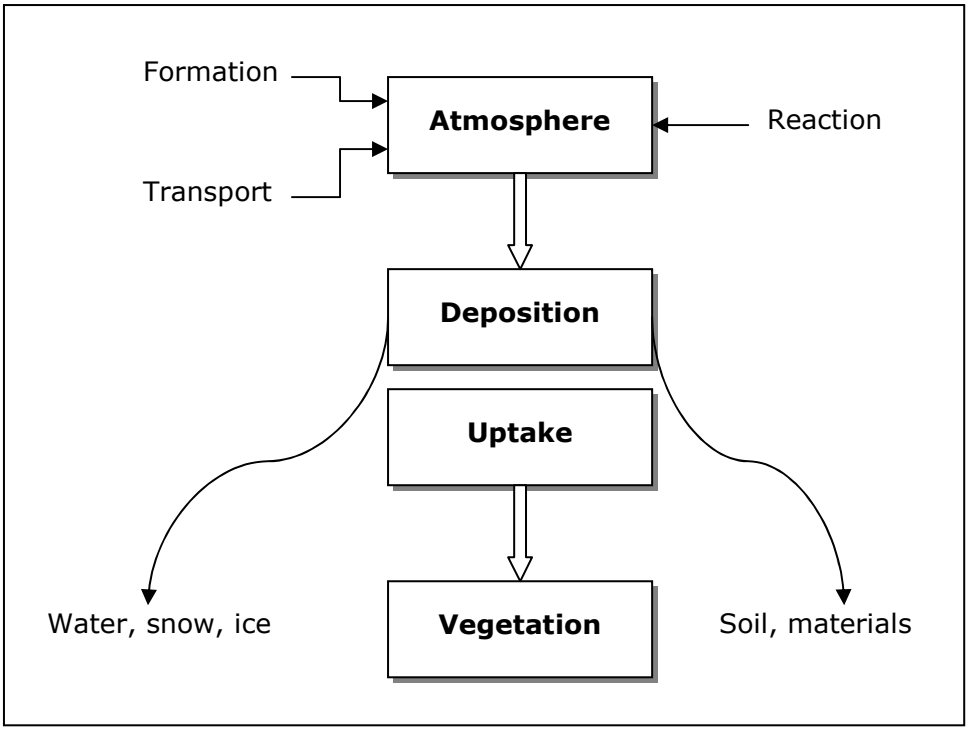


Fig. 1: Overall scheme of gaseous pollutant uptake and deposition (Lefohn, 1992).

Fig.1 also recognizes that the system is dynamic: ozone is entering or being formed (and destroyed) within this reservoir and is leaving it via various routes. These routes of depletion are summarized in the term „deposition", best reserved for describing transfer from the ambient air, regardless of which sinks are involved, leaving „uptake" for when the focus is on the plant response. For uptake is concerned not only with sorption on the leaf and entry into its interior, but with transport to, and utilization at, the various reaction sites within it (Lefohn, 1992).

The relative importance of the three major sinks depicted in Fig. 1 is obviously dependent on location, meteorology, and the extent and nature of the vegetative cover. Appreciable deposition of ozone may occur on soil surfaces. On the other hand, deposition on water bodies or snow is usually one order of magnitude less than on vegetation; roughness of the water surface may double such deposition. Transfer of ozone to foliage does not require rainfall, as in the "wet deposition" of several other air pollutants, although the presence of water on leaf surfaces can reduce uptake (Lefohn, 1992, WHO Regional Office for Europe, 2000, Kohut, 2007).

With regards to uptake by plants, numerous studies have led to the description of more detailed models, but it is appropriate to summarize the models in terms of three major components: the free air above the vegetation; the layer of air immediately above the vegetation (the boundary layer); and the vegetation itself, with its canopy of foliage (Lefohn, 1992).

While these three components provide a simplistic view of uptake, their use can nevertheless provide insights into their individual importance to plant uptake and the conditions under which their roles may be modified.

Hicks and Matt (1988) have suggested that conditions above and within the canopy are more important than those within the boundary layer in affecting the uptake of gaseous sulphur dioxide; the same may well be true for ozone. They point out that their treatment is based upon the "big leaf" concept, which regards the canopy as a homogeneous entity rather than as a heterogeneous assemblage of foliar and other surfaces and structures. The "big leaf" concept has provided a useful means of investigating uptake and its regulation, at the population and community levels (Lefohn, 1992).

2.1.3.2 The canopy, the plant, and the pathways of uptake

Since foliage is a major sink for ozone, the structure of the canopy and the density of the foliage within it will have a pronounced effect upon the concentration of ozone to which individual leaves will be exposed. It has been clearly shown that the concentration of ozone diminishes as one passes down through a canopy to soil level. As a result, leaves

within a dense canopy will tend to be exposed to lower concentrations than those on the surface or at its edges.

Other features of the environment of leaves within a canopy are also modified in relation to those on the outside, e.g., reduced light intensity, increased temperature, and increased humidity, so that the impact of a given ambient ozone concentration outside the canopy on such leaves will be a function of all of these factors.

In many crop situations the spacing of rows opens up the canopy to provide optimal leaf-area indices, permitting light penetration. However, such spacing also tends to reduce the resistance to the transfer of ozone between different layers of the canopy, thereby reducing the gradient within the crop and maintaining the potential for uptake down to the lowest leaves.

Canopy structure and density become particularly important when attempting to extrapolate to field situations from results obtained under laboratory or controlled conditions utilizing individual plants, though in some situations, comparable responses have been reported. Furthermore, canopy structure influences the magnitude of the response of different species in mixtures, whether of agricultural crops or of natural vegetation. In such mixed vegetation, some species may well "protect" others by virtue of their relative locations within the layers of the canopy. Their capacity to act as sinks (thereby influencing the ozone concentration) and to modify other environmental factors may influence the responses of the other species.

The combination of leaf anatomy and physical conditions therefore leads to several possibilities with regard to the fate of ozone molecules in the ambient air surrounding a leaf (In: Lefohn, 1992):

- Sorption and reaction on the cuticular surface
- Passage through the cuticle into the epidermis
- Reaction in the epidermal cells or passage to the interior of the leaf
- Diffusion through the stomata into the substomatal cavities
- Reaction with gases within the substomatal cavities and other air spaces
- Sorption, partition into the liquid phase, and reaction on the epidermal and mesophyll cell surfaces within the substomatal cavities
- Diffusion of ozone or its reaction products through the air spaces between the mesophyll cells
- Following sorption and partition into the liquid phase, movement of ozone or Products of its reaction through the cell walls to the cell membrane

- Movement of ozone or its reaction products through the cell membrane to the Interior of the cell and its organelles (nucleus, chloroplasts, mitochondria, vacuole)

It is not known whether ozone itself or the products of its reaction within the air spaces or with cell wall constituents and the cell membrane are the chemical species that are directly responsible for many of the various phytotoxic effects attributed to ozone. It may well be that some effects are caused directly by ozone, while others are caused by its reaction or decomposition products. The relatively low solubility of ozone suggests that it is likely to penetrate deep into the interior air spaces. This, together with the high palisade surface area interfacing the air spaces, may help explain why the acute effects of ozone injury that results in cell necrosis typically occur in the palisade layer (Lefohn, 1992, Nussbaum, 2003, Massman, 2004, Zhang, 2006).

2.1.3.3 Models of pollutant uptake

Following the early work of Gaastra (1959), numerous models of photosynthetic gas exchange based on the electrical circuit analogue of a network of resistances to gas flow have been described and reviewed. The concepts involved have been applied to the development of models of gaseous air pollutant uptake by numerous workers (Lefohn, 1992).

A common feature of models applied to the uptake of ozone (or any gas) is that they are essentially extrapolations from the theory developed to describe the movement of water vapour out of transpiring leaves and, to a lesser degree, the exchange of CO_2 between the atmosphere and the leaf. The two sets of basic assumptions made are (1) that the eddy and molecular diffusivities of the gas of interest are inversely proportional to molecular weight: the greater the molecular weight, the lower the diffusivity; and (2) that the water vapour gradient starts from near saturation within the leaf's air spaces, particularly the substomatal cavities. The values reported for the various resistances to the uptake of ozone are, for the most part, obtained by calculation from porometric measurements of water vapour transfer.

Some of the uptake models described are concerned with overall dry deposition and, hence, incorporate soil and other sinks in addition to vegetation. Most are based upon the electrical resistance analogue in which total pollutant flux, F_t (g m^{-2} s^{-1} units), onto and into the foliage, is treated as the result of the concentration difference between the ambient air, C_a (g m^{-3} units) and the internal sinks (C_i; usually C_i is considered to equal C_0=0), such that

$$F_t = \frac{(C_a - C_0)}{r_t}$$

Where r_t (s m^{-1} units) is the overall resistance to gas movement (In: Lefohn, 1992).

Such models are conventionally described in terms of networks of resistances and therefore appear to describe the steady-state situation with regard to the fluxes resulting from a particular set of conditions occurring at a particular instant, the networks are dynamic and some or all of the resistances involved may vary appreciably over time.

Furthermore, although most models have utilized the concentration of pollutant (expressed in g m^{-3} units) as the equivalent of the electrical potential across the network, the transfer process may also be viewed as being the outcome of differences in the partial pressure of the pollutant, or differences in mole fraction. Since mole fraction (or mixing ratio) is dimensionless, resistance, on this basis, is expressed in m^2 s mol^{-1} units.

Its reciprocal, conductance, has the same units as flux (mol m^{-2} s^{-1}). The use of mole fractions offers the advantage that the resistance values obtained are independent of concentration and pressure and less dependent on temperature than those based on absolute concentrations. And mole fraction is equivalent to the widely used parts per million by volume (ppmv) of expressing concentration.

The resistance, r_b, external to the leaf surface, limits transfer through boundary layers. The other resistances relate to diffusion through the stomata (r_s) and cuticule/epidermis (r_c) and to sorption and reaction on the leaf surface (r_p). Since the resistance analogue is strictly appropriate only for gas-phase processes, the resistances of the parts of the pathway that involve internal gas-phase diffusion, partition into the liquid phase, diffusion in the liquid phase, and the quasiresistances of any chemical reactions that a pollutant enters into, are amalgamated for convenience into "residual" or "internal" resistances in the mesophyll (r_i) and the cuticule/epidermis (r_{ci}) (Lefohn, 1992).

Several models have incorporated the concept of deposition velocity, v_g (m s^{-1} units), defined as $v_g = F/C$, for an average driving concentration, C, expressed in absolute rather than mole fraction units. Deposition velocity is thus equivalent to conductance and is the reciprocal of the overall resistance:

$$v_g = [r_t]^{-1}$$

Unsworth (1981) has pointed out, one should not assume that v_g is constant, since it clearly depends upon wind speed, physiological responses, and chemical reactivity and may vary by at least an order of magnitude, depending on the canopy environment.

Application of these models has led to certain general conclusions. With regard to uptake by foliage itself, the most important resistances are usually those affecting flux to the foliar surfaces (i.e., the boundary layer resistance, r_b, and external aerodynamic resistances affecting transport to the boundary layer) and the flux into the foliage (i.e., the stomatal resistance, r_s). The composite internal resistances, r_i and r_{ic}, may also be significant.

Leuning (1983) has pointed out that the net influx of a gas such as ozone is somewhat impeded by the outward flux of water vapour, as a result of intermolecular collision, the net result of which would be to raise r_s by a few percent.

Chameides (1989) argues that, while flux to the leaf is largely dependent upon stomatal resistance, flux to the plasmalemma, F_{pl}, is highly dependent upon the ascorbate content of the mesophyll cell walls and, hence, upon the reactive resistance of ascorbate oxidation by ozone, in the cell walls. This resistance can vary from about 100 to 1500 s m^{-1} as the ascorbate content decreases from 10^{-3} ascorbate concentration to zero.

In spite of the differences that may exist in r_i among species in different environmental conditions, there is no question that stomatal resistance exercises a major control of ozone uptake, as shown by the daily rise and fall in flux that accompanies the typical daily cycle of stomatal opening and closing, regardless of any changes in ambient ozone levels. However, stomatal response is itself capable of being modified by exposure to ozone. While most reports indicate that ozone causes reductions in aperture (Winner et al., 1988), the response may be significantly modified by the leaf's previous history of exposure (In: Lefohn, 1992).

An alternative approach to the determination of the overall deposition of ozone involves the use of micrometeorological measurements alone, without specific information about the component resistances to ozone flux. Measurements of the temperature and humidity gradients above a canopy permits computation of Bowen ratios,

B: $$B = \frac{j \cdot (T_2 - T_1)}{e_2 - e_1}$$

Where j is the psychrometer constant (Pa °C^{-1} units), and ($T_2 - T_1$) and ($e_2 - e_1$) are measured gradients of temperature (T; °C) and water pressure (e; Pa units), respectively. Since the net radiation at the canopy, R, may be partitioned into sensible heat flux, H, latent heat flux, LE, and soil heat flux, G (all in W m^{-2} units),

$$LE = \frac{R - G}{1 + B}$$

Assuming that the eddy diffusivities of ozone and water vapour are the same above the canopy, the combined flux of ozone to the canopy and the soil is given by

$$F = \frac{f \cdot j}{d \cdot C_p} \cdot LE \cdot \frac{x_2 - x_1}{e_2 - e_1}$$

Where f is the conversion factor relating the ozone mole fraction, X (m^3 m^{-3} units), to density (kg m^{-3} units) (approximately 2.0 kg m^{-3} at 20°C and 101.3 kPa; d is the density of air (kg m^{-3} units); and C_p is the specific heat of air (J kg^{-1} °C^{-1} units). (x_2-x_1) and (e_2-e_1) are measured over the same height interval.

Leuning et al. (1979a) point out that the main shortcoming of this approach is that it does not distinguish between flux to the vegetation and flux to the soil and, hence, cannot be used to explore specific plant uptake-response relationships unless the usually appreciable deposition to the soil is also determined. Nevertheless, in spite of this need to be able to partition the major fluxes, the micrometeorological approach has distinct advantages when dealing with the diversity of species found in many natural habitats.

Recognition of the roles of environmental conditions in determining uptake has led to a few reports in which such information was used as an alternative to stomatal resistance measurements in order to provide better estimates of dose. Mukammal et al. (1982) applied the concept of utilizing meteorological data as an alternative to direct stomatal information in developing an empirical linear relationship between ozone-induced injury on white beans (Phaseolus vulgaris). In this they used a modified exposure term given by $D = R \cdot CHU \cdot O \cdot \frac{M}{E}$

Where D is the modified dose, R is a relative rainfall factor to normalize different sites, CHU is the ratio of corn heat units accumulated to the time of assessment of injury to the accumulated corn heat units required for crop maturity, O is a weighted cumulative daily ozone exposure (ppmv-h), E is the weighted cumulative pan evaporation, and M is the minimum weighted pan evaporation observed during the July-August season, also used to normalize E. The weighting function, running backwards in time, used to derive O, E, and M is given by

$$F = e^{-(x-1)/k}$$

Where x=1 is the day before assessment, and k is a time constant found to equal 4 d for the best fit of the data.

Resistance models have led to improved understanding of the importance of the different pathways of uptake. However, such information has so far seen little application in response modelling. Runeckles (1974) introduced the concept of "effective dose", which incorporates uptake, as the basis for establishing dose-response relationships. As a development of this concept, and by analogy with the medical usage of the RAD as the unit of absorbed dosage of ionizing radiation, Fowler and Cape (1982) proposed the dose unit PAD (pollutant absorbed dose or cumulative uptake, g m^{-2} units), defined as the product of ambient concentration, time, and stomatal (or canopy) conductance:

$$PAD = C_a^* \cdot \left(\frac{1}{r_s^*} \right) \cdot t$$

or

$$PAD = \sum_{t=1}^{N} C_a \cdot \left(\frac{1}{r_s} \right)$$

Where t is the time interval over which n measurements are taken, and C_a and r_s are mean concentrations and resistances for the period t.

There is no doubt that changes in stomatal aperture during the day can result in dramatic differences in flux and uptake, and Leuning et al. (1979a) expressed high confidence in the fluxes calculated from ozone concentration and stomatal resistance measurements. Furthermore, Reich (1987), in his review of ozone effects on photosynthetic rates and growth, justified his computation of ozone uptakes as the products of dose and mean diffusive conductance even when dose-response and conductance data were only available (for a given species) from unrelated experiments, because the error is likely to be small compared to interspecific variation in leaf conductance which covers a 10-fold range.

Several workers have suggested that flux (g m^{-2} s^{-1}) has to be used to define dose. The definition of PAD is in accordance with this concept, since it is essentially the accumulated flux over time. Experiments in which various species were subjected to different types of exposure to ozone failed to reveal any simple relationship between degree of foliar injury and PAD, based solely on stomatal resistance, although the magnitude of the response decreased dramatically the later the peak concentration occurred during the exposure period.

Short-term variations in uptake computed in PAD units will reflect variation in both concentration and canopy (stomatal) resistance. The daily changes in the plant's external atmospheric environment are stochastic and are governed by meteorology, photochemical synthesis, chemical reaction, and transport. In many locations, the overall consequences of photochemical formation of ozone lead to the typical daily pattern of rising concentrations during the daylight hours and a subsequent decline during the night, with minima usually occurring between 04:00 and 07:00 h.

Stomatal resistance (over which the plant has limited control) similarly follows a general cycle of daytime opening and night time closure. The more closely the cycles are in phase, the greater will be the flux and the greater the uptake. The cumulative uptake-curves clearly show the degree to which phase differences dictate the cumulative uptake. The curves may be used to illustrate several ways in which differences in uptake patterns may dictate plant response. Thus, although the fluxes are greatest overall when the concentration and stomatal conductance cycles are synchronized, the steady-state situation, or one with a much less pronounced early morning minimum concentration than any of the three cycles depicted may result in greater flux values for the first few hours following stomatal opening than those in the synchronous case. Since plants

appear to be most sensitive to ozone at the beginning of an exposure period (Bicak, 1978), the occurrence of such situations may have a significant bearing on response.

Although knowledge of uptake is essential in attempting to predict response, information about flux rates and their rates of change may be equally important (In: Lefohn, 1992).

2.1.4 Crop responses to ozone

Since first identified as a significant, phytotoxic, gaseous air pollutant half a century ago, ozone has progressively become the major air pollutant in many parts of the world.

Several general reviews of the effects of air pollutants on vegetation including some discussion of the biochemical, metabolic, and physiological consequences of exposure to ozone, have appeared over the past decades (e.g., Darrall (1989) and Roberts (1984), Koziol and Whatley, 1984; Heck et al., 1988; Treshow, 1984; Unsworth and Ormrod, 1982; Ashenden et al., 1995; Lefohn, 1992, Martin et al., 2000; Chevone et al., 1998; Fiscus, 2005, Cape, 2008).

There is no clear dividing line between many of the biochemical and physiological effects of ozone, because the latter have their origins in the chemical reactions of ozone (or its reaction products) with cellular constituents. The effects may be the result of reactions with proteins and other components of various cell membranes, or may be on soluble enzymes and substrates, resulting in modifications of normal metabolic processes.

Observations of such effects are merely observations of the results of the collective integration of cellular events, and while they may lead to an empirical knowledge of the effects of ozone and such empirical knowledge is not un-useful in applications such as the estimation of the magnitudes of crop losses by ozone, but much remains to be learned about the ways in which such losses are modified by other environmental factors. Ongoing research is needed because the current levels of tropospheric ozone are largely anthropogenic in origin and will continue to impose an unwanted stress on crops and other vegetation for the foreseeable future (Lefohn, 1992, Cape, 2008, Xu, 2007).

2.1.4.1 Reactions of Ozone with biological materials

Ozone can react with a diverse array of biological compounds and metabolites that are normally present in plant cells. Historically, ozone toxicity has been attributed to lipid peroxidation and/or ozonolysis of the plasma membrane (Tomlinson and Rich, 1969), followed by increases in cell permeability and subsequent failure of chemiosmoregulatory processes (Chimiklis and Heath, 1975; Sutton and Ting, 1977). While this series of events may represent conditions at extreme concentrations (>0,5 ppmv), reviews by Mudd (1982), Heath (1984), and Heath and Castillo (1988) have emphasized the importance of the interactions of ozone with the plant cell, which involves the oxidation of reactive sulfhydryl groups located on membrane and other proteins or the oxidation of

other cellular scavenging compounds. They present convincing arguments against an initial direct attack of membrane lipids (In: Lefohn, 1992).

A wide range of cellular constituents can be directly oxidized by ozone *in vitro.* The rate constants for the reaction of ozone with biological compounds *in vitro* are presented in Tab.1. The most reactive compound is the sulfhydryl-containing tripeptide, glutathione (c-glutamylcysteinylglycine), with a rate constant $>1.0\cdot 10^{9}$ M^{-1} s^{-1} at pH 7.0. Cysteine, the sulfhydryl-containing amino acid of glutathione, has a similar rate constant, which suggests that other sulfhydryl - rich peptides and proteins are highly susceptible to ozone attack. The reactivity of ascorbate is relatively high and is one to two orders of magnitude greater than the reaction rates with simple polyunsaturated fatty acids. Amines, amides, alcohols, and carboxylic acids are among the least reactive compounds.

The localization of the different reactions with ozone will influence the terminal effect of ozone on the plant cell. Glutathione and ascorbate may be considered among the primary reactants and scavengers of ozone because of their high rate constants for oxidation, molecular mobility, high cytoplasmic (and chloroplastic) concentrations, their generalized dispersion throughout the cell, and, in the case of ascorbate, its occurrence within the cell wall (Lefohn, 1992, Lerner, 2003, VanderHeyden, 2001).

Compound	Rate constant ($M^{-1}s^{-1}$)	References
Glutathione (pH 7.0)	$>1.0 \cdot 10^9$	Pryor et al., 1984
Free amino acids		
cysteine (pH 7.0)	$>1.0 \cdot 10^9$	Pryor et al., 1984
trytophan (pH 7.0)	$7.0 \cdot 10^6$	Pryor et al., 1984
methionine	$4.0 \cdot 10^6$	Pryor et al., 1984
proline	$4.3 \cdot 10^6$	Pryor et al., 1984
Ascorbic acid (pH 7.0)	$5.6 \cdot 10^7$	Giamalva et al., 1985
α-tocopherol (pH 7.0)	$7.5 \cdot 10^5$	Giamalva et al., 1985
Simple polyunsaturated fatty acids (PUFA)	$\approx 1.0 \cdot 10^6$	Giamalva et al., 1985
oleic acid	$9\ 8 \cdot 10^5$	Giamalva et al., 1985
methyl oleate	$8\ 7 \cdot 10^5$	Giamalva et al., 1985
linoleic acid	$1.0 \cdot 10^6$	Giamalva et al., 1985
2°, 3° Amines	$\approx 1.0 \cdot 10^2$	Pryor et al., 1984
Water	$\approx 5.0 \cdot 10^1$	Staehelin and Hoigne 1982, 1985
1°, 3° Alcohols	$\approx 5.0 \cdot 10^0$	Pryor et al., 1984
Carboxylic acids	0.0	Pryor et al., 1984
Amides	0.0	Pryor et al., 1984

Tab. 1: Rate Constants for the Reaction of Ozone with Biological Compounds *in vitro* (Lefohn, 1992)

2.1.4.2 Ozone and the production of oxyradicals

The interactions of ozone with substrates that yield hydrogen peroxide can lead to increased levels of O_2- and to the subsequent generation of the highly reactive hydroxyl radical. Hydrogen peroxide itself combines with superoxide to produce the OH radical through the Haber-Weiss reaction:

$O_2^- + H_2O_2 \rightarrow OH + OH^- + O_2$

The potential importance of systems that result in the production of OH radicals needs to be viewed in the context of the extreme reactivity of such radicals and their concomitant brief lifetimes (1.2ns) and diffusion path lengths (3.5nm). In particular, the diffusion path length is comparable to such intracellular dimensions as the thickness of phospholipid bilayers (5nm), membrane globular proteins (7.5nm), and the mean water thickness around biomolecules in general (3nm). These considerations led Saran et al. (1988) to conclude that such radicals could only react with specific target biomolecules in the

immediate proximity of the site of their production. In contrast, the less reactive superoxide anion (with an approximate lifetime of 1 ms) could travel several molecular distances to reach a specific target site.

The production of the superoxide anion and hydroxyl radicals thus presents an attractive hypothesis for the initial effects of ozone that lead to cellular perturbations.

Cassab and Varner (1988) have speculated that histidine residues within cell wall protein may be attacked by oxygen radicals (Rivett, 1986), which would result in changes in cell-wall pH and the interrelationships of ionized wall constituents such as proteins, and Ca^{2+}. Oxyradicals have also been implicated in the chemical modification of DNA. In the case of hydrogen peroxide toxicity to Escherichia coli, which is largely attributable to DNA damage resulting from the reaction of H_2O_2 with D N A – bound iron in the presence of a source of reducing equivalents, oxygen itself may work together with SOD (superoxide dismutase) and catalase to form a scavenging system that converts other oxyradicals to O_2^-, which is then destroyed (In: Lefohn, 1992).

2.1.4.3 The extent of ozone penetration into the cell

The observation that the ozone concentration in the intercellular airspace of sunflower leaves is close to zero, even when the leaves are exposed to ozone concentrations as high as 1 ppmv (Laisk et al. 1989), suggests that the apoplastic space and the plasmalemma are major sinks for ozone, at least during the first few minutes of exposure, and prevents the penetration of ozone per se into the deeper layers of the cell.

There have been many reports of ultrastructural changes to internal membranes, such as identations of chloroplast envelope, the swelling of chloroplast thylakoids, golgi body cisternae, the endoplasmatic reticulum, the nuclear envelope, the shrink of mitochondrial cristae, and the appearance of crystalline bodies within the chloroplast.

Many of these effects of ozone might be interpreted as indicating disturbed osmotic relationships within the cell, caused by changes in the plasmalemma, since they resulted from exposures to relatively high ozone concentrations (0.4 to 0.5 ppmv) which led to the appearance of visible symptoms of injury (Lefohn, 1992, UNEP, 2000).

2.1.4.4 Intercellular reactions

Ozone may react with olefins, such as ethylene, and terpenoids, released the intercellular spaces. Mehlhorn and Wellburn (1987) suggested that the foliar injury attributed to ozone is the result of ozonolysis of stress-induced ethylene, leading to the formation of free radicals such as the superoxide anion, since injury and rate of ethylene production were found to be directly correlated. At levels of ozone insufficient to cause acute injury or necrosis, Taylor et al. (1988) similarly observed that the reduction in CO_2 assimilation

rates and stomatal conductance caused by ozone were diminished or eliminated when stress-ethylene production was metabolically inhibited (In: Lefohn, 1992).

On the other hand, there is increasing evidence for ozone-induced changes in apoplastic constituents, especially ascorbate.

The increased levels of extra cellular ascorbate could act as direct sinks for ozone, as suggested by Chameides (1988). However, in order to function as an enhanced detoxification system for ozone, the increased activity of ascorbate peroxidase requires that ozone is converted to hydrogenperoxide either directly or via the superoxide anion. As a result of the observations, it becomes apparent that, although many of the effects of ozone observed in leaves involve intracellular processes such as photosynthesis and respiration, the actions of ozone on non-structural constituents of the cell wall outside the plasmalemma are important indirect determinants of the magnitudes of these internal effects (Lefohn, 1992, Loreto F., 2007, Smirnoff, 2007, Altimir, 2008).

2.1.4.5 Potential intracellular ozone-scavenging systems

Oxyradical formation is an inevitable consequence of life in an oxygen-rich environment (Halliwell, 1974). As a consequence, numerous scavenging systems have evolved in order to minimize their harmful effects.

In addition to the ability of ascorbate to act as a sink for ozone prior to its reaching the plasmalemma, both ascorbate and glutathione have the potential to scavenge ozone within the cell. There are numerous reports of changes in ascorbate and glutathione levels during and following exposures to ozone.

The initial reaction between reduced ascorbate (AA) and hydrogenperoxide is catalysed by ascorbate peroxidase:

$H_2O_2 + AA \rightarrow dHAA + 2H_2O$

with the monodehydro ascorbate radicals as an intermediate (In: Lefohn, 1992).

The monodehydroascorbate radical can dismutate to dehydroascorbate (dHAA) or be reduced to ascorbate by NADHP-dependent monodehydroascorbate reductase. The oxidized dehydroascorbate is reduced back to ascorbate by dehydroascorbate reductase, using two molecules of glutathione:

$dHAA + 2GSH \rightarrow AA + GSSG.$

The oxidized glutathione (GSSG) is then reduced to GSH by NADPH-dependent glutathione reductase:

$GSSG + NADPH + H^+ \rightarrow 2\ GSH + NADPH^+$

(In: Lefohn, 1992).

Normal electron flow arises from the photolytic cleavage of water associated with photosystem II. The electrons are ultimately accepted by oxidized ferredoxin, transferred to $NADPH^+$, and utilized in carbon dioxide reduction. Ferredoxin can donate a single electron to oxygen, forming the superoxide anion. A subsequent series of reactions involves the production of hydrogenperoxide and its reduction to water through ascorbate, glutathione, and NADPH. The NADPH is then generated by normal electron flow through ferredoxin. Hydrogenperoxide is a pivotal metabolite in this alternate pathway of electron flow and, if not removed, will damage chloroplast function. In addition, the reaction of the superoxide anion with hydrogenperoxide produces the hydroxyl radical (*OH), which is a potent initiator of lipid peroxidation. In this respect, it may not be surprising that Alpha-Tocopherol, which has been found to be predominantly associated with chloroplast membranes, is an efficient scavenger of lipid radicals (Lefohn, 1992, Moldau, 1998).

2.1.4.6 Effects of ozone on photorespiration on respiration

Photorespiration involves reactions within the chloroplast, the cytoplasmic glyoxysomes, and the mitochondria and results from the action of the oxygenase function of rubisco.

The oxygenase function of rubisco has been suggested to involve the formation of the superoxide anion, which is retained within the "cage" of the catalytic site of the enzyme.

There appear to have been few studies of specific effects of ozone, probably because of the difficulties in making quantitative measurements.

Normal respiration is primarily a function of the mitochondria. As in the case of ozone effects on chloroplast ultrastructure, changes to mitochondrial structure have been reported. Black (1984) has pointed out that it is difficult to differentiate between direct effects on the respiratory processes and changes due to leaf injury. Earlier evidence supporting the latter view comes from the observation that a high concentration of ozone (0.5 ppmv) increased glucose-6-phosphate dehydrogenase and decreased glyceraldehydes-3-phosphate dehydrogenase activities in soybean (Lefohn, 1992, McKee, 1997, Madhoolika, 2003, Hay, 2006).

2.1.4.7 Effects of ozone on carbohydrate metabolism

The key roles that carbohydrates play in the carbon and energy economies of the plant, therefore, also lead to effects on almost all other aspects of metabolism and growth.

Among the reported effects of ozone on carbohydrate levels, a common finding has been a general decrease in free sugars and storage polysaccharides in roots although the levels of individual sugars, such as fructose and sucrose, have been found to increase (Lefohn, 1992, Sild, 2002).

2.1.4.8 Effects of ozone on organic acids and lipids

Because of the key role of lipids in membrane structure and function and the potential for their direct chemical reactions with ozone, much early work was directed towards relating plant injury to lipid decomposition and the formation of malondialdehyde.

Various lipid and fatty acid fractions have been shown to undergo changes as a result of exposure to ozone. A decrease in wheat leaf phospholipids was accompanied by an increase in free fatty acids (Lefohn, 1992, Loreto, 2001, Sild, 2002, Goodman, 2003, Agrawal, 2007).

2.1.4.9 Effects of ozone on secondary metabolism

The effects of ozone on the levels of phenolic compounds have received the most attention, possibly because of their known involvement with enzymatic browning reactions and the frequent observation of pigmentation as a visual symptom of ozone injury.

The conclusion of the different reports was drawn that the increases in phenolics resulted from the activity of polyphenoloxidases acting upon precursors released as a result of membrane breakdown. In some instances, the changes were detectable without the subsequent appearance of visible injury symptoms (Lefohn, 1992, Loreto, 2001, Sild, 2002, Agrawal, 2007, Goodman, 2003).

2.1.4.10 Effects of ozone on the physiology of plant cells and tissues

An important physical feature of membranes is their semipermeability to many solutes. The plasmalemma that surrounds the cell is particularly vulnerable, it separates the apoplast and symplast and is the first membrane likely to be encountered by ozone following its entry into the leaf. Hence, the reactions of ozone with its protein and lipids components will have far-reaching consequences for the plasmalemma's physico-chemical functions. These in turn will affect intracellular activities and may lead to effects at the tissue level. Heath (1987a) made the important distinction between chronic responses, which may be reversible and acute responses, which are not. The intitial acute response is the appearance of waterlogging within the palisade mesophyll tissues of exposed leaves, indicating a breakdown of membrane function and leakage into the apoplastic space.

Ozone can also alter the physical condition of membranes. Dominy and Heath (1985) and Finchk and Heath found that ozone inactivated the Mg^{2+}-dependent and K^{+}-stimulated plasmalemma ATPases that are thought to be associated with the ion pumps on the membrane, presumably by oxidizing sulhydryl groups (Lefohn, 1992, Loreto, 2001, Sild, 2002, Goodman, 2003, Agrawal, 2007).

2.1.4.11 Permeability and leakage

Heath and Castillo (1988) stress the potential importance of membrane disturbances that affect the transport of K^+ and Ca^{2+} ions, because of their important regulatory roles in a wide range of cellular functions. They point out that the cell expends considerably energy in maintaining low levels of free Ca^{2+} in the cytoplasma ($<10^{-6}M$) vs. higher levels outside the plasmalemma (in the cell wall) and within organelles such as the mitochondria and the vacuole. Heath and Castillo (1988) suggest that ozone may cause in free Ca^{2+} in the cytoplasm. If excessive free Ca^{2+} levels occur, the recovery processes would be overwhelmed, leading to irreversible and fatal changes. They further write that Ca^{2+} imbalance may disturb the normal functioning of scavenger system for ozone and its products. Peroxidases are activated by Ca^{2+}, and Castillo et al. (1984) observed ozone-induced increases in extracellular peroxidase activity, because of greater secretion from the cell caused by an increase in cytoplasmic Ca^{2+} levels, which is the result of the cell membranes' inability to maintain normal Ca^{2+} balances. There is abundant evidence at the tissue level for the ozone-induced breakdown of general membrane integrity in leaf cells, as revealed by an increased ability to leach a range of ionic and non-ionic solutes from treated leaves. It is important to recognize that many effects may be mediated by changes in membrane structure and function that profoundly influences the physiology of the affected cells, the tissues in which they occur, and the plant as a whole. Plant responses that result in visual changes in foliar characteristics are secondary processes of ozone toxicity, which appear after initial defence mechanisms are overrun. Cellular, biochemical and physiological alterations occur without visible injury symptoms appearing, and these modifications affect critical metabolic functions capable of limiting oxidative stress and ozone toxicity both directly and via more complex physiological interactions within the cell. It is the integrated cellular system that confers and determines plant sensitivity to ozone (Lefohn, 1992, Loreto, 2001, Sild, 2002, Goodman, 2003, Agrawal, 2007).

2.1.4.12 Effects of ozone on plant physiology and growth

2.1.4.12.1 Photosynthetic gas exchange

The general finding has been that ozone inhibits photosynthetic gas exchange, although the degree of inhibition is highly dependent upon the concentration of ozone, the duration of the exposure, and the species or cultivar tested. From the earliest studies it was recognized that the effects of ozone on CO_2 exchange rates could be modified by numerous environmental variables (light, humidity, ambient CO_2, other gaseous pollutants), and other biological factors (leaf age, plant water status, respiration rate), and that it was necessary to distinguish between the effects of short duration exposures to high ozone levels and to prolonged or repeated exposures to lower levels. In particular, at low exposure levels, reduction in CO_2 exchange rates are usually only

observed during the exposure to ozone, with almost full recovery afterwards. At higher levels (e.g., greater than 0.2 ppmv for 4h - broad bean, In: Lefohn, 1992), lack of subsequent recovery indicated permanent damage to the photosynthetic system.

It is apparent that although there are many independent observations to the effect that ozone causes transient or permanent inhibitions of photosynthetic rates, the diversity or responses reported for different species (and cultivars), environmental conditions, and experimental procedures for conducting the exposures to ozone has yet to lead to a clear picture of the nature of the inhibition and its mechanism. This result, in part, from our inability to relate short-term responses (which are usually reversible provided that the level of ozone stress is not excessive) to long-term, chronic responses that may include some degree of adaptation, and, in part from the interaction between effects of photosynthesis, photorespiration, and stomatal function. It is nevertheless clear that in chronic exposure situations, impaired photosynthetic ability is closely related to accelerated senescence and decreased overall growth (Lefohn, 1992, McKee, 2001, Fiscus, 2005).

2.1.4.12.2 Interaction of photosynthesis and stomatal conductance

There are several reports that indicate that reductions in photosynthesis (P_N) cannot be accounted for solely by stomatal closure and its consequences. Nevertheless, the development and maintenance of a reduced steady-state photosynthesic rate observed during short-term ozone exposure (for example, barley, Rowland-Bamford et al., 1988; eastern white pine, Yang et al., 1983, In: Lefohn, 1992) suggests the attainment of an equilibrium condition between ozone influx and P_N inhibition.

Fig. 2 represents a model system that summarizes the physiological and biochemical processes that are likely to be important in the development of such an equilibrium state. In the upper sequence of events, ozone influx to the intercellular spaces ($O_{3\ int(o)}$) is controlled by the initial stomatal conductance ($g_{s(o)}$) and the external ozone concentration ($O_{3\ ext}$). Within the leaf tissue, ozone molecules can undergo a variety of physico-chemical interactions with cellular components. Some of the reactions result in the production of less damaging, or non-toxic, metabolites (e.g., water or molecular oxygen), whereas other reactions may generate chemical entities as toxic as ozone or more (e.g., hydrogen peroxide, hydroxylradical).

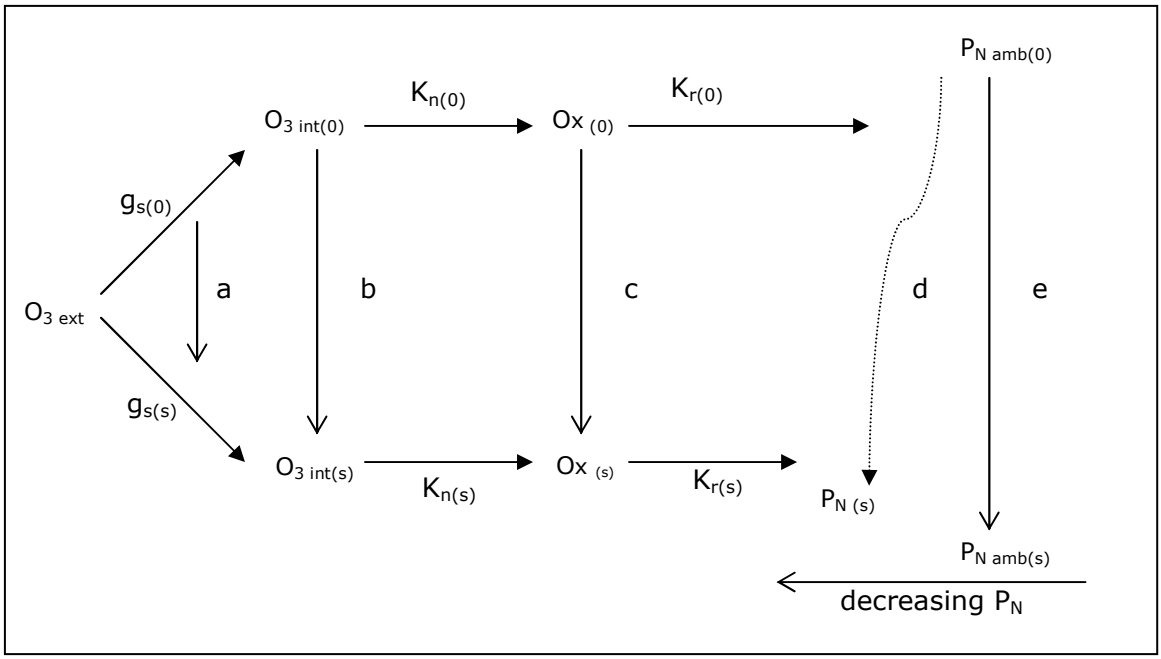


Fig. 2: Schematic model representing interactive processes involved in an ozone-induced steady-state inhibition of net photosynthesis $\mathbf{O_{3\ ext}}$=ambient ozone concentration external to the leaf; $\mathbf{O_{3\ int}}$=intercellular leaf ozone concentration; **Ox**= ozone or metabolites that inhibit $\mathbf{P_N}$; $\mathbf{P_{N\ amb}}$= net photosynthetic rate in ambient air containing no ozone; $\mathbf{g_s}$=stomatal conductance to ozone; $\mathbf{k_n}$=net accumulation rate of ozone or toxic metabolites; $\mathbf{k_r}$=inhibition rate of **Ox** on $\mathbf{P_N}$; **T**=time; **(0)** subscripts represent initial events in ozone fumigation; **(s)** subscripts represent steady-state events (Lefohn, 1992).

The mesophyll flux of ozone and toxic metabolites ($Ox_{(o)}$) ($k_{n(o)}$) is the resultant of those processes producing toxic substances and those involved in detoxification.

As $Ox_{(o)}$ accumulates, the rate of photosynthesis in unpolluted air ($P_{Namb(o)}$) declines along curve d. The characteristics of P_{Namb} reduction depend upon the mechanisms involved, the net rate of the inhibition process (e_s) ($k_{r(o)}$), which also depends upon the cell's (and its organelle's) capacity for repair of biochemical and cytological lesions. The decrease in CO_2 fixation results in an increase in light saturation (C_i) and a subsequent change in stomatal opening through feedback. Stomatal conductance will therefore gradually decrease $\vec{a}$ to a new steady-state level ($g_{s(s)}$). The intercellular ozone flux will also diminish $\vec{b}$ from $O_{3int(o)}$ to the equilibrium rate, $O_{3int(s)}$. Assuming that $k_{n(o)}$ remains unchanged, the mesophyll flux of toxic compounds will then decrease $\vec{c}$ from $Ox_{(o)}$ to $Ox_{(s)}$. The decreased flux ($Ox_{(s)}$) will establish a new net inhibition rate ($K_{r(s)}$), and net photosynthesis will attain an equilibrium rate at $P_{N\ amb(s)}$, determining both $g_{s(s)}$ and $k_{r(s)}$.

The development of a reduced, steady-state $P_{N\ amb(s)}$ rate, caused by ozone exposure, can therefore be viewed as a series of interactive processes whereby ozone influx is regulated by the inhibition of net photosynthesis, the increase in C_i, and the subsequent decrease in g_s (In: Lefohn, 1992).

The model is useful in providing an explanation for differences in tolerance and response. A tolerant genotype may possess a relatively low $g_{s(s)}$, resulting in low intercellular flux of ozone ($O_{3int(s)}$). In addition, high endogenous concentrations of ascorbate, which are all scavengers of ozone or toxic oxygen species, may favor a low accumulation rate (k_n) of intermediate toxic metabolites (Ox). The concentration of Ox may never rise sufficiently to surpass the maintenance/repair capacity within the chloroplast, and net photosynthesis will remain unaffected by ozone exposure (Lefohn, 1992, McKee, 2001, Fiscus, 2005).

2.1.4.13 Effects on Transpiration

Ozone-induced changes in stomatal diffusive resistances will affect the efflux of water vapour as well as the exchange of other gases. Stomatal closure has the immediate effect of decreasing the transpiration rate (E). Stomatal closure induced by ozone may thus serve to conserve water as well as reduce ozone stress by reducing ozone flux. Conversely, there is considerable evidence indicating that drought-stressed plants are less susceptible to ozone than are well-watered plants, because of their increased diffusive resistance, regardless of the mechanism responsible for stomatal closure.

Where E is reduced by stomatal closure, CO_2 flux is reduced, but the flux of H_2O changes proportionally more with changes in g_s. Hence, ozone-induced decreases in g_s may result in increased water-use efficiency (WUE, expressed as g CO_2 per kg H_2O). Although WUE may increase, concomitant reductions in CO_2 assimilation resulting from decreased flux of CO_2 will inevitably lead to reduced growth (Lefohn, 1992, McKee, 2001, Fiscus, 2005).

2.1.4.14 Effects on growth

Plant growth results from the integration and coordination of the processes of photosynthesis, respiration, translocation, and biosynthesis, controlled by the plant's genetic composition and modified by environmental factors (Runeckles and Chevone, 1992). Growth can be readily determined as a gain in weight or size of the plant as a whole or of any of its parts (e.g. fruits, seeds or tubers). Extensive series of field studies in the framework of the U.S. National Crop Loss Assessment Network (NCLAN)-program (summarized in Heck et al., 1988, In: Lefohn, 1992) have provided abundant evidence that exposure to increased ozone levels usually results in decreased growth and yield, if exposure results in decreased photosynthesis. The relative impact on the growth of the different parts of the plant is the result of differential effects on the translocation of assimilates from the leaves (Reich and Amundson, 1985). A frequently observed whole-plant response to gaseous air pollutants in general is that root growth is reduced more than shoot growth, because of reduced translocation to the root, and a consequence is the reduction in the supply of metabolites and energy to heterotrophic microorganisms.

These include both symbionts, such as rhizobium and mycorrhiza, and soil microorganisms in the rhizosphere (Lefohn, 1992, McKee, 2001, Fiscus, 2005).

2.1.4.14.1 The analysis on the effects of ozone on growth dynamics

Many studies of the effects of ozone have been concerned with harvest yields. Haas (1970) appears to have been the first to apply some of the plant growth-analysis-techniques (in his investigation of the relationship between ozone-induced foliar injury and overall growth of bean plants in the field).

Bennett and Runeckles (1977a, In: Lefohn, 1992) examined such relationships in their studies of the long-term effects of ozone on growth of clover (Trifolium incarnatum) and annual ryegrass (Lolium multiflorum). They observed that root growth was most severely impaired (increased S/R ratios). In both species, specific leaf area (SLA) and leaf area ratio (LAR) were reduced, indicating smaller heavier leaves. However, there was no effect on overall relative growth rate.

(R)·LAR and R are linked with unit leaf rate E:

R = E·LAR

In spite of the ozone-induced decrease in LAR with no change in R, no significant increase in E was observed (In: Lefohn, 1992).

Walmsley et al. (1980) partitioned E in order to show that the partitioning of assimilates was persistently reduced by ozone, whereas partitioning of the leaves was significantly enhanced during the middle of the growth period. This implies that the accelerated senescence of the earlier leaves induced physiological changes in the plant which caused more of the available assimilate to be used for the production of new leaf tissue (Lefohn, 1992, McKee, 2001, Fiscus, 2005).

Furthermore they observed that under relatively severe and continuing ozone stress, increased leaf senescence was more than offset by the accelerated development of new leaves. Ozone caused a shift in the occurrence of the maximum LAR, and the new leaves were acclimated to ozone, but the acclimation was not related to stomatal function.

Accelerated senescence and necrosis of leaves are plant responses to various environmental stresses, including air pollutants. Runeckles (1982) used growth analysis concepts in developing the relative death rate, R_d, that defines the dynamics of senescence as a function of the healthy tissue present, according to the relationship:

$$R_d = \frac{1}{M_1} \cdot \frac{dM_d}{dt}$$

Where M_1 and M_d are measures of the living and senescing tissues or organs, respectively, and can reveal how the senescence response varies over time (In: Lefohn, 1992).

Other approaches to the analysis of effects of environmental variables on long-term growth and its dynamics include demographic analysis, sequential yield component analysis, and their combination with plant growth analysis (Lefohn, 1992, McKee, 2001, Fiscus, 2005).

2.1.4.15 Effects of ozone on reproduction

The reproductive yield of many crops is reduced by ozone stress. In some cases because of changes in partitioning, but also because of reduced floral initiation and development, and adverse effects on the success of fertilization, for example, in wheat (Heagle et al., 1979b), and soybean (Heagle and Letchworth, 1982). Kress and Miller (1983) reported decreases in filled pods per plant and seeds per filled pods with increased ozone stress (Lefohn, 1992, Heagle, 2000).

2.1.4.16 Exposure-yield relationships

From the earliest studies of the effects of air pollution on plants, there has been an ongoing interest in defining "dose-response"-relationships for all pollutants. The elucidation of quantitative relationships between exposure and crop yields is essential for purposes of estimating the magnitude of the adverse effects of tropospheric, ground-level ozone pollution on agricultural production, and as an important contribution to the process of establishing standards of air quality.

In defining a relationship between exposure to a gaseous pollutant, such as ozone, and a plant response, two points need to be emphasized:

1. "Exposure" and "dose" are not synonymous (Lefohn, 1992). The former merely defines the condition of the ambient air in which the plant is growing. The latter concerns the amount of pollutant or its products that reacts of the reactive sites within the plant tissue that are adversely affected and

2. exposure in field conditions constitutes a sequence of different instantaneous concentrations that are a consequence of the fluid nature of the atmosphere and the influence of meteorological factors, particularly wind and turbulence.

Approximations of ozone flux rates have been found to lead to statistically improved empirical relationships. Simple linear repressions may be used to define yield responses, but suffer from their inability to reflect the curvilinear nature of the responses observed in most crops.

Runeckles and Wright (1989) proposed a gamma-function model of comparable flexibility to the Weibull model to overcome its limitation, as it is a monotonic-decreasing function,

and the literature contains reports of stimulations of various growth measures caused by exposures to low ozone levels. Their 3-parameter model has the form:

$Y = \alpha \cdot (X+1) \cdot \gamma \cdot e^{-\beta x}$

Where Y is yield, and X is exposure index, Parameter Alpha is the yield at zero exposure. The function does not require that increases in yield occur at low exposures, but can respond to their presence (Lefohn, 1992, Nali, 2002, Elliingsen, 2008).

2.1.4.17 Ozone-flux-dose

Especially in the context of establishing control strategies based on flux-oriented dose-response relationships, O_3 flux measurements and O_3 exchange simulations are needed for representative ecosystems. Meanwhile, it is accepted in the United States and in Europe that there is a need to establish a basis for control strategies based on critical O_3 doses. Any perturbation of plant metabolism depends on the amount of molecular diffusion of O_3 into the leaf interior (uptake or absorbed flux density, $F_{absorbed}$), the integral of which over time t is the pollutant absorbed dose, PAD:

$$PAD(O_3) = \int_{t_1}^{t_2} IF_{absorbed}(O_3) \cdot I \cdot x \cdot dt$$

O_3 uptake, $F_{absorbed}(O_3)$ is only one part of the total flux to the canopy, $F_{total}(O_3)$, which also comprises deposition on external plant surfaces, $F_{external}$ plant surfaces(O_3), and on the soil surface, $F_{soil}(O_3)$.

Because O_3 stomatal uptake cannot be measured directly, it must be modelled. Generally, stomatal resistance for trace gas exchange is parameterised as a function of water vapour or carbon dioxide (CO_2) exchange. Therefore, such models of trace gas exchange between phytosphere and atmosphere need a basis validation by quantification of water vapour and/or CO_2 exchange on canopy level.

In models with multi-layered resolution of vegetation the transfer mechanism are resolved more explicitly than in single source models. As stated by, for example, Raupach and Finnigan (1988) models with a vertical resolution of the canopy are more precise but less useful than models without it. As shown by several authors in the last years water vapour and O_3 exchange simulated by more or less sophisticated big leaf models seem to be able to reasonably reproduce the observed response of canopy (Lefohn, 1992, Nali, 2002, Elliingsen, 2008).

Because any administrative/political measure on European level must be based on risk evaluations as accurate as possible, flux model parameterisations need a validation in two steps:

1. Validation of the parameterisation of stomatal conductance via micrometeorological flux measurements of water vapour.

2. Validation of the parameterisation of O_3 stomatal uptake and non-stomatal deposition via micrometeorological O_3 flux measurements.

This concept requires a limited number of micrometeorological flux measurements sites distributed over Europe.

As mentioned by Grünhage and Jäger (2002) flux-response relationships based on chamber experiments are biased in principle. Exposure-response relationships deduced from chamber experiments show increasing intensities of plant responses with increasing O_3 exposure concentration due to the experimental design (Grünhage, 2002). The chamber environment may affect plant and plant community responses independently of a pollution stress due to the differences in radiation, air temperature and air humidity between chamber and ambient microclimate (Grünhage et al., 1990, Grünhage, 2002).

Taking into account NO emissions from fertilised arable land and their impacts on O_3 deposition, it might be reasonable to improve gas exchange parameterisations based on the big-leaf approach by multi-layer models at some flux measurements sites (Fig.3) Grünhage, 2002). The European O_3 risk assessments have to be based on relatively generalised concepts. Such a generalised approach must be carefully calibrated by well validated models for site level risk assessments flux-based approaches for local scale risk evaluations for crops and natural and semi-natural vegetation are ranked by the degree of their uncertainty as described in Fig. 3 (Grünhage et al., 2002).

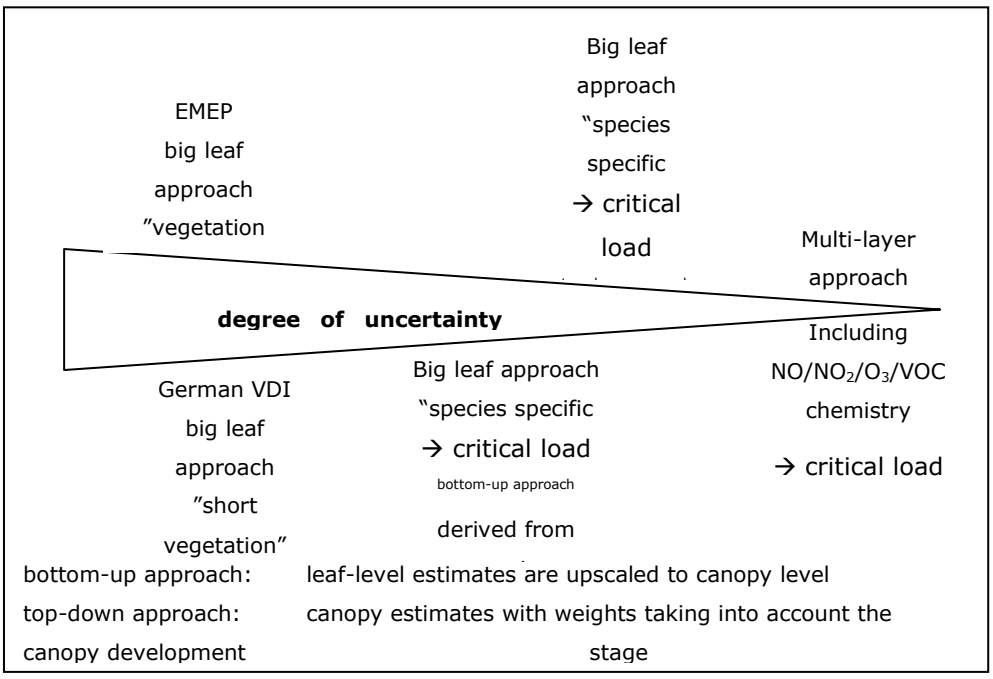


Fig. 3: Conceptual degree of uncertainty in flux-based O_3 risk assessments for crops, natural and semi natural vegetation using measured data (Grünhage, 2002).

2.1.4.18 Effects of ozone coupled with other environmental factors

The effects of ozone on plants are functions of both the severity of the stress induced by ozone itself, and a wide range of factors that influence susceptibility or modify the plant's metabolism and physiology. Some of these factors are inherent, including those dictated by genetic composition and developmental stage at which the stress is imposed, while others relate to abiotic and biotic components of the plant's environment.

2.1.4.18.1 Other pollutants

Lefohn and Tingey (1984) and Lefohn et al. (1987) found that simple co-occurrences of exposure to elevated levels of ozone and SO_2 in ambient air in urban and rural areas were less frequent than sequential or combined sequential/concurrent exposures.

However, where exposure conditions have been realistic in terms of their likelihood of occurrence in ambient air, certain responses are clear: For example, ozone and SO_2, at sufficiently high levels, act synergistically in causing acute injury (In: Lefohn, 1992). The symptoms resembled those of ozone rather than those of SO_2.

Runeckles and Palmer (1987) exposed bean, mint, radish and wheat plants to NO_2 followed by ozone. With radish and wheat the sequence acted synergistically, reducing shoot growth more than ozone alone (NO_2 alone increased shoot growth). On the other hand, pre-exposure to NO_2 reduced the negative impact of ozone (Lefohn, 1992).

2.1.4.18.2 Plant nutrition

It has long been known that plant response to ozone can be influenced by nutritional status. The non-specific term "influenced" is appropriate because of the diversity of effects that have been reported. Reports exist that indicate that sensitivity increases with both increasing and decreasing levels of general soil fertility and that adverse effects of ozone on growth may be ameliorated or enhanced. Nevertheless the bulk of the evidence suggests that optimal nitrogen minimizes, and nitrogen deficiency increases, foliar injury.

Cowling and Koziol (1982) felt that there was sufficient evidence to suggest that, in spite of the discrepancies among the various findings, the interactive effects of ozone and fertility were probably caused by different levels of fertility shifting the plant's soluble carbohydrate pool away from the optimum. Fertility may also influence response of ozone indirectly, as in field situations, adequate nutrition results in denser canopies that will affect uptake, which could lead to compensatory effects favouring the growth of less-sensitive individual genotypes (Lefohn, 1992, Loreto, 2001, Sild, 2002, Goodman, 2003, Agrawal, 2007).

2.1.4.18.3 Drought stress

Drought stress is frequently an important modifier of plant response to ozone. Susceptibility has generally been found to decrease inversely with the level of stress, as reviewed by Heagle et al. (1988).

Where interactions occurred, growth was usually more severely reduced by ozone in plots with adequate water supply, but in some study years, there was no significant influence of drought stress on yield reductions due to ozone. Greenhouse studies with potted plants have also shown the reduced impact of ozone on plants provided with limited water supply.

Several studies examined the effects on components of growth and yield, and Moser et al. (1988) developed ozone-response curves for various components, to further demonstrate the importance of the time of occurrence of drought stress during plant development on the nature and magnitude of the interaction.

Where drought stress has been observed to reduce the adverse effect of ozone on yield, the weight of the evidence indicates that this is related to stomatal closure (Lefohn, 1992, Bender, 1999, Khan, 2005, Hassan, 2006).

2.1.4.18.4 Other abiotic factors

Darrall (1989) has reviewed the effects of light intensity and CO_2 levels and pointed out that, although it is under high light intensities that the potential for the endogenous production of harmful oxyradicals is greatest, and they may combine with ozone derived radicals to overcome scavenging capacity, at low intensities, overall CO_2 assimilation may limit the scavenging of energy for maintenance and repair (In: Lefohn, 1992).

Ambient humidity can play a role in determining ozone uptake. There are several reports of increased susceptibility to ozone, both short and long term, resulting from increased humidity (Lefohn, 1992).

2.1.4.18.5 Biotic factors

Various biotic and ecological factors play important roles: the incidence and severity of disease and pest infestations, the establishment of symbiotic relationships, and the competition for resources. These relationships may involve individuals or populations of different species, each of which may be differentially affected by ozone.

2.1.4.18.5.1 Diseases

Plant-pathogen relationships may also be affected by ozone as a result of effects on the host plant, on the pathogen, or on both. Such effects may lead to stimulations or inhibitions of disease incidence or severity.

The diverse interactions between fungal pathogens and ozone on the leaf surface have been discussed by Dowding (1988) and include effects on cuticular chemistry and surface properties, exuded materials, and stomatal response. The growth and development of the host may be inhibited directly by ozone, since ozone toxicity has been observed in axenic culture.

Following infection, ozone may influence the severity of the disease through its effects on the host plant. Conversely, the development of the pathogen may affect the susceptibility of the host plant.

There have been numerous reports of reduced susceptibility to ozone being conferred by infection with viruses and with fungi: for example Puccinia graminis on wheat (In Lefohn, 1992).

There is considerable evidence indicating that exposure to ozone can reduce infection, invasion, and sporulation of fungal pathogens. However, examples also exist of increased infection of ozone-injured plants. This diversity of response is not surprising in view of the complex interrelationships involved in which the genetics of the host and pathogen play important roles, but one generalization can be made about the impact of ozone on diseases, pathogens that can benefit from injured host cells and disordered transport mechanism will be enhanced by earlier exposure of the host to ozone, while those that depend on "healthy" host tissues will be disadvantaged (Lefohn, 1992).

2.1.4.18.5.2 Insects and related pests

The topic can be sub-dividend into the influence of ozone on insect attack and population dynamics, and the converse effects of insect attack on plant response to ozone. Host-plant resistance to attack may be modified through metabolic changes that affect feeding preference and insect behaviour, development, and fecundity. Ozone-induced changes in both major and secondary metabolites may be qualitative and quantitative, and while there is abundant evidence that such changes can influence insect growth and development, there have been few experimental investigations of the specific effects of ozone (Lefohn, 1992).

For example Trumble et al. (1987) reported that the tomato pinworm (Keiferia Lycopersicella) developed faster on ozone-injured tomato plants. The Mexican bean beetle (Epilachna varivestis) preferentially selected soybean foliage that had been exposed to ozone (Lefohn, 1992).

2.1.4.18.6 Weed and plant competition

Although in many crop situations, competition from weeds contributes more to yield losses than any other factor, there have been few studies of its effects in crop mixtures.

Grass-legume studies have confirmed the fact that ozone exposure results in a shift in mixture biomass in favour of the grass species (Bunce, 2000). Rebbeck et al. (1988) observed that a 40% reduction of the ambient ozone level (averaged 0,048 ppmv over 12 daytime hours) resulted in a shift towards dominance of clover (Lefohn, 1992).

2.1.4.19 Genetic variability and protection against ozone

The diverse responses to ozone exhibited by different species and cultivars are phenotypic expressions of genotypes that have evolved naturally or have been selected by plant breeders. The variation in susceptibility to ozone that is under genetic control permits the selection of resistant types as a means of minimizing the adverse effects of tropospheric ozone. Furthermore, since sensitivity is also influenced by numerous environmental factors, many of which can be managed (e.g., fertility, water availability), the judicious selection of appropriate cultural practices may also minimize the adverse impact of ozone on crops. Normal crop production practices continue to make frequent use of various agricultural chemicals in weed, disease, and pest management programs, and studies showed that several fungicides and insecticides had antioxidant properties that could provide some degree of protection against ozone field. Natural and synthetic compounds have been found to be capable of conferring some degree of protection against ozone. These have ranged from the plant growth substances, kinetin and abscisic acid, to commercial antioxidants such as ascorbic acid derivatives and n-propyl gallate, the experimental ethylene diurea, which has to be developed for commercial use (Lefohn, 1992, Rötter, 1999, Black, 2000, Smirnoff, 2007).

2.1.5 Ozone standards and their relevance for protecting vegetation:

In order to develop ozone standard(s) that provide an adequate measure of protection to vegetation, it is necessary to define, in as precise terms as possible, the relationship between ozone exposures and the potential for adverse effects on vegetation (Lefohn, 1992). It is important to define clearly what the proposed standard is designed to protect. Tingey et al. (1989) have discussed the definition of "adverse" as the word relates to the standard-setting process, and point out that injury encompasses all measurable plant reactions, such as reversible changes in metabolism, reduced photosynthesis, leaf necrosis, leaf drop, altered quality, or reduced growth, that do not influence agronomic yield (Lefohn, 1992).

Damage, on the other hand, includes all effects that reduce the intended human use or the value of the plant ecosystem.

Exposure indices are important because they form the linkage between air quality standards that are promulgated to protect specific targets and the actual dose that is responsible for eliciting an effect (Lefohn, 1992).

Long-term seasonal average concentrations (e.g., 7- or 12 h average concentrations) do not correlate strongly, at most ozone monitoring sites, with the components of exposure regimes that are most important in affecting vegetation. Before an ozone standard can be established to protect vegetation, the actual pollutant levels below which plants will be protected must be identified. Guderian et al. (1985) have pointed out that plant growth is influenced more by concentration than by exposure duration, when similar products of concentration and time are used (Lefohn, 1992).

Although all plants are capable of being adversely affected by exposure to phytotoxic gases and particulates in polluted air, the nature of the response can be extremely variable.

Any ozone exposure index that is used to describe those regimes that cause vegetation effects must be able to characterize adequately the upper tail of the hourly average-distribution curve. It appears that a combination of indices may be required to adequately describe the upper end of the distribution curve.

Krupa and Nosal (1989) have discussed the use of the application of spectral coherence analysis to develop multiple-parameter indices to describe the relationships between ozone exposure and crop growth (Lefohn, 1992).

By addressing simultaneously acidification, eutrophication and ground-level ozone, the 1999 Gothenburg Protocol (Kärenlampi and Skärby, 1996) was the most comprehensive protocol under the Convention on Long-range Transboundary Air Pollution (CLRTAP) and the first that specifically addressed the control of ozone pollution. The basis of effects-based approach used for the Protocol was the bringing together of quantitative information on effects (critical loads and levels), emissions (from reported information), pollutant loads and levels (calculated from emissions using atmospheric transport models) and costs of abatement technology (described in cost curves for emission abatement for each country). Hence, relatively simple critical levels of ozone (level I) were used for integrated assessment modelling, even if it was recognized that they were provided only an approximate solution. In 1999, several options for a level II approach were identified at the workshop in Gerzensee, Switzerland. There was general agreement that the development of a flux-based approach should be the long-term goal of a level II approach. The Executive Body for the Convention at its nineteenth session (2001) decided "to schedule the review of the Gothenburg Protocol, in view of its expected entry into force in 2003, to commence in 2004" (ECE/EB.AIR/75, para. 69 (a)). The Working Group on Effects at its twenty-first session (2002) approved the updated medium-term work-plan for the future development of the effect-oriented activities, containing, inter alia, the following tasks (EB.AIR/WG.1/2002/4, para 8).

1. Further develop the ozone flux-effect model for clover, including validation with data from the 2001 and 2002 seasons, and finalize flux-effect models for wheat and potato;

2. Establish new (level II) or revised (level I) critical levels of ozone for crops, semi-natural vegetation and trees.

The decisions are taken must be applicable to a range of vegetation types across the different climates of Europe and consistent with transport model outputs and must lend themselves to integrated assessment modelling and economic assessments (Radovan Chrast, Keith Bull, Heinz-Detlef Gregor, 2002).

2.2 Relevant examples of ozone injury on crops in wheat

Pleijel et al. (1996) found that ozone exposure is much more effective in decreasing the grain yield of wheat between the onset of anthesis and end of grain filling than before anthesis. The increase in the protein content of the grain, which has been associated with ozone effects on the grain yield of wheat in a number of studies, was found only in the NF post treatment. This confirms the conclusion that the post-anthesis period is more sensitive to ozone exposure in wheat than the pre-anthesis period.

Soja et al. (1996) studied the interaction of ozone exposure with drought stress in winter wheat. Abundant water supply made the plants most sensitive to ozone exposure, and AOT 40 ozone dose of 27.7 ppmh caused a reduction of 35% in grain yield. At intermediate water availability the same ozone dose reduced yield only by about 15%, whereas under severe drought stress no ozone-induced yield reduction at all could be observed. They concluded that soil water availability should be considered in the definition of critical level of ozone.

Krupa et al. (1993) examined 73 crop harvests-data from OTC-experiments carried out from the National Crop Loss Assessment Network (U.S. NCLAN). 77% of the examined harvests showed no statistically significant yield differences between NF and AA treatments. Univariate linear regressions between various O_3 exposure parameters and per cent yield losses in NF showed that the cumulative frequency of occurrence of O_3 concentrations between 50 and 87 ppb was the best predictor (adjusted R_2=0.712, p=0.011).

Fangmeier et al. (1992) examined spring wheat, which was exposed to different levels of O_3 and water supply in OTC. The plants were grown either in CF, NF and in CF-air with proportional addition of ambient ozone (CF_1) as well as with twice proportional addition of ambient ozone (CF_2). O_3 enhanced senescence and reduced growth and yield. At final harvest, dry weight reductions were mainly due to reductions in ear weight. Grain yield loss by ozone mainly resulted from depressions of 1000 grain weight, whereas numbers

of ears per plant and of grains per ear remained unchanged. Water stress alone did not enhance senescence, but also reduced growth and yield.

Fuhrer (1995) derived a correlation factor (f_{water}) for soil water availability in spring wheat by applying a simple model to predict the mean available soil water content (%FC) from precipitation and potential evapotranspiration, an empirical relationship between %FC and yield, and by assuming that the decrease in grain yield due to reduced soil water is proportional to the reduction in ozone sensitivity. The model was also used to analyze the variability of f_{water} across Europe. It is concluded that without introducing a correlation factor, under dry conditions potential yield losses may be significantly overestimated.

2.3 Critical levels for ozone

A critical level is defined as the "concentration of a pollutant in the atmosphere above which direct adverse effects on receptors may occur according to present knowledge" (UBA, 1996).

Critical levels for ozone have been established in recent years (Fuhrer and Achermann, 1994, Kärenlampi and Skärby, 1996), and maps showing the spatial distribution and the extent of exceedance have been produced both at the national and international level. As such maps become available, two questions must be raised:

1. Are there negative effects on plants in areas where the critical level is exceeded?
2. How certain can we are that the prediction of negative impacts is correct?

What is needed to answer these questions is called *Ecological Risk Analysis*, which can be defined as "process that evaluates the likelihood that adverse effects occur or are occurring as a result of exposure to one or more stressors". In this context, "stressors must have (1) the ability to cause adverse effects and (2) to co-occur with or contact an ecological component long enough and at sufficient concentrations to cause the identified adverse effects" (USEPA, 1992). Adverse effects can be detected as changes at any level of biological organisation, ranging from molecular to the ecosystem level. According to Guderian et al. (1985) adverse effects can be classified into injury and damage. Injury includes all measurable effects that do not influence yield or biomass, and damage includes all effects that reduce the intended value. In the context of ozone risk analysis, Tingey et al. (1990) considered adverse effects as a loss in (i) economic production, (ii) ecological structure, (iii) genetic resources, and (iv) cultural values. In the framework of the UN/ECE Convention on LRTAP, and as the basic for the revised Air Quality Guidelines of the WHO, the main adverse effects presently considered are:

1. loss of economic yield (crops)
2. reduction in biomass or growth (trees)

3. change in the relative species composition or seed yield (semi-natural vegetation)

The choice of specific adverse effects is mainly based on the effects of ozone observed in experiments on the various receptors, and on the ecological and economic importance of changes in these parameters. The term "effect" implies that a particular experimental treatment causes a change in the selected parameter, as compared to the control treatment which should reflect the unpolluted reference. Hence, not only the choice of the assessment endpoint, but also the choice of the reference is an important determinant for the outcome of the risk assessment. For ozone, there is considerable difficulty in defining the "unpolluted" reference; it is likely that the reference varies with latitude and altitude.

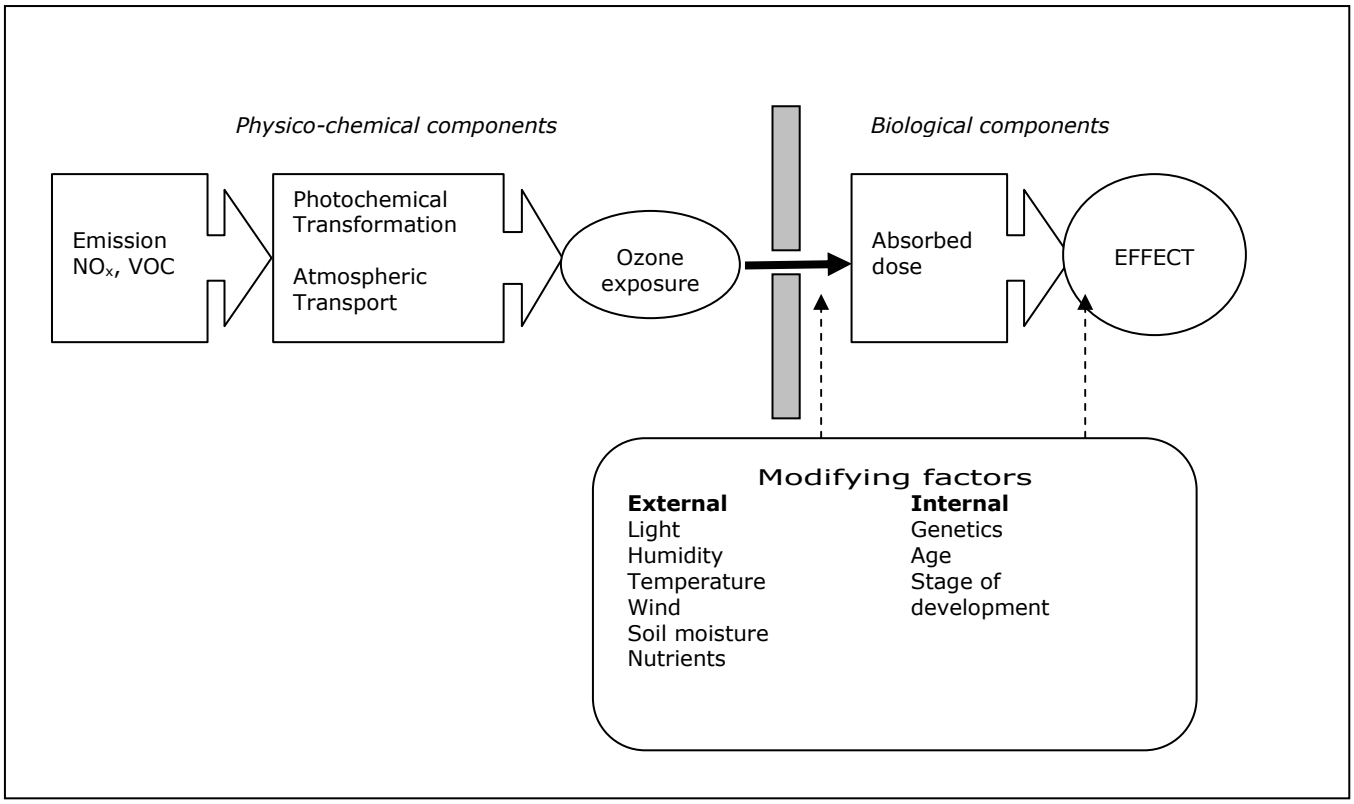


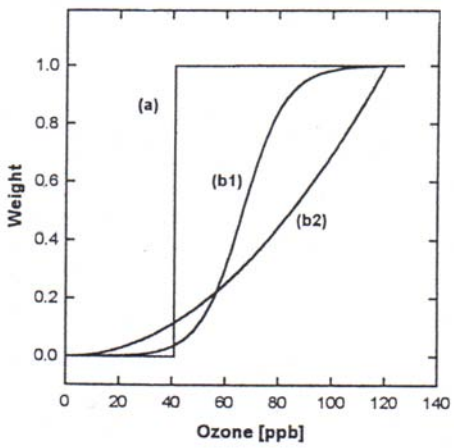
Fig. 4: Conceptual model for ozone risk analysis (Fuhrer, 1993)

Fig. 4 shows a conceptual model for ozone risk analysis, which is composed of physical-chemical components, including ozone exposure, and biological components, including ozone uptake, ozone effects, and the influence of modifying factors on both uptake of ozone and effect. Physical-chemical and biological components are separated by the boundary between outside and inside of leaves, i.e., the leaf surface, and the flux across the boundary is controlled by stomatal conductance (Fuhrer, 1996).

In order to define the critical level for a particular pollutant-receptor combination, exposure-response information is essential. This can be obtained experimentally by using different exposure systems, or by field observation (Sanders et al., 1995). In order to

construct exposure-response relationships it is necessary to characterize ozone exposure by using a single statistical index, which efficiently captures those components of exposure which are biologically most effective. The most suitable exposure indices to be related to long-term effects are cumulative, i.e., they integrate exposure over time. This was shown both for crops and for forest trees.

Previous air quality guidelines and critical levels for long-term effects have been based on mean concentrations over a fixed period of time, e.g., the arithmetic mean over the growing season (e.g., April to September) of the daily mean concentrations for a specific 7-h period (usually 09.00-16.00). The use of a mean concentration implicitly gives equal weight to all concentrations. However, experimental exposure-response studies with ozone suggest that this is not appropriate; it was shown that it is the intermittent exposure to higher concentrations which is most important in causing long-term effects (Lefohn, 1992). This can be explained physiologically by the capacity of the plant to detoxify ozone and other oxidants; it is only when the concentration of flux, of ozone exceeds this capacity that adverse effects result.



Discontinuous and continuous weighting of ozone concentrations.
Discontinuous weighting of concentrations depends on the choice of the cut-off concentration, i.e. 40 ppb.
Continuous weighting depends on either a sigmoid weighting function (b1) or an allometric weighting function (b2).

Fig. 5: Discontinuous and continuous weighting of ozone concentrations (UBA, 1996)

This phenomenon can be incorporated into the exposure index by using procedures for continuous or discontinuous weighting of concentrations. Fig. 5 illustrates two possibilities: (a) The use of a discontinuous weighting procedure. This approach assigns the weight of 0 to all concentrations below a cut-off value and a weight of 1 to all concentrations above the cut-off (b). The concentration can be weighted with a sigmoid function, i.e. a multiplicative weighting factor between 0 and 1, which depends on concentration, is used for each hourly O_3 concentration (b1). Alternatively, by using an allometric weighting function the concentration is raised to a power (b2). In theory the latter procedure, using continuous weighting, gives in theory a better representation of

exposure. An index based on a sigmoid function which has been used successfully to fit exposure-response data is referred to as W126 and considers concentrations above 30 ppb (Lefohn et al., 1988). In order to calculate a cumulative exposure index, such as the AOT (accumulated over threshold), the positive differences between the actual hourly mean concentration and the cut-off concentration are summed for the exposure period of interest.

An alternative cumulative-type index often used in the US is the sum of concentrations equal or higher than a cut-off, for instance 60 ppb (SUM06) (Lefohn and Foley, 1992). As opposed to the calculation of an AOT, a SUM index considers the total hourly concentrations instead of the difference between the cut-off and the measured value. Hence, the AOT index focuses more directly on the portion of exposure which is thought to be biologically effective. This concept was adopted at the UN/ECE workshop at Egham in 1992, when a threshold concentration of 40 ppb was tentatively suggested (Ashmore and Wilson, 1993). The exposure index AOT40 is expressed in units of ppb.h or ppm.h. Subsequent statistical analysis of yield data from European open-top chamber experiments has demonstrated that the use of these thresholds generally provides better linear fits to exposure-response data than the use of higher thresholds (Fuhrer, 1994). A linear exposure-response relationship provides a sounder statistical basis to define critical levels corresponding to a specific effect than do other types of exposure-response relationships (Sanders et al., 1995).

Effects of ozone depend on ozone uptake, and it has been suggested that ozone exposure indices should be replaced by flux estimates (Grünhage et al., 1993). In OTCs, ozone flux measurements have been carried out (Grandjean Grimm and Fuhrer, 1992) and it is possible to relate the mean ozone flux density, or mean ozone uptake, to AOT40. The results indicate a direct relationship between the two parameters suggesting that AOT40 can be used as a surrogate for ozone flux. Under ambient conditions, ozone flux is controlled by atmospheric and boundary layer resistance, and high ozone concentrations may or may not be associated with high ozone flux (estimations show an about 20% higher ozone flux in OTCs than under field conditions) (Grünhage and Jäger, 1994). But it has been recognized, that the ozone flux concept could not be applied to critical levels because of a lack of quantitative relationships between ozone flux and long-term ozone effects under field conditions.

Based on the suggestion made at the Egham UN/ECE Workshop, results from recent European OTC experiments with a limited number of agricultural crop species were used to test the relationship between the cumulative exposure index using a threshold of 40 ppb (AOT40) and grain or seed yield. Indices with other cut-offs, such as AOT30 and AOT60, were included for comparisons. These experiments (data from eight experiments were used; Fuhrer, 1994) represented a range of site conditions with locations from

southern Sweden to Switzerland. The data from all experiments were combined and subjected to linear regression analysis. Based on the resulting regression equation, the AOT corresponding to different levels of relative yield reductions were calculated. For a 10%-reduction in grain, the corresponding AOT40 value was 5300 ppb.h, the corresponding AOT30 and AOT60 values were 6800 ppb.h, and 2700 ppb.h, respectively. The coefficient of determination was not different when using AOT30 (r_2=0.91) or AOT40 (r_2=0.91), but it was lower when using AOT50 (r_2=0.88) and much lower when using AOT60 (r_2=0.79). This shows that concentrations between 40 and 60 ppb make an important contribution to the observed change in yield and should not be ignored. An introduction of additional data did not change the regression equation, hence, using the AOT40 proved to be a robust exposure index for grain yield of wheat. This may not imply that relationships with other plant traits would be equally good, whereas the yield of grass-clover mixtures was best related to an AOT with a very high cut-off (AOT110) (Nussbaum et al., 1995, Fuhrer, 1996)

But already in the Mapping Manual (1996) it is stated that this level-I critical level is only a minimum requirement. Local factors such as crop species and cultivar, phenological stage, soil moisture deficit, co-occurrence with other pollutants and climatic factors (vapour pressure deficit and wind speed) should be taken into account in calculating site-specific critical levels of ozone (Posch and Fuhrer, 1999).

The existence of differences in sensitivity to ozone at contrasting stages of plant development, as well as to other environmental stresses, has been recognized for many years (Soja, 1996; Lyons and Barnes, 1998). Consequently, "weightings" for phenological growth stage were included in the Mapping Manuel of critical levels as one of the factors desirable in a Level II approach (Kärenlampi and Skärby, 1996, Soja, 1999).

The role of the United Nations Economic Commission for Europe International Cooperative Programme on effects of air pollution and other stresses on crops and non-wood plants (UN/ECE) ICP-Crops is to monitor and document the effects of ambient air pollution on crop and more recently, non-wood plant species, throughout Europe. Since its initiation, 22 Parties of the Convention on LRTAP-Convention have participated in the programme. The crops of primary interest to the ICP-Crops are from the leguminosae family (Trifolium repens). Characteristic injury symptoms such as bronzing, chlorosis and necrosis of leaf foliage have been widely reported throughout Europe on these and other ozone sensitive species (Sanders and Benton, 1995).

Two critical levels for ozone were defined for agricultural crops, natural vegetation and forest trees at level I at the UN/ECE Critical Levels for Ozone Workshop held in Kuopio, Finland 1996. These were (i) a long-term critical level which would cause reductions in

biomass or yield if exceeded and (ii) a short-term critical level which, when exceeded, would cause visible injury.

For agricultural crops the long-term critical level at level I was defined as an AOT40 of 3000 ppb.h calculated for daylight hours, defined as calculated global clear-sky radiation above 50W/m^2, and for three months. If a fixed period is needed for the assessment then the months should be May-July. Otherwise the running three months ozone AOT40 should be calculated, and the most appropriate value for the crop of interest should be used.

The short-term critical levels for agricultural crops were defined on the basis of visible injury and set at AOT40 values of:
500 ppb.h over 5 days for high vapour pressure deficit conditions
200 ppb.h over 5 days for low vapour pressure deficit conditions
based on the analysis of data from white and subterranean clover (Benton et al., 1996).

The Level I critical level is applied to ensure protection of all crops.

A Level II approach is essential for any accurate assessment of the impacts caused by exceedance of critical levels of ozone, e.g., yield loss.

At the workshop in Gothenburg, 2002, it was concluded that for the time being, it is only possible to derive flux-based ozone critical levels for the crops of wheat and potato, using the multiplicative algorithm based on the methodology described by Emberson et al. (2000b).

During the workshop on critical levels of ozone 2006, organized by the Economic Commission for Europe, the flux-based concept was further developed. The workshop proposed the flux-based approach as a common method to assess the risk of effects of ozone on ecosystems in integrated assessment modelling.

The quantitative indicator for flux is $AF_{st}Y$, the accumulated stomatal flux of ozone above a flux threshold of Y nmol m^{-2} s^{-1} per unit projected leaf area (Economic and Social Council, 2006).

Stomatal flux-based critical levels (Cle_f) for ozone take into account the varying influences of temperature, water vapour pressure deficit, light, soil water potential, ozone concentration and plant development on the stomatal flux of ozone and therefore provide a estimate of the critical amount of ozone entering through the stomata and reaching the sites of action inside the plant. The hourly mean stomatal flux of ozone based on the projected leaf area, F_{st} (in nmol m^{-2} s^{-1} PLA), is accumulated over a stomatal flux threshold of Y nmol m^{-2} s^{-1}.

The accumulated stomatal flux of ozone above a flux threshold of Y ($AF_{st}Y$), is calculated for the appropriate time-window as the sum over time of the differences between hourly

mean values of F_{st} and Y nmol m^{-2} s^{-1} PLA for the periods when F_{st} exceeds Y. The stomatal flux-based critical level of ozone, Cle_f mmol m^{-2} PAL, is then the cumulated stomatal flux of ozone, $AF_{st}Y$, above which direct adverse effects may occur according to present knowledge. Values of Cle_f have been identified for wheat and potato (Mapping Manual, 2004).

2.3.1 Criteria of assessment

In order to protect human life and the vegetation the legal emission-limit values for O_3 and other air pollutants in Austria are established as follows:

a) Ozone-threshold values according to the "Ozongesetz" BGBl.Nr.210/1992/2003

		Human Health	Vegetation
	MW1 (µg/m^3)	MW8 (µg/m^3)	AOT40 (µg/m^3.h)
Information Threshold	180		
Alarm Threshold	240		
Target Threshold (from 2010 onwards)		120*	18000
Target Threshold (from 2020 onwards)		120	6000

*May not exceed in the mean over three years on not more than 25 days/year.

b) Austrian "Immissionsschutzgesetz Luft" (IG-L) BGBl. 1997/115, BGBl. 2001/62, BGBl. 2003/34, BGBl. 2006/34

The Austrian „Immissionsschutzgesetz Luft" states the extreme value for the following emissions in kt/year:

SO_2	39
NO_x	103
VOC	159
Ammonia	66

Alarm values:

SO_2: MW3-500 µg/m^3

NO_x: MW3-400 µg/m^3

Target values:

PM10: TMW-50 µg/m^3 (max. 7x per year to be exceed): JMW: 20 µg/m^3

NO_2: TMW-80 µg/m^3

Ozone:

Information Threshold: 180 µg/m^3 as MW1

Alarm Threshold: 240 µg/m^3 as MW1

Target Threshold for a permanent protection of human health:

MW8 (floating) 120 µg/m^3
(may not exceed in the mean over three years on not more than 25 days/year)

Target Threshold for the protection of vegetation:

AOT40 of 18,000 µg/m^3.h, calculated from MW1 between May and July, as a mean over 5 years.

Long-term target for the protection of vegetation:

AOT40 of 6,000 µg/m^3.h, calculated from MW1 between May and July.

2.3.2 Open Top Chambers (OTCs)

In response to the observed excessive increase in daytime temperature and the lack of exposure to ambient rainfall in closed field chambers (the first version on field chambers developed from Thompson and Taylor, 1969), Heagle et al. (1973) and Mandl et al. (1973) developed large cylindrical OTCs. Charcaol-filtered ambient air is blown into the lower part of the OTCs at a velocity that permits it to rise within the chamber and exit through the open top. This reduces ingress of ambient air from above the OTC and prevents problems with daytime increases in temperature (Skärby et al., 1986). The Heagle-design has been the most popular one, in use for more than 25 years and also used in the Austrian Research Centers Seibersdorf.

The Heagle OTC is a large cylinder (3.05m in diameter, 2.44m high, made of channelled aluminium framework, and covered with upper and lower panels of clear, flexible polyvinylchloride film. The upper panel is a single layer. The lower panel is double layered, with six rows of 2.5 cm diameter holes at 17 to 19 cm intervals on the inner layer of the panel. An air inlet duct is attached to the lower double panel. Air entering from a fanbox is blown into this lower panel via the inlet duct. This inflates the lower

double panel; the air passes through the holes of the inner panel into the chamber, and then moves upward within and out of the chamber through the open top. Approximate velocity of air movement through the chamber is 43 m^3/m, with two or three exchanges per minute. Incoming air is passed through a particle (dust) filter and is designed as nonfiltered air (NF). Activated charcoal elements can be added to the fanbox to produce charcoal-filtered air (CF). Under conditions of low wind velocity, the CFs may revove 50-60% of the ambient O_3. Downdraft incursions of ambient air into OTCs increase with wind velocity, causing problems (Skärby et al., 1986).

OTCs have been used for many years, allowing the accumulation of considerable information about their performance under a variety of conditions. Some advantages and disadvantages of the use of OTCs are summarized in Tab. 2.

Advantages	**Disadvantages**
Widely used with 15+ years historical record, especially for CF/NF comparison	Problems with comparison of results from CF, NF, and AA[a]
Crops can be grown to maturity in the field	Limited space in chambers Long-term use may mask effects in perennial plants and trees
Dose-response studies at concentrations above ambient can be made by O_3 additions	Microclimate effects may affect results, e.g., soil moisture problems, changes in insect and disease incidence
Each OTC is cost effective, portable and durable	The many OTCs required for field work are expensive and labor-intensive. Each chamber requires a 20-amp circuit. Use leads to increased plant growth in cool seasons and winter, compared to AA plants

[a]CF = charcoal filtered; NF = nonfiltered; AA = ambient air plot.

Tab. 2: Some Advantages and Disadvantages of the Use of Open-Top-Chambers (OTCs) (Lefohn, 1992)

OTCs were originally used to compare plant responses to CF and NF under field conditions (Heagle, 1989). CF was found to increase yields of crops, such as wheat or field corn, when comparisons were made with yields from NF. Proportional or fixed concentrations of O_3, separately or combined with other pollutants, can be added to OTCs. This allows dose-response studies in the field with a range of concentrations of O_3 about the ambient (In: Lefohn, 1992).

OTC-dose-response studies have been used to predict how changes in future, elevated ambient O_3 concentrations will affect plant yield.

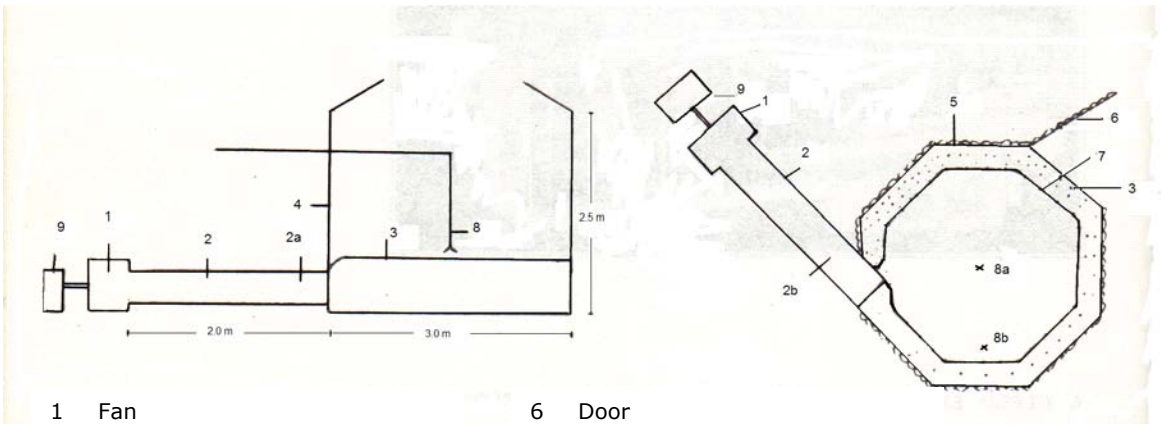
Chamber effects have been intensively investigated by Heagle et al. (1988), Manning and Keane (1988), Heagle (1989). A condensed summary of chamber effects is presented in Tab. 3.

In terms of plant response, decreases in light and changes in rainfall distribution seem to be most important variables. Chamber effects need to be investigated in a systematic way to determine their influence on plant responses (In: Lefohn, 1992).

Parameters measured	**Changes**
Activated charcoal (CF)	50 – 75% reduction in ambient O_3,increase in NO over ambient, and considerable reductions in NO_2, PAN, and SO_2
Particles (dust) (NF)	5 – 10% reduction in ambient O_3, some reduction in SO_2
Gas exchange	
Canopy resistance	
CO_2 uptake	
Leaf boundary layer resistance	
Stomatal conductance	
Microclimate	
Air turbulence (due to ingress of ambient air)	Greater in downwind half of the chamber
Dew point	0.5° – 2.0°C higher
Light	Decrease of 12 – 20%, especially with dirty plastic, sun shadows possible
Relative humidity	5 – 10% increase or decrease, usually less than 5%
Temperatures air	2.0° – 3.7°C increase, usually less than 2.0°
Leaf	Slight increase
Wind speed	Decrease
Factors	Changes
Microclimate effects (not defined)	Plants in NF may yield less than in AA
	Plant in NF are often taller than in AA; chambers can delay leaf senescence and shoot maturity in grapevines
Pesticide usage (usually increased)	Unusually low occurrences of diseases, insects and mites
Pollination problems	Fewer bees in chambers reduces pod set and seed yields in broadbeans
Position effects in chambers	Higher yields may occur in northern rather than southern half of chambers

Tab. 3: Open-Top Chamber Effects (Lefohn, 1992)

2.3.2.1 Technical data



1	Fan	6	Door
2	Duct	7	Sheep- fence
2a	Monitoring point for air flow	8	Monitoring point
2b	„ „ „	8a	Ozone and NO_2-concentration, temperature, relative humidity
3	Manifold (polyethylene)		
4	Aluminium frame	8b	Ozone and NO_2-concentration
5	PVC corrugated hard plastic	9	Ozone generator

Fig.: 6 Schematic picture of an Open Top Chamber (Air Pollution Research Report 5, 1986)

The OTCs used for this project have a total height of 3.1m with a rain-exclusion cap, which was used to reduce the ingress of rain and are 2.95 in diameter made of channelled aluminium framework, and covered with upper and lower panels of clear, flexible polyvinylchloride film. From 9^{00} to 17^{00}/7 days the week the plants were fumigated with additional ozone of 50ppb. All chambers were unfiltered.

ad a) For the project, OTCs with axial-blade fans, 2000m^3/h, 0.5kW were used;

ad b) The duct was made of galvanized iron, 3m long and 0.3m in diameter. To measure air flow it was necessary to have a laminar air flow. This was obtained by putting a blade inside the duct;

ad c) The manifold was made of polyethylene. There were 5 holes distributed above each other and 45 around the OTC with a diameter of 3cm. Around the manifold there were no holes, the height above ground was 0.4m and the upper height 0.7m. The final height of the manifold was 1.1m.

ad d) Additional ozone was produced by a generator from Fischer 502 D**.** Ozone was delivered into the air stream between the fan box and the chamber via a Teflon-tube (inside-diameter of 4mm, outside-diameter of 6mm). The air stream entered through a double-walled section of the cover which encircled the chamber to a height of 1.2m. Air left the chamber through the top opening at positive pressure. The ventilator also worked in those OTCs, which were not fumigated in order to avoid stagnant air.

ad e) In two chambers air sampling was done from two different positions at canopy height. The chamber ozone concentration is determined by sequential sampling of the

chambers. The ozone data are collected by an automated data acquisition system and recorded as a mean value. From a point outside the OTCs, nearby the chamber, an ambient air sample was taken**.** The air temperature was measured in two OTCs as well as outside nearby the chambers in the shade. General weather and air pollution data were measured and recorded at a monitoring station on the site of the ARC Seibersdorf.

ad f) The ozone concentration in the OTCs, as well as the ambient air samples were controlled by a Horiba APOA 350E. The graph recorder Endress + Hauser (Chroma-logger, Line- graph recorder) recorded constantly the O_3-values within the OTCs as well as date and time. Primary-bottle relief, secondary-bottle relief, the pressure within the ozone-generator**,** gas-throughput within the ozone-generator and governor setting in ppb were controlled and recorded one time a day. The ambient air samples were constantly recorded by the Datalogger DL 2 Delta-t devices, Cambridge, England. O_2 was supplied by Linde Co.

2.3.3 Stomata

Stomata (singular stoma), sometimes anglicised as stomatas, provide an essential connection between the internal air spaces of plants and the external atmosphere. The external surfaces of most herbaceous plants and the leaves of woody plants are covered with a waxy layer of cutin, which is relatively impermeable to water vapour and carbon dioxide. This enables plants to conserve water in dry air, but it also hinders the entrance of the CO_2 essential for photosynthesis. Stomata are pores in the epidermis and associated cuticle bordered by pairs of structurally and physiologically specialized guard cells and adjacent epidermal cells termed subsidiary cells. This group of cells forms the stomatal complex and facilitates gas movement through the epidermis (Kramer and Boyer, 1995). In the absence of stomata the supply of CO_2 for photosynthesis would be inadequate for survival of most plants, but at the same time the unavoidable loss of water vapour through them creates the danger of dehydration. Thus, the ability of stomata to adjust their aperture is extremely important to the success of plants (Lefohn, 1992, Barnes, 2002, Taylor & Francis, 2004, Chen, 2005, Wiley & Sons, 2006).

2.3.3.1 Historical Review

Malpighi observed the presence of pores in leaves in 1674 and in 1682 Crew pictured them in his plant anatomy. The study of stomatal behaviour began with von Mohl about the middle of the 19th century.

Stalfelt (1932, 1956a) and Bange (1953) showed that in moving air, where the boundary layer resistance is low, transpiration is closely correlated with stomatal aperture. Various investigations (Mansfield, 1986, p.202) showed that ABA increases in water-deficient leaves and that an external application of ABA usually causes stomatal closure. This led to the concept that stomatal closure in water-deficient plants often is caused by chemical

signals from the roots. This was reviewed by Davies and Zhang (1991), and Davies et al. 1994 (In: Lefohn, 1992).

2.3.3.2 Occurrence and frequency

Stomata occur on stems, leaves, flowers, and fruits, but not on aerial roots. They occur on both surfaces of many leaves (amphistomatous) or on only one surface, usually the lower (hypostomatous), especially in woody plants. Common exceptions among woody plants are poplar and willow, which are amphistomatous. Stomata vary widely in size and frequency and species with smaller stomata usually have a higher frequency. The frequency ranges from 60 to 80 per mm^2 in corn to 150 in clover, 300 in apple, and over 1000 in scarlet oak. There often are variations in the number in various parts of a leaf (Smith et al., 1989) and among genotypes of a species. In monocots, conifers, and some dicots, stomata occur in parallel rows, but in leaves with netted venation they are scattered and usually they open into substomatal cavities in the mesophyll tissue. They are easily visible on leaf surfaces under magnification because of the peculiar shape of the guard cells and the fact that guard cells, unlike other epidermal cells, usually contain chloroplasts (Kramer and Boyer, 1995). Specialized epidermal cells, called subsidiary cells, are associated with the guard cells and play a role in guard cell functioning. According to Meidner (1990) and Stalfelt (1956a), the full opening of stomata is associated with a slight decrease in turgor of epidermal cells (Lefohn, 1992, Taylor & Francis, 2004, Wiley & Sons, 2006).

2.3.3.3 Stomatal functioning

2.3.3.3.1 Guard cells

The walls of guard cells bordering the pores usually are thickened and sometimes have ledges and projections that extend into the pores. The thickening of the inner walls was supposed to play an essential role in causing turgid guard cells to bulge and separate, opening the stomatal pores, but Aylor et al. (1973) concluded that the micellar structure of the cell wall is more important than the thickening (Mansfield, 1986). Guard cells are often described as kiney or bean shaped, but those of grasses are elongated and the ends are enlarged, resembling dumbbells, and various other shapes occur. When wide open the stomatal pores occupy from less than 1% to 2% or more of the leaf surface (Lefohn, 1992, Kramer and Boyer, 1995, Taylor & Francis, 2004, Wiley & Sons, 2006).

2.3.3.3.2 Stomatal behaviour

The most important characteristic of stomata is that they open and close and the change in size of their aperture regulates gas exchange. In general they are open in the light and closed in darkness, although the stomata of plants with Crassulacean acid metabolism (CAM plants) behave in the opposite manner, being largely closed during the day and

open at night to reduce water loss without an equivalent decrease in dry matter production because the rate of transpiration is low at night. It is found in a number of succulents and other plants of dry habitats (Lefohn, 1992, Kramer and Boyer, 1995, Taylor & Francis, 2004, Wiley & Sons, 2006).

2.3.3.3.3 Mechanism of stomatal opening and closing

The opening of stomata requires an increase in turgor of guard cells while closing requires a decrease in turgor. Originally changes in turgor were attributed to changes in proportions of starch and sugar in guard cells (In: Lefohn, 1992). Fischer and Hsiao (1968), and Fischer (1971) showed that the transport of K^+ in and out of guard cells is chiefly responsible for changes in turgor. Macallum observed in 1905 that the K^+ concentration was much higher in guard cells of open stomata than in those of closed stomata, but the significance of this early observation was neglected for more than half a century in favour of Lloyds' explanation (Kramer and Boyer, 1995, Taylor & Francis, 2004, Wiley & Sons, 2006).

It now seems that there appears to be a good correlation between the K^+ content of guard cells and stomatal aperture. In the cell, the K^+ is accompanied by various anions that balance the positive charge on K^+. Some guard cells take up Cl^- as a balancing anion but organic acids also can be synthesized internally and serve the same function (Kramer and Boyer, 1995, Taylor & Francis, 2004, Wiley & Sons, 2006).

The guard cell chloroplasts exhibit fluorescence transients resembling those of mesophyll chloroplasts, and K^+ and abscisic acid afferts the transient as though energy from guard cell chloroplasts is used to accumulate K^+ (Lefohn, 1992, Taylor & Francis, 2004, Wiley & Sons, 2006).

According to Cardon and Berry (1992), guard cell chloroplasts probably carry on photosynthesis that is similar to that occurring in mesophyll cells, although it may be slow. It now seems most likely that CO_2 is fixed mainly by the enzyme phosphoenolpyruvate carboxylase and that the oxaloacetate product is reduced to malate (Scheibe et al., 1990) that balances some of the charge of the incoming K^+. The malate together with incoming Cl^- thus forms osmoticum that adds substantially to the osmotic effect of the incoming K^+. It is likely that mitochondrial respiration can supply the energy for opening in the absence of guard cell photophosphorylation and photolysis since opening can occur in the dark under certain conditions, particulary low CO_2 (Fischer, 1968a, Raschke, 1972). The starch of guard cell chloroplasts probably serves as a store of carbon compounds that can be used for energy as well as for organic counterions for K^+ (Raschke, 1975; Zeiger, 1983). The K^+ available in fertile soils appears to be sufficient for guard cell function (Lefohn, 1992, Taylor & Francis, 2004, Wiley & Sons, 2006).

Mansfield et al. (1990) suggests that there could be more than one process competing for CO_2 one of which is inhibitory and the other stimulatory for opening (Lefohn, 1992, Kramer and Boyer, 1995, Taylor & Francis, 2004, Wiley & Sons, 2006).

The loss of K^+ that results in stomatal closure can be brought about by elevated levels of abscisic acid around the guard cells and this probably is the main means of closure. Because the guard cells can metabolise and thus inactivate abscisic acid, they exert considerable local control over the opening and closing process. This suggests that there could be some variability in stomatal aperture across a leaf because of variable rates of local breakdown of the abscisic acid, so that rarely all stomata are at the same aperture (Lefohn, 1992, Taylor & Francis, 2004, Wiley & Sons, 2006).

The loss of K^+ that also occurs during stomatal closure in water-deficient leaves is found whether the roots are present or not and further indicates that local synthesis and metabolism of abscisic acid probably accounts for much of the opening and closing response during water deficits (Lefohn, 1992, Kramer and Boyer, 1995, Taylor & Francis, 2004, Wiley & Sons, 2006).

2.3.3.4 Factors affecting stomatal aperture

The changes in guard cell turgor that bring about stomatal opening and closing are dependent on a number of environmental factors, including light, CO_2-concentration, humidity, and temperature, and on internal factors such as tissue water status and the level of such plant growth regulators as ABA and cytokinins. Complex interactions often exist among these factors which make it difficult to distinguish the relative importance of individual factors such as light and CO_2 or water status and ABA.

However, it is not entirely clear how stomatal conductance responds to these environmental signals. Collatz et al. (1991) suggested that responses of stomata to environmental factors be divided into two groups, those dependent on photosynthesis and those independent of photosynthesis, but there are important interactions between the two groups. Farquhar and Sharkey (1982) concluded that although stomatal conductance substantially limits transpiration, it rarely seriously limits photosynthesis because the latter is limited by other factors in addition to those contributing to stomatal closure (Lefohn, 1992, Kramer and Boyer, 1995, Taylor & Francis, 2004, Wiley & Sons, 2006).

2.3.3.4.1 Light

Although it has been known for many years that stomata usually open in the light, it has been difficult to determine whether this is a direct effect of light or whether it occurs because photosynthesis decreases the internal concentration of CO_2.

It is now believed that two photoreceptors are involved; one that is sensitive to red and far red light and another that absorbs in the blue and ultraviolet (Hsiao et al., 1973; Ogawa et al., 1978). These seem similar, or possibly identical, to the systems controlling photomorphogenetic processes such as photoperiod and phototropism. They must operate by affecting the amount and direction of ion transport across guard cell membranes (Lefohn, 1992, Taylor & Francis, 2004, Wiley & Sons, 2006).

Cardon et al. (1994) reported that oscillations in light intensity cause stomatal conductance to vary widely, either above or below that in steady light, depending on the frequency of the oscillations, and they also reported that oscillations in the carbon dioxide concentration of the atmosphere, such as those caused by air turbulence, can cause oscillations in stomatal conductance (Lefohn, 1992, Kramer and Boyer, 1995, Taylor & Francis, 2004, Wiley & Sons, 2006).

2.3.3.4.2 Carbon dioxide

The stomatal aperture of many kinds of plants is approximately inversely proportional to the CO_2 concentration in both light and darkness, increase in CO_2 in the intercellular or ambient air causing closure and decrease causing opening. According to Mott (1988), stomata respond only to changes in the intercellular CO_2 concentration, but this is effected by the external concentration. Ball and Berry (1982) suggested that the ratio of internal to external concentration of CO_2 is important in controlling stomatal aperture.

The reaction to CO_2 seems to vary with light intensity, temperature, humidity, and presence of ABA (Raschke, 1986). As CO_2 concentration affects photosynthesis and the latter affects stomatal aperture, there may be indirect effects through photosynthesis on stomatal conductance. Penuelas and Matamala (1990) reported that the stomatal density and nitrogen content of leaves have decreased since the CO_2 concentration of the atmosphere has increased. This is based on a study of leaves of various ages preserved in herbaria (Lefohn, 1992, Kramer and Boyer, 1995, Cousson, 2002, Bunce, 2004, Taylor & Francis, 2004, Wiley & Sons, 2006).

2.3.3.4.3 Humidity

Mansfield (1986) reported that the effect of humidity on evaporation from guard cells is important. However, Nonami and Schulze (1989) found that the water potential of the mesophyll cells in transpiring leaves was lower than that of epidermal cells. This suggests that transpiration from epidermal and guard cells is less important than sometimes claimed (Kramer and Boyer, 1995, Taylor & Francis, 2004, Wiley & Sons, 2006).

Aphalo and Jarvis (1991) found that stomatal conductance is better correlated with a vapour pressure deficit than with humidity in Hedera helix, and Assmann and Grantz (1990) found a similar situation in sugarcane and sorghum. Of course the vapour

pressure gradient is greatly affected by temperature (Lefohn, 1992, Taylor & Francis, 2004, Wiley & Sons, 2006).

2.3.3.4.4 Temperature

The effects of temperature on stomatal aperture and the rate of response to stimuli vary among different kinds of plants. It is difficult to separate direct effects of temperature from indirect effects caused by larger vapour pressure deficits associated with increasing temperature. Wuenscher and Kozlowski (1971) found that in five Wisconsin tree species stomatal aperture decreased and stomatal resistance increased significantly as the temperature increased from 20 to 40°C. Pereira and Kozlowski (1977) found considerable interaction between light intensity and temperature on stomatal aperture of sugar maple (Lefohn, 1992, Kramer and Boyer, 1995)

2.3.3.4.5 Wind

The effect of wind on transpiration and the varying effects on transpiration of plants of different species probably results at least in part from varying effects on stomata and in part from effects on the boundary layer conductance. The few studies show a decrease of stomatal conductance with increasing wind velocity (Lefohn, 1992).

According to Kitano and Eguchi (1992b), a sudden increase in wind velocity caused strong cycling in absorption, transpiration, and stomatal conductance of cucumber plants in bright lights, but less in low light and none in darkness. It has also been reported that shaking plants or branches causes stomatal closure, and both dehydration and shaking may be involved in decreased stomatal conductance in wind (Lefohn, 1992, Kramer and Boyer, 1995, Taylor & Francis, 2004, Wiley & Sons, 2006).

2.3.3.4.6 Stomata and air pollution

The effect of air pollutants such as SO_2, ozone, and fluorides on stomata is important because stomata are the pathway for the entrance of most gaseous pollutants into leaves. Olszyk and Tingey (1986) found ozone twice as effective as SO_2 in causing stomatal closure in pea and saw evidence of synergistic effects between the two. In preliminary experiments on young beech trees, Pearson and Mansfield found that ozone causes stomatal closure on well-watered trees, but keeps them open on water-deficient trees. Data from both short-and long-term experiments suggest stomatal limitation of photosynthesis in polluted air (Lefohn, 1992, Kramer and Boyer, 1995, Gerosa, 2003, Taylor & Francis, 2004, Wiley & Sons, 2006).

2.3.3.4.7 Internal factors affecting stomata

Guard cell behaviour and stomatal aperture are affected by internal factors such as leaf water status, internal CO_2 concentration, and growth regulators, especially ABA and cytokinins.

Experiments involving split root systems and use of pressure to keep the shoots turgid, although part of the root system is water deficient, indicate that signals might include decreases in amino acids, ions, and cytokinins, but an increase in ABA probably is the chief signal. Generally the ABA concentration increases in roots of water-deficient plants, and Davies and Zhang (1991) and Davies et al. (1994) discuss in detail the possible role of roots as detectors of increasing soil water deficit and sources of chemical signals to the shoots that can modify or override the effects of shoot water status. Stomata often fail to reopen immediately after water-deficient plants are re-watered and this has been attributed to persistence of a high concentration of ABA. On the other hand, cytokinins promote opening and interact with ABA.

In their review Mansfield et al. (1990) suggest that calcium ions may also affect stomatal aperture, as Ca^{2+} ions reduce it on epidermal strips. It is even suggested that such varied stimuli as darkness, ABA, and cytokinins use calcium ions as second messengers. Ehret and Boyer (1979) reported that large losses of K^+ occur from guard cells of leaves on slowly dehydrated plants and suggested that this contributes to loss of guard cell turgor in water-deficient plants (Lefohn, 1992, Kramer and Boyer, 1995, Taylor & Francis, 2004, Wiley & Sons, 2006).

2.3.3.5 Anomalous behaviour of stomata

Stomata do not always behave as expected. Opening and closing sometimes continue for several days after plants are placed in continuous darkness and they sometimes cycle during the day. Also, not all of the stomata on a leaf behave in the same manner (Lefohn, 1992, Taylor & Francis, 2004, Wiley & Sons, 2006).

2.3.3.5.1 Cycling

Under some circumstances stomata show cycling or oscillation between the open and closed condition. Cycling most commonly occurs in water-stressed plants with relatively high root resistance and can be initiated by a sudden shock such as a short period of darkness, cooling the soil, or changes in humidity or temperature. The cycling has a periodicity ranging from minutes to hours, but most often occurs in range of 15 to 120 min. It has been observed in several kinds of herbaceous plants (Lefohn, 1992, Taylor & Francis, 2004, Wiley & Sons, 2006).

Various leaves on a plant can be at different phases of the oscillation cycle at the same time. Cowan (1972) proposed that cycling optimises the conflicting requirements for

carbon dioxide uptake and control of water loss (Lefohn, 1992, Taylor & Francis, 2004, Wiley & Sons, 2006).

These anomalies increase the difficulty of developing a general theory of stomatal behaviour, so that the possible occurrence of short-term cycling and daily rhythms should always be considered (Kramer and Boyer, 1995, Taylor & Francis, 2004, Wiley & Sons, 2006).

2.3.3.5.2 Heterogeneity in stomatal response

The stomata on the upper and lower surfaces of leaves sometimes behave differently. The reaction of stomata to environmental factors varies with the age of leaves and their past treatment and it often is difficult to determine how much of the difference among experimental results is intrinsic and how much is caused by differences in methods, age, and previous treatment of the experimental plants.

As leaves grow older the stomata often become less responsive and may open only partly, even at midday (Brown and Pratt, 1965), and Tazaki et al. reported that stomata on older leaves of mulberry do not close. According to Ackerson and Krieg (1977), although stomata of corn and sorghum close when they are water deficient during the vegetative stage, they do not close in similar conditions in the reproductive stage (Lefohn, 1992).

Schulze and Hall (1982) reviewed the effects of environmental factors on stomatal behaviour and urged that short- and long-term effects are differentiated.

The effects of age and past treatment probably account for many of the differences in behaviour found in the literature and increase the difficulty in generalizing about stomatal behaviour (Lefohn, 1992, Kramer and Boyer, 1995, Taylor & Francis, 2004, Wiley & Sons, 2006).

2.3.3.6 Measurement of stomatal aperture and conductance

Because of the importance of stomata in controlling water loss, CO_2 and pollutant uptake, there has been much interest in the measurement of stomatal aperture or the conductance of stomata. Some investigators have expressed the stomatal opening in terms of conductance, others as resistance which is the reciprocal of conductance, resulting in hyperbolic curves (Lefohn, 1992, Kramer and Boyer, 1995, Taylor & Francis, 2004, Wiley & Sons, 2006).

2.3.3.6.1 Visual observations

Most of the early observations were made by stripping off bits of epidermis and fixing them in absolute alcohol before observing them under the microscope. This method was used for the classical studies of Loftfield (1921), but it is difficult to strip epidermis from

some leaves and stripping sometimes causes change in stomatal aperture (Kramer and Boyer, 1995).

Another method is to make impressions of the epidermis of attached leaves in collodion (Clements and Long, 1934), silicone rubber (Zelitch, 1961), or dental paste (Kuraishi et al. in Hashimoto et al., 1990; Weyers and Meidner, 1990), or even nail polish, strip them off, and examine them under the microscope. Vazzana et al. (1988) reported a new technique which creates imprints of the leaf surface on pieces of Cellulose-di-acetate softened by acetone (Lefohn, 1992). Impressions can be sputtered and investigated in the electron-microscope. For examinations under the light-microscope, slide-shaped rectangles should be cut from 2.0 mm thick sheets, which are costly (Meister, 2007).

Meister (2007) found that Perspex (Poly-Methy-MetAcrylate), being offered by many suppliers and at comparably low price, can easily be deployed, which proves to be a considerable advantage especially in field trials. The result is a permanent impression of the epidermis' surface, perfect for long-term storage (Meister 2001). This method is described as follows and also used in this work: Microscopic slides were cut from a 1 mm thick PMMA sheet using a Stanley-knife or a guillotine. Ethyl-methyl-ketone was used instead of acetone to soften the surface of the slide. A drop of solvent was placed on the surface of the Perspex slide. While the surface was left to soften (10-20 seconds), leaf-disks (diameter 6-7 mm) were punched out with a cutter (cork borer). Imprints of leaf surface were simply created by gently impressing a leaf disk onto the softened surface (finger tip). To achieve imprints of higher quality, another drop of solvent was placed on the slide before the leaf-disk was impressed on the softened surface by a lead-cylinder (diameter 10 mm, 30g). The leaf-disks were removed after 3-10 minutes, depending on the amount of solvent used.

Modern technology has made better visual observations of stomata possible. Hashimoto et al. (1984), Omasa et al. (1985a) and Hashimoto (1990), described the use of a remote-controlled light microscope, television camera, and image processing system to observe the effects of stresses such as water deficit and air pollutants on stomata in different parts of a leaf. This method showed that stomata in different parts of a leaf sometimes responded differently. They concluded that visual observations under a microscope are desirable to observe the effects of air pollutants on stomata in different parts of a leaf (Kramer and Boyer, 1995, Taylor & Francis, 2004, Wiley & Sons, 2006).

2.3.3.6.2 Porometers

Visual methods have been largely supplemented by porometers that measure the movement of gas out of leaves in such a manner that readings can be converted into diffusion resistance of the leaf in $sec \cdot m^{-1}$ or its reciprocal conductance in $m \cdot sec^{-1}$, mmol $H_2O/m^2 \cdot s$. This method measures total leaf resistance, but if cuticular resistance is

high it gives a good approximation of stomatal resistance. There are two types of porometers: pressure or bulk flow, and diffusion porometers.

a. Pressure or bulk flow porometers:

Francis Darwin, the plant physiologist son of Charles Darwin, is credited with developing the porometer and Darwin and Pertz (1911) described a simple pressure flow porometer. Numerous modifications have been made, including recording porometers (Gregory and Pearse, 1934; Wilson, 1947) and portable models such as that developed by Alvim (1965).

Fiscus et al. (1984) and Fiscus (in Hashimoto et al., 1990) described a viscous flow porometer that is computer controlled and can be used to schedule irrigation of a crop. Viscous flow porometers are not suitable for use on leaves with stomata on only one surface (many trees) and with bundle extensions that extend to the epidermis and prevent free lateral movement of gases through the intercellular spaces. Also a pressure greater than 10 cm of water can cause alterations in stomatal aperture (Raschke, 1975). Meidner (1992) discussed some problems with bulk flow porometers. These types of porometers indicate aperture effects rather than diffusion through the leaf, and are highly responsive to changes in aperture (Eckles, R. D., 1982, Lefohn, 1992, Kramer and Boyer, 1995).

b. Diffusion porometers:

These usually are small cuvettes that can be attached to leaves that measure the time required for a predetermined change in humidity to occur. They have undergone many modifications, but most of the earlier ones used a cuvette containing a humidity sensor connected to a meter to read humidity and a timing device.

Beardsell et al. (1972) introduced the zero point porometer that measures the steady-state rate of transpiration of a leaf enclosed in a cuvette into which dry air is blown to maintain a constant humidity. This eliminates some calibration problems and the lag caused by adsorption of water vapour on cuvette walls.

Kaufmann (1981) used ventilated cuvettes, approximately 15 litres in volume and large enough to enclose leafy twigs. They were closed from time to time long enough to measure transpiration. A computer controlled opening and closing and data acquisition and processing. With this apparatus Kaufmann was able to estimate canopy transpiration from measurements of leaf conductance.

In this work, a PMR-1-steady-state porometer from PP-Systems was used to measure the stomatal conductivity. The porometer delivers the actual stomata conductance for water vapour of individual leaves rapidly and allow therefore quantifications of their dependence on environmental plant-endogenous factors.

One of the limitations on use of diffusion porometers is that they average the behaviour of stomata in a relatively large area of leaf surface, but provide no information concerning the behaviour of individual stomata or differences in different parts of leaves. Thus, for some purposes visual observations may be desirable (Omasa et al., 1985a; and in Hashimoto et al., 1990). However, the data provided by larger samples generally are useful. Diffusion porometers are affected by any factor controlling water loss by leaves; stomatal aperture is only one. Differences in cuticle thickness, leaf thickness, intercellular space dimensions, and other factors can contribute to diffusion differences but do not indicate differences in stomatal aperture (Eckles, R. D., 1982, Lefohn, 1992, Kramer and Boyer, 1995).

3 MATERIALS AND METHODS

3.1 Accumulated stomatal ozone flux

Stomatal flux-based critical levels (Cle_f) for ozone take into account the varying influences of temperature, water vapour deficit (VPD), light (irradiance), SMD (soil moisture deficit), ozone concentration and plant development (phenology) on the stomatal flux of ozone and therefore provide an estimate of the critical amount of ozone entering through the stomata and reaching the sites of action inside the plant. This is interesting and important as it shows that the stomatal flux for a given ozone concentration in warm, humid conditions with moist soil can be much greater than that in hot, dry conditions with dry soil because stomata will be more widely open. Exposure based critical levels do not differentiate between such climatic conditions and do not indicate the increased risk of damage in warm, humid conditions (Mapping Manual, 2004).

The hourly mean stomatal flux of ozone based on the projected leaf area (PLA), F_{st} (in nmol m^{-2} PLA s^{-1}) is accumulated over a stomatal flux threshold of Y nmol m^{-2} s^{-1}. The accumulated stomatal flux of ozone above a flux threshold of Y ($AF_{st}Y$) , is calculated for the appropriate time-window as the sum over time of the differences between hourly mean values of F_{st} and Y nmol m^{-2} PLA s^{-1} for the periods when F_{st} exceeds Y. The stomatal flux-based critical level of ozone, Cle_f mmol m^{-2} PLA is then the cumulative stomatal flux of ozone, $AF_{st}Y$, above which direct adverse effects may occur (Mapping Manual, 2004).

The ozone fluxes through the stomata of leaves found at the top of the canopy are calculated using a multiplicative algorithm based on the methodology described by Emberson *et al.* (2000b).

The strongest relationship between yield effects and $AF_{st}Y$ were obtained using Y=6 nmol m^{-2} s^{-1} (Pleijel, 2002, Danielsson, 2003). Ozone exposure started to contribute to $AF_{st}Y$ at an ozone concentration at the top of the crop canopy of approximately 22 ppb. As such, the flux based critical level, Cle_f of ozone for wheat is an $AF_{st}6$ of 1 mmol O_3 per m^2 projected leaf area, accumulated during an effective temperature sum period starting 270°C days before anthesis and ending 700°C days after anthesis (Mapping Manual, 2004). $AF_{st}6$ relationship has an intercept of unity, indicating that this exposure index can explain the full range of effects (Pleijel, 2006).

The core of the leaf ozone flux model is the stomatal conductance (g_{sto}) multiplicative algorithm which has been developed by Emberson *et al*. (2000b).

All g_{sto} measurements used to derive g_{max} and other parameters were made on the flag leaves. Measurements were made during those times of the day and year when g_{max} would be expected to occur.

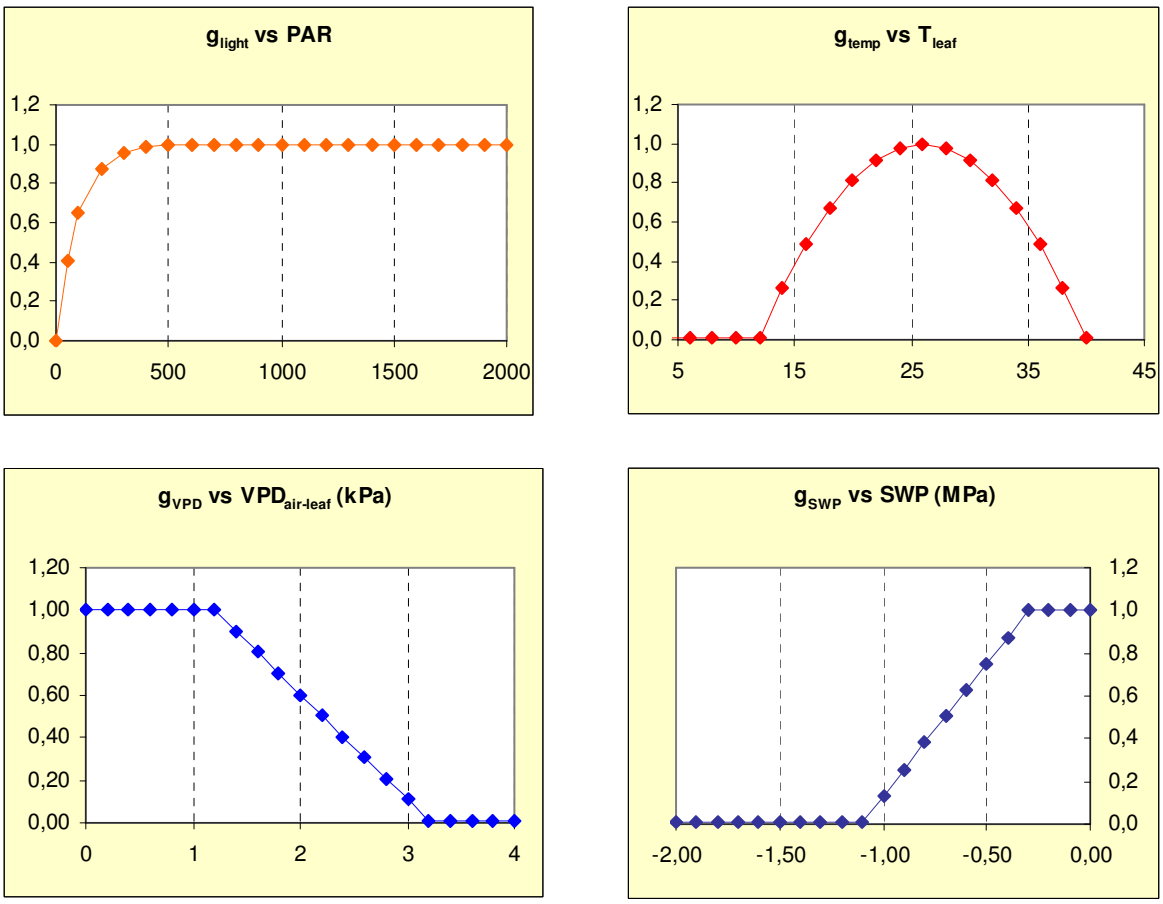
$g_{sto} = g_{max} *[\min(f_{phen}, f_{O3})]*f_{light}*\max\{f_{min},(f_{temp}*f_{vpd}`*f_{swp})\}$

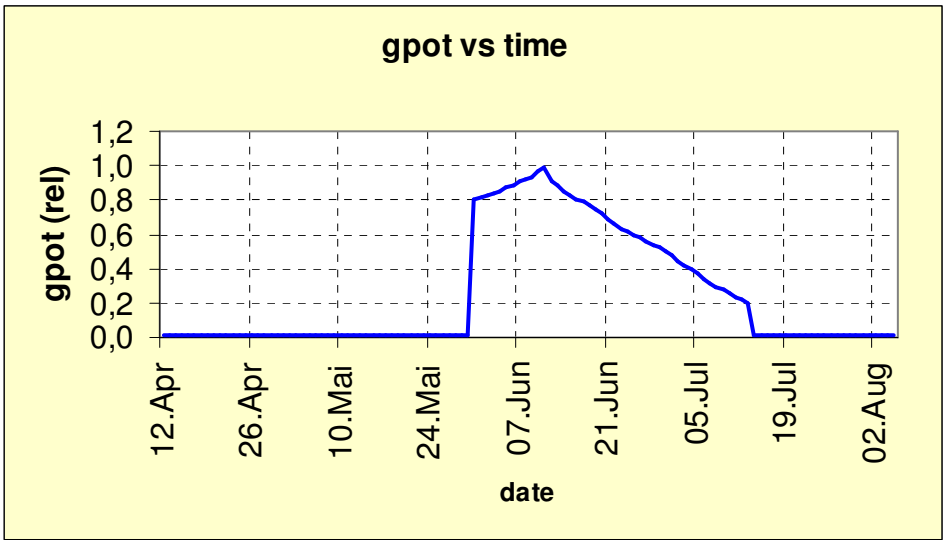
where g_{sto} is the actual stomatal conductance (mmol O_3 m^{-2} PLA s^{-1}) and g_{max} is the species-specific maximum stomatal conductance (mmol O_3 m^{-2} PLA s^{-1}). The parameters f_{phen}, f_{o3}, f_{light}, f_{temp}, f_{vpd} and f_{swp} are all expressed in relative terms as a proportion of g_{max}.

3.1.1 Parameterisation (g_{sto} functions) of the stomatal conductance model:

These listed parameters allow for the modifying influence of phenology, ozone, and environmental variables on stomatal conductance to be estimated.

Fig. 7 shows the parameterisation of the stomatal conductance model used for the g_{light} function, the g_{temp} function, the g_{VPD} function, the g_{SWP} function, and the g_{pot} function vs $_{time}$. All data points used are shown as well as the envelopes used to define the boundary lines of the multiplicative g_{sto} model.




gpot vs time
gpot (rel)
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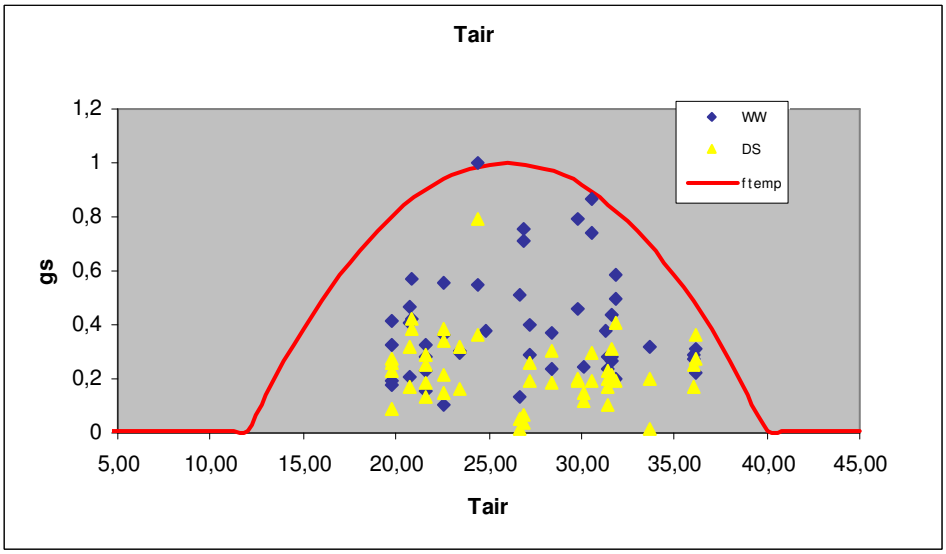
date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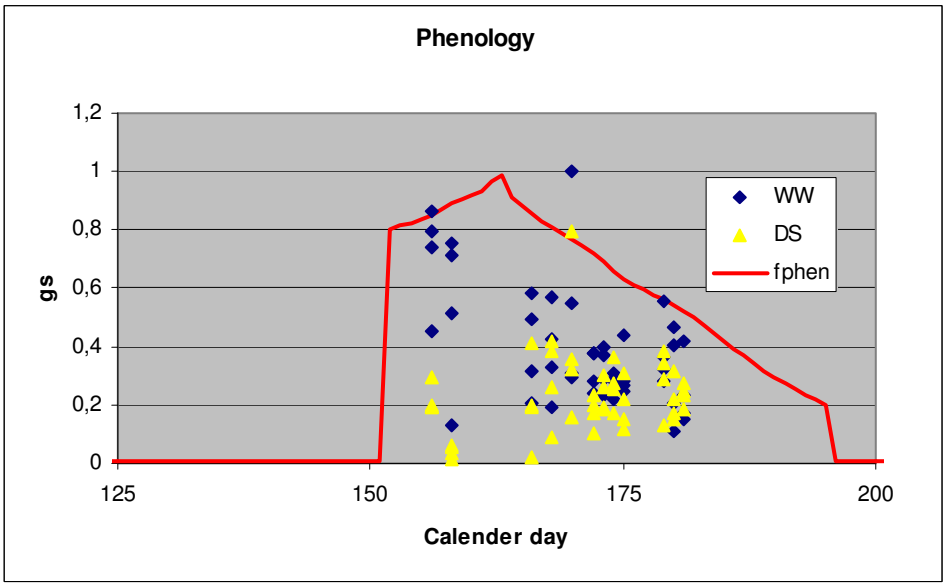
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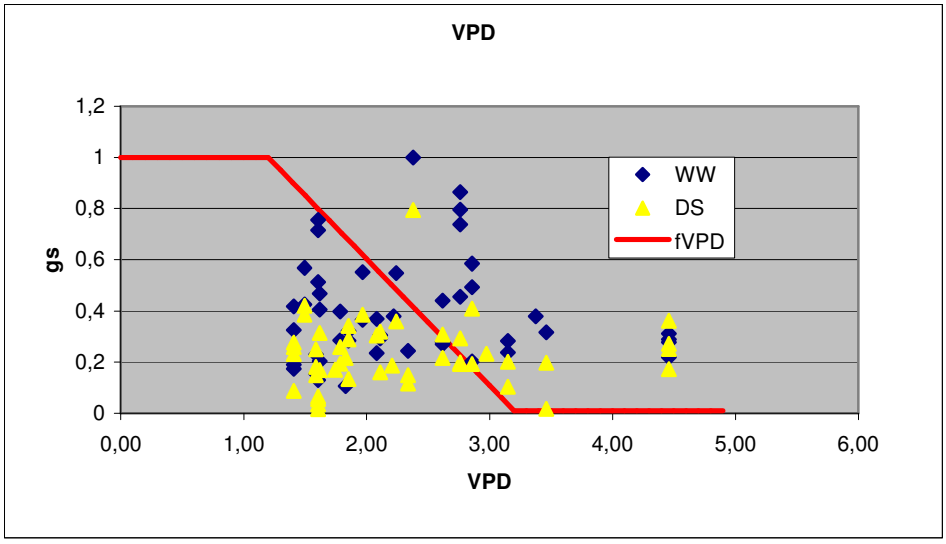


Fig.: 7 Parameterisation of the stomatal conductance model

3.1.2 Wheat input parameters

Tab. 4: Wheat input parameters

	Value	Units
Boundary layer conductance	4.7339826	mol m^{-2} s^{-1}
Stomatal flux		
g_{max}	397	(mmol O_3 m^{-2} PLA s^{-1})
f_{min}	0.01	fraction
SGS (start growing season)	not used	Julian day
EGS (end growing seasons)	not used	Julian day
f_{phen}_a	0.8	fraction
f_{phen}_b	0.2	fraction
f_{phen}_c	15	days
f_{phen}_d	40	days
f_{phen}_e	270	°C days
f_{phen}_f	700	°C days
light_a	0.0105	constant
T_{min}	12	°C
T_{opt}	26	°C
T_{max}	40	°C
b_t	1.00	
VPD_{max}	1.2	kPa
VPD_{min}	3.2	kPa
Sum VPD_{crit}	8	kPa
SWP_{max}	-0.3	MPa
SWP_{min}	-1.1	MPa

G_{max} and f_{min}

Receptor-specific values are provided for g_{max} and f_{min} based on stomatal conductance measurements on the experimental site during the two experimental years 2000 and 2001.

F_{phen}

Crop leaves do not attain maximum stomatal conductance until a significant period of development, while stomatal conductance declines later along with senescence (Jones, 1994, Pleijel, 2006). In wheat leaves, higher conductance at anthesis have been reported (Lehnherr et al., 1988, Frederick, 1997, Pleijel, 2006).

The phenology function uses a fixed number of days.

When $A_{start} < yd\ y\ (A_{start} + f_{phen\_c})$

$F_{phen} = (1-f_{phen\_a}) * ((yd-A_{start})/f_{phen\_c})+f_{phen\_a}$

When $(A_{start} + f_{phen\_c}) < yd < (A_{end}-f_{phen\_d})$

$F_{phen} = 1$

When $(A_{end}-f_{phen\_d}) < yd < A_{end}$

$F_{phen} = (1-f_{phen\_b}) * ((A_{end}-yd)/f_{phen\_d}) + f_{phen\_b}$

Where yd is the year day; A_{start} and A_{end} are the year days fort he start and end of ozone accumulated period respectively.

f_{light}

$f_{light} = 1\ EXP((-light\_a)*PFD)$

where PFD represents the photosynthetic photon flux density in units of μmol m^{-2} s^{-1}.

f_{temp}

The main effect of f_{temp} is to limit stomatal conductance at low temperatures since, high temperatures are commonly associated with high VPD values, which cause very low conductance through the f_{vpd} function (Pleijel, 2006).

When $T_{min} <T<T_{max}$

$f_{temp} = \max \{f_{min},[(T-T_{min})/(T_{opt}-T_{min})]*[(T_{max}-T)/(T_{max}-T_{opt})]^{bt}\}$

when $T_{min}>T<T_{max}$

$f_{temp} = f_{min}$

Where T is the air temperature in °C, T_{min} and T_{max} are the minimum and maximum temperatures at which stomatal closure occurs to f_{min}, T_{opt} is the optimum temperature and bt is defined as follows:

$bt = (T_{max}-T_{opt})/(T_{opt}-T_{min})$

f_{VPD}

$f_{vpd} = \min \{1, \max\{f_{min}, ((1-f_{min})*(VPD_{min}-VPD)/(VPD_{min}-VPD_{max}))+f_{min}\}\}$

There is more or less instantaneous effect of high VPD levels on stomata resulting in stomatal closure which reduces the high rate of transpiration water vapour flux rates out of the leaf under such conditions. Under dry and hot conditions such limitation of VPD may occur early during the day after the sunrise. If there is a large VPD sum it is likely to be related to a larger amount of transpiration and if the accumulated amount of transpiration during the course of the day exceeds a certain critical level, then stomatal re-opening in the afternoon is assumed not to occur (Pleijel, 2006).

The instantaneous response of the stomata to VPD is described by the f_{VPD} function.

f_{SWP}

$f_{SWP} = \min\{1, \max\{f_{min}, ((1-f_{min})*(SWP_{min}-SWP)\ (SWP_{min}-SWP_{max}))+f_{min}\}\}$

f_{O3}

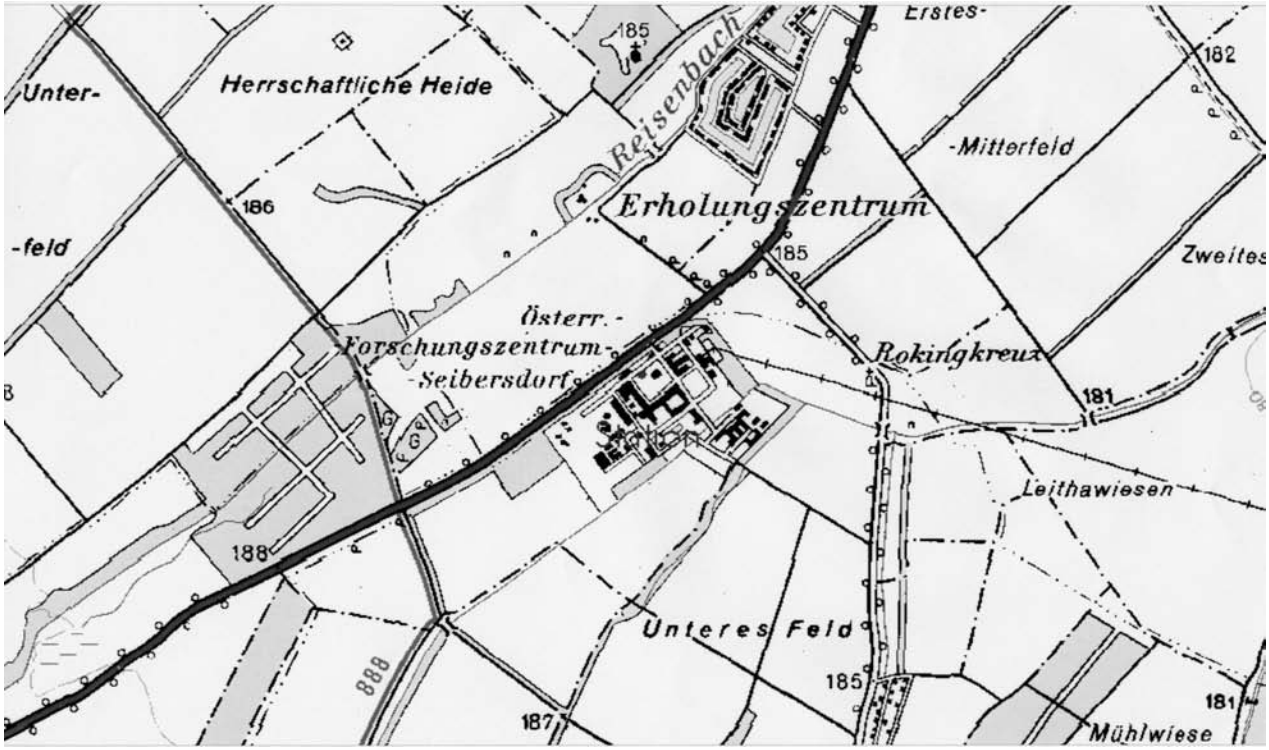
$f_{O3} = 1/(1+(AF_{st}0/11.5)^{10})$

where $AF_{st}0$ is accumulated from A_{start}.

This function allows the inclusion of ozone concentration on stomatal conductance via the onset of early senescence. This function is used in association with the f_{phen} function to estimate g_{sto}. The f_{O3} function typically operates over a one-month period and only comes into operation if it has a stronger senescence-promoting effect than normal senescence.

Post-anthesis ozone induced decline in stomatal conductance is proportional to ozone induced leaf senescence (Pleijel, 2006).

3.2 Meteorological measurements



3.2.1 Climate Data:

Site: Station Seibersdorf:

Altitude above the see: 185.00

Longitude: 163025

Latitude: 475839

Climate observation-times: 6:54, 13:54, 18:54 MEZ

Time difference: 7:00 MEZ=7:06 MOZ

3.2.2 Physical climate:

Air Temperature

Type: YSI 44020

Producer: Yellow Springs Instr., USA

Measuring range: -50 bis +50°C

Exactitude: ±0.1°C

Time factor: 10s for stagnant air

Air Pressure:

Type: Barogeber 317S/Barometer GB1

Producer: Kroneis, Vienna/Meteolabor, CH

Measuring range: 100hP (to choose between 825-1050hP)/630-1060hP

Exactitude: ± 0.2hP/

Measuring principle: Pressure capsule (analogue signal 0-2V)/Frequency 15-32 kHz

Relative humidity:

Type: Pernix800L100

Producer: Lambrecht KG, BRD

Measuring range: 5-100%

Exactitude: ±3%

Rainfall:

Type: AP23

Producer: Paar, Graz

Funnel: 500cm^2

Analysis: 0.1mm

Measuring principle: floating contact

Irrigation:

Type: Regensensor

Producer: Kroneis, Wien

Collection surface: 40cm^2

Analysis: approximately 5min

Measuring principle: capacity with floating contact

Wind:

Type: 263 PR

Producer: Kroneis, Wien

Measuring range: 0.5-50 m/s/0-359 Grad

Exactitude: WG ± 0.5 m/s/WR ± 1 Grad

Measuring principle: WG pulse generator**/**WR Potentiometer

Global radiation:

Type: Sternpyranometer 8101

Producer: Schenk, Vienna

Measuring range: 300-3000nm

Exactitude: 2.5 mV/J cm^{-2} min^{-1}

Measuring principle: Thermocouple

Mean concentrations of primary pollutants:

Site: Stixneusiedl / Lower Austria

Approximate distance of weather station from field site: 25km

3.2.3 Environmental conditions during the experiments

Tab. 5: Mean temperature, relative humidity, irradiance and global radiation during the experimental periods 2000/01

	Mean temperature (°C)	**relative humidity (%)**	**irradiance ( )**	**global radiation ( )**
Vegetative growth to Development of flag leaf				
2000: 03.20.-06.02. (75d)	14.2	72.5	0.3	231
2001: 03.16.-05.24. (70d)	11.4	70.6	0.2	200.2
Appearance of flag leaf to Anthesis				
2000: 06.03.-06.10.Na/06.16.Ex	21.8/22.5	58/60.3	0.5/0.4	306.2/295.2
2001: 05.25.-05.30.Na/06.06.Ex	23.6/18.6	65.5/66	0.4/0.29	314.3/259.5
Onset flowering to Ripening				
2000: 06.11./06.17.-07.15.	20.2/19.6	62.9/62.8	0.31/0.3	259.7/254.,6
2001: 05.31./06.07.-07.03.	17.7/19.4	65.4/67.4	0.26/0.3	241.5/262.2
Ripening to Harvest				
2000: 07.16.-08.05.	19.1	71.6	0.2	206.3
2001: 07.04.-07.24.	20.4	68.2	0.3	223

Tab. 6: Mean concentrations in mg/m^3 of the major air pollutants during the growing periods 2000/2001

	NO (ppb)	**NO_2 (ppb)**	**SO_2 (mg/m^3)**
Vegetative growth to Development of flag leaf			
2000: 03.20.-06.02. (75d) *(data from 04.01.)**	2.74	7.38	0.01
2001: 03.16.-05.24. (70d) *(data from 04.01.)**	3.83	3.98	0.005
Appearance of flag leaf to Anthesis			
2000: 06.03.-06.10.Na/06.16.Ex	2.45/2.48	6.43/6.72	0.01/0.01
2001: 05.25.-05.30.Na/06.06.Ex	6.21/6.16	5.03/4.91	0.006/0.005
Onset flowering to Ripening			
2000: 06.11./06.17.-07.15.	2.51/2.507	7.079/7.08	0.01/0.01
2001: 05.31./06.07.-07.03.	6.12/6.12	4.82/4.83	0.005/0.005
Ripening to Harvest			
2000: 07.16.-08.05.	2.71	8.01	0.006
2001: 07.04.-07.24.	5.46	4.04	0.003

*) In the first three weeks we neglected the uptake of pollutants, as the plants were only in their earliest growth stages. So we started the calculations from the beginning of April. As the sensitivity of wheat towards air pollutant changes as a function of development, the average concentrations during those specific growth stages are listed below.

Tab. 7: Ozone concentrations during the experiments in 2000/2001 (24-h mean concentration)

	24h mean	**7°° - 17°°**
Vegetative growth to Development of flag leaf		
2000: 03.20.-06.02. (75d) *(data from 04.11.)**	39.5	47.9
2001: 03.16.-05.24. (70d) *(data from 04.12.)**	35.3	37.9
Appearance of flag leaf to Anthesis		
2000: 06.03.-06.10.Na/06.16.Ex	43.4/49.0	53.7/61.3
2001: 05.25.-05.30.Na/06.06.Ex	45.0/43.5	50.5/44.0
Onset flowering to Ripening		
2000: 06.11./06.17.-07.15.	38.5/37.0	48.9/47.0
2001: 05.31./06.07.-07.03.	37.8/38.3	41.4/42.4
Ripening to Harvest		
2000: 07.16.-08.05.	35.4	43.0
2001: 07.04.-07.24.	35.5	38.2

3.3 The wheat cultivars Extradur and Nandu

In the Austrian cultivar list 1997 (BFL-Bundesamt und Forschungszentrum für Landwirtschaft, Ministry of Environment) one can find the following description of the two wheat cultivars EXTRADUR (Triticum durum) and NANDU (Triticum aestivum) we chose for our experiments:

1 = favourable

9 = unfavourable

except baking quality:

9 = very high

1 = very low

Tab. 8: Austrian cultivar list 1997 (BFL-Bundesamt und Forschungszentrum für Landwirtschaft)

Note	Heading, Anthesis, Ripening	Growth Height	Youth development
1	very early	very short	very good
2	early	short	good/quick
3	early to medium	short to medium	good to medium
4	medium to early	medium to short	medium to good
5	medium	medium	medium
6	medium to late	medium to tall	medium to little
7	late to medium	tall to medium	little to medium
8	late	tall	little to slow
9	very late	very tall	slow

Note	Storing, out growth susceptibility for diseases	Yield, Quality	Crude fiber, Glucosinolat
1	none, very little	very high	very low
2	little	high	low
3	little to medium	high to medium	low to medium
4	medium to little	medium to high	medium to low
5	medium	medium	medium
6	medium to high	medium to low	medium to high
7	high to medium	low to medium	high to medium
8	high	low	high
9	very high	very low	very high

	EXTRADUR	NANDU
Year of admission:	91	92
Awn/Spadix:		K
Heading:	4	7
Ripening:	6	7
Growth height:	3	5
Storage:	3	4
Grain loss:	2	
Outgrowth:	5	5
Mildew:	6	5

Leag rust:	8	3
Black rust:	4	5
Septoria Nodorum-leaf:	7	5
Glume blotch:		6
Ear fusarium rot:	7	5
Yellow rust:	4	
Yield-dry area:	6	
Yield-wetland:		5
Cultivation property:	T	
Thousand grain weight:	2 (49.1g)	6 (38.8g)
Hektolitermasse:	5	4
Crude protein:	2.5	4
Wet gluten:	4	3
Swelling capacity Q0:	7	5
Sedimentation value:		4
Falling number:	3	3
Viscosity (Amyl.):	2	
Pope-yield:	5	
Vitreousity:	4	
Coloring matter:	3	
Baking quality:	7	

3.4 Experimental requirements

a) Experimental Plot: The wheat plants were exposed to two different levels of ozone in OTCs. Air was non-filtered (A) and A plus 50ppb addition of ozone (O). Each treatment was replicated three times (O_1, O_2, O_3/A_1, A_2, A_3).

b) Pots and Wicks: The plants in separate chambers were exposed to two different levels of water supply:
Dry-treatment: 1x130cm wick of fibreglass; these plants were irrigated insufficiently to show typical symptoms of drought stress (-40% of soil water capacity).
Well watered-treatment: 4x70cm + 1x130cm wicks; these plants were irrigated sufficiently not to show any symptoms of drought stress.

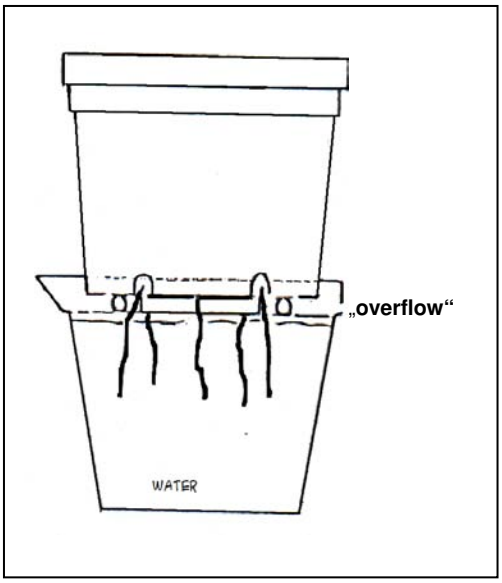


Fig. 7: Water supply of the wheat cultivars

c) In the experimental year 2000 we exposed both cultivars (Nandu and Extradur) to the two levels of water supply. As the cultivar Nandu had proven to be more sensitive to acute ozone damage during comparative fumigation experiments in closed chambers we decided to expose only this cultivar to both levels of water supply in the second experimental year 2001. The cultivar Extradur was irrigated sufficiently.

d) Soil mixture and fertilizer: In this study we used a commercial mix of ED 63 (Einheitserde- und Humuswerke, Gebrüder Patzer GmbH Waldsiedlung 4, D-36391 Sinntal-Jossa/pH 5.5-6.5, 50-300 mg/N, 80-300 mg/Phosphor, 80-400mg/K, Salt < 1.5g/l).

3.4.1 Establishing the wheat plants

a) Planting procedure:

The seeds of spring wheat Nandu and Extradur were sown on the 13.03.01 in 10.6l pots of 24.5cm diameter and 20 cm height after they had been pre-germinated for 48 hours in a dark, warm (20°C) greenhouse. Before germination they had to be soaked in water for 7 hours. After sowing they had been placed immediately in field environment. We planted 15 seeds per pot in three rows.

To avoid the spread of viruses and diseases we kept insects off the plants by using a reticle**.**

Through fibreglass wicks the plants had access to a water reservoir, whereas the distance between the pot base and the water level had to be approximately 3 cm and the pot base may not have been immersed in water.

On the 05.09.2001 we placed the pots of the two cultivars Nandu and Extradur cultivars in the OTCs and started ozone fumigation.

3.4.2 Care of wheat plants

In the experimental year 2000 we sprayed Pronto Plus (250ml/l) twice against Mildew: On the 06.09.2000 and 07.06.2000.

Regularly we removed weeds from the pots and around the pots; we observed the plants carefully for insect pests and sprayed in the experimental year 2001:

06.11.2001: Bulgadis (5ml - 0.1% + 2.5ml wetting agent) because of aphids.

We observed the plants as well for viruses and fungal pathogens and sprayed in the experimental year 2001 three times because of Mildew:

05.10.2001: Pronto Plus (250ml/l)**.**

05.26.2001: Corbel (12.5ml) and Decis (2.5ml + 2.5ml wetting agent).

06.28.2001: Decis (2.5ml + 2.5ml wetting agent) and Corbel (12.5ml).

And we made periodically observations of the wheat plants to record the growth stages:

Tab. 9: Development stages of both cultivars during the experimental years 2000 and 2001

	2000	**2001**
	Nandu/Extradur	**Nandu/Extradur**
Emergence /11	03.20.2000	03.16.2001 both
Appearance of flag leaf /49	06.02.2000	05.24.2001 both
Onset of flowering /61	06.10.2000/06.16.2000	05.30.2001/06.06.2001
Start of Ripening/91	07.15.2000	07.03.2001 both
Harvest/97	08.05.2000	24.7.2001 both

Cereal growth stages after the descriptions of the principal and secondary growth stages:

The decimal code for the growth stages of cereals, devised by Zadoks, Chang & Konzak (1974), is reproduced with stylised drawings of selected stages of wheat, barley and oat plants. Expanded definitions of some of the descriptive phrases are designed to assist the application of the code to agrochemical research, development and use in the UK. Each of the principal stages has provision for sub-division into 10 secondary stages. These are denoted by a second series of digits extending the scale from 00 to 99. Many people and organisations, in particular the ICP-crops, have contributed to the evolution and presentation of this version of the decimal code for cereal growth stages.

b) Stomatal conductance measurements:

To enable ozone flux modelling to wheat canopies, g_s parameters and relationships with environmental conditions need to be made (i.e. g_{max}, g_{pot}, g_{min}, g_{light}, g_{temp}, g_{vpd}). To date, flux modelling had been performed to assess the ozone uptake to a single flag leaf of the upper canopy. To enable estimates to be made of wheat yield loss it is necessary to estimate ozone uptake to the entire wheat canopy. This requires knowledge of how g_s varies throughout the canopy. The leaf on which g_s measurements are made is also important, i.e. its position within the canopy.

We had many measurement periods: All over the day on sunny days from appearance of flag leaf (02.06.00/24.05.01) to senescence, and additionally in the morning and afternoon hours, otherwise the input PAR would have been under presented in the dose-response model.

The measurements were made on both leaf surfaces (upper and lower surface), which represents the total leaf gas exchange.

Additionally we recorded the PAR (in µmol $m^{-2}s^{-1}$), as well as the time of the day for each measurement and the associated environmental conditions.

c) Destructive harvests:

The final harvest was in the first experimental year 07.05.2000 and in the second experimental year 07.24.2001.

Before harvesting, we counted the numbers of ears, and measured the height of straw per pot and chamber.

After harvesting each pot separately we dried the ears and the straw less than 80°C until weight consistency to determine the dry mass/pot**.** We weighted the straw and ears per pot.

The ears/pot were threshed and the kernels counted to define the grain yield per pot. Then we defined the thousand grain weight (TGW)/pot, which was given at the water content at harvest.

4 RESULTS

4.1.1 Calculation of $AF_{st}6$ mmol m^{-2} PLA

Growing season: 970°C days, starting 200°C days before mid-anthesis till 07/14.

Effect: Yield reduction

Tab. 10: Accumulated stomatal flux of ozone based on the projected leaf area above the flux threshold of 6 mmol m^{-2} s^{-1}, calculated as the sum over the vegetation period (April to August) of the differences between hourly mean values of stomatal flux and 6 nmol m^{-2} s^{-1} projected leaf area for the periods when the stomatal flux exceeds 6.

2000	**Extradur**	**Well watered**		**$AF_{st}6$** (mmol m^{-2} PLA) of NF treatment in growing season 2000	**3.39**
			+50 ppb	**$AF_{st}6$** (mmol m^{-2} PLA) of NF+50 ppb treatment in growing season 2000	**8.84**
		Drought stressed		**$AF_{st}6$** (mmol m^{-2} PLA) of NF treatment in growing season 2000	**1.11**
			+50 ppb	**$AF_{st}6$** (mmol m^{-2} PLA) of NF+50 ppb treatment in growing season 2000	**5.61**
	Nandu	**Well watered**		**$AF_{st}6$** (mmol m^{-2} PLA) of NF treatment in growing season 2000	**1.00**
			+50 ppb	**$AF_{st}6$** (mmol m^{-2} PLA) of NF+50 ppb treatment in growing season 2000	**3.77**
		Drought stressed		**$AF_{st}6$** (mmol m^{-2} PLA) of NF treatment in growing season 2000	**0.06**
			+50 ppb	**$AF_{st}6$** (mmol m^{-2} PLA) of NF+50 ppb treatment in growing season 2000	**0.59**
2001	**Extradur**	**Well watered**		**$AF_{st}6$** (mmol m^{-2} PLA) of NF treatment in growing season 2001	**2.12**
			+50pb	**$AF_{st}6$** (mmol m^{-2} PLA) of NF+50 ppb treatment in growing season 2001	**5.37**
	Nandu	**Well watered**		**$AF_{st}6$** (mmol m^{-2} PLA) of NF treatment in growing season 2001	**0,55**
			+50 ppb	**$AF_{st}6$** (mmol m^{-2} PLA) of NF+50 ppb treatment in growing season 2001	**2,26**
		Drought stressed		**$AF_{st}6$** (mmol m^{-2} PLA) of NF treatment in growing season 2001	**0,01**
			+50 ppb	**$AF_{st}6$** (mmol m^{-2} PLA) of NF+50 ppb treatment in growing season 2001	**0,27**

The comparison of the flux maps of both cultivars showed a higher flux into Extradur in both experimental years 2000 and 2001.

One of the main findings from the experiments is the reduced ozone flux into both cultivars under drought stress. This is also more apparent for the ozone sensitive cultivar Nandu. It is apparent that a closure of stomata caused by moderate drought stress reduces ozone uptake through the stomata.

4.1.2 Extradur

In the experimental year 2000, the accumulated stomatal flux with the critical threshold of 6 nmol m^{-2} s^{-1} into Extradur under well watered conditions was **3.39** mmol m^{-2}. The stomatal flux of well watered cultivars under fumigated conditions was 161% higher (**8.84** mmol m^{-2}). Compared with drought stressed plants, the $AF_{st}6$ value was 67% lower (**1.11** mmol m^{-2}) than the stomatal flux under well watered conditions (**3.39** mmol m^{-2}). The accumulated stomatal flux under ozone fumigation was under drought stressed conditions 80% higher than under ozone fumigated and well watered conditions.

In the experimental year 2001, the accumulated stomatal flux of the well watered non fumigated cultivars into Extradur was **2.12** mmol m^{-2}, which was 37% lower than in 2000. Under fumigated conditions, the $AF_{st}6$ of the well watered plants was in 2001 39% lower than in 2000 (**5.37** mmolm^{-2}).

4.1.3 Nandu:

In the experimental year 2000, the accumulated stomatal flux with the critical threshold of 6 under well watered conditions was **1.00** mmol m^{-2}, which was 70% lower than the $AF_{st}6$ of Extradur under the same non fumigated and well watered conditions. The ozone sensitive cultivar showed an increase of 276% under fumigated conditions (**3.77** mmol m^{-2}).

This increase was 1.7 times higher compared with the less ozone sensitive cultivar Extradur.

The drought stressed plants of the cultivar Nandu showed a 94% lower flux value than the well watered plants (**0.06** mmol m^{-2}) under non fumigated conditions. Compared with the cultivar Extradur this difference was 1.4 times lower. The fumigated and drought stressed treatment showed an increase of the accumulated ozone flux of **0.59** mmol m^{-2}. The stomatal ozone flux of the non fumigated treatment was 89% lower compared with the fumigated treatment.

In the experimental year 2001, the stomatal flux of ozone into the well watered Nandu plants was **0.55** mmol m^{-2} and **2.26** mmol m^{-2} under fumigated conditions. This was a very similar result compared with the stomatal ozone flux in the experimental year 2000 and again 1.2 times lower compared with Extradur.

The drought stressed and non fumigated treatments in the experimental year 2001 were with **0.01** mmol m^{-2} 81% lower than in 2000 and 98% lower than the well watered plants. The $AF_{st}6$ of the ozone fumigated plants under drought stress showed a decrease of 88% with **0.27** mmol m^{-2} compared with the well watered plants.

The stomatal flux curves showed an increase of ozone uptake from appearance of the flag leaf until anthesis and represented the full development of flag leaf.

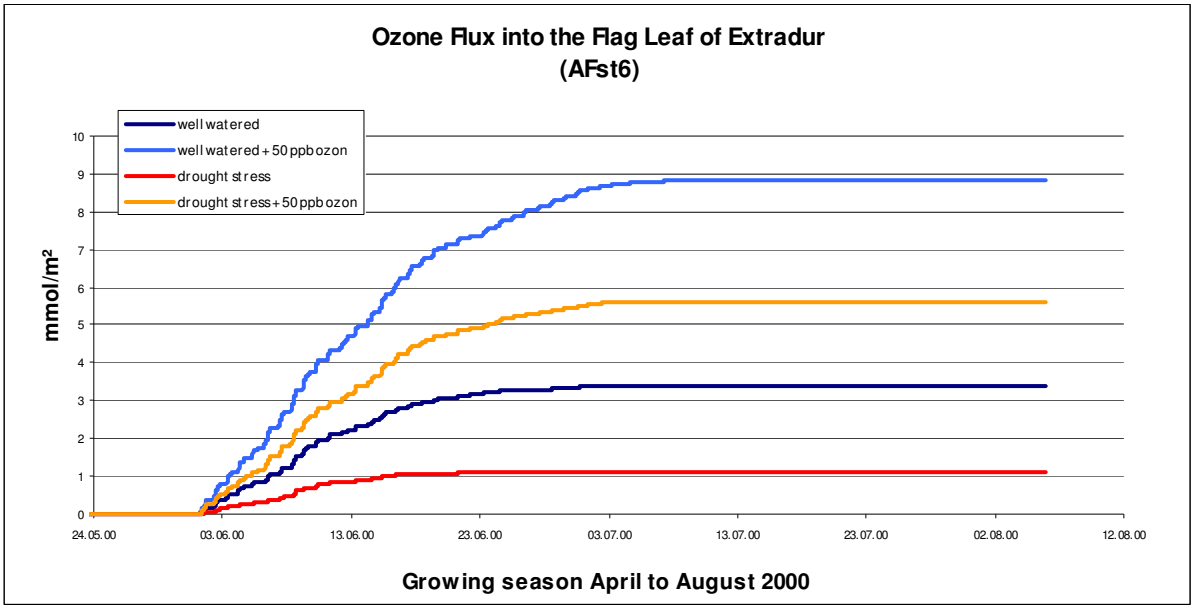


Fig. 8: Stomatal ozone flux into the cultivar Extradur in the first experimental year 2000.

In Fig. 8 the $AF_{st}6$ into the well watered plants of Extradur under fumigated conditions showed an increase of 57% compared with the fumigated and drought stressed plants, and was nearly 7 times higher than the stomatal ozone flux into the Extradur cultivar compared with the drought stressed plants without fumigation.

The stomatal ozone flux of the well watered treatment without fumigation was two times higher than the drought stressed treatment without fumigation but 66% lower than the drought stressed treatment with fumigation and 62% lower than the well watered treatment with fumigation.

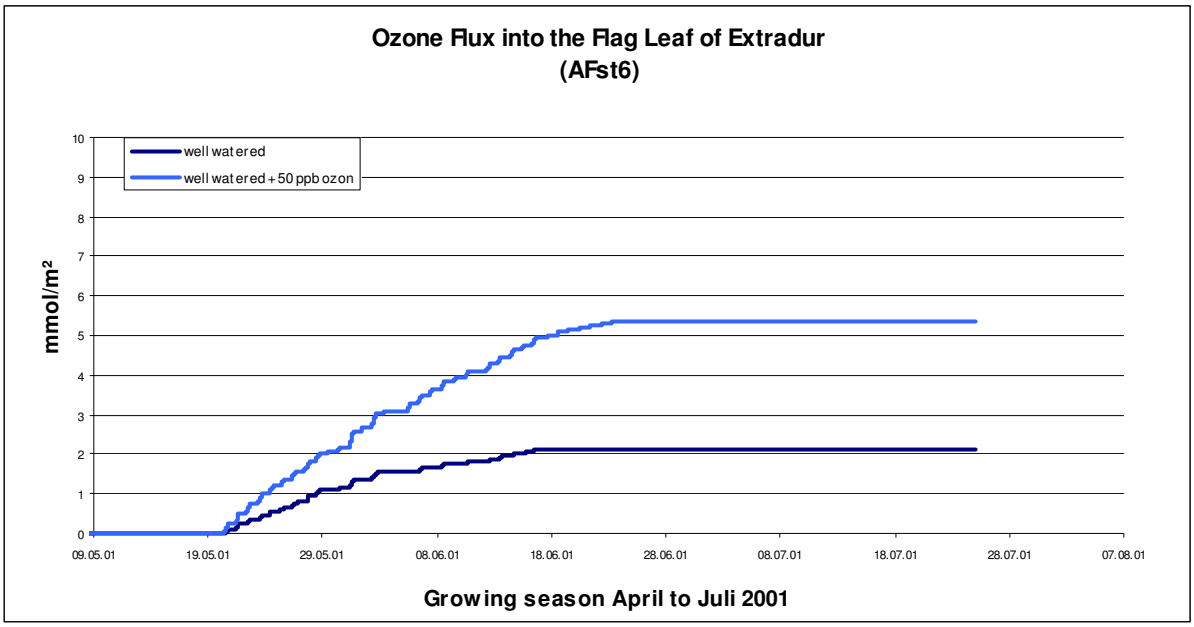


Fig. 9: Ozone flux into Extradur in the experimental year 2001.

In the experimental year 2001 the $AF_{st}6$ of the well watered plants with fumigation was 1.5 times higher than the stomatal flux of the plants without fumigation as can be seen in Fig. 9.

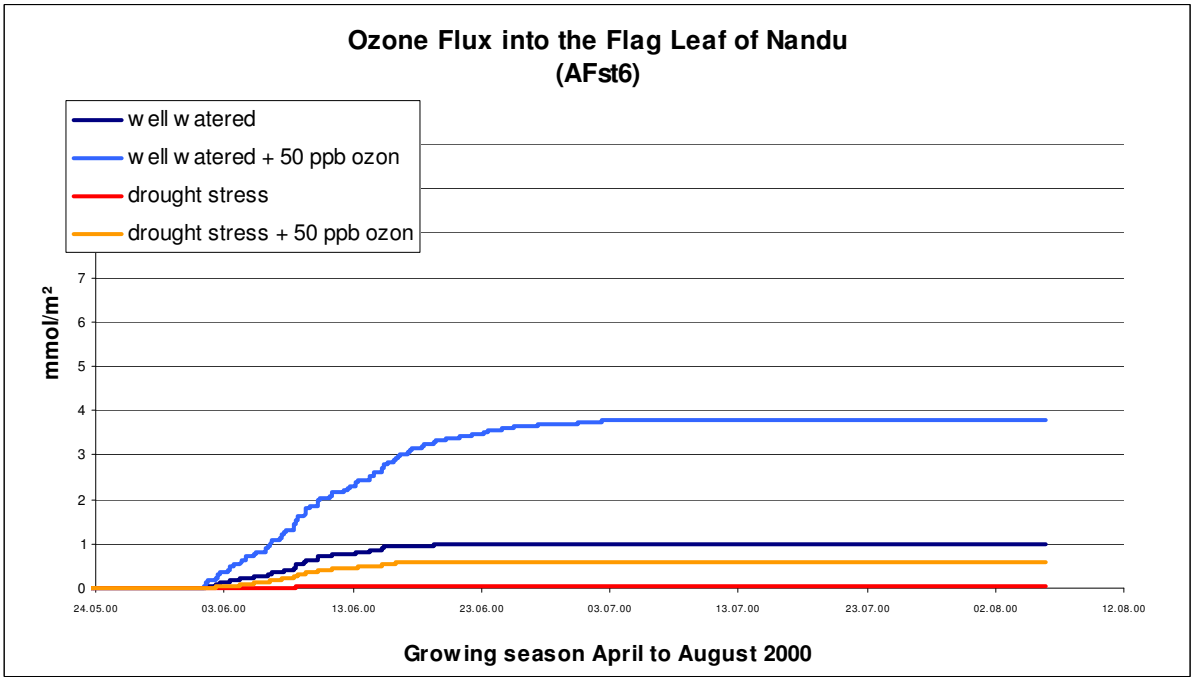


Fig. 10: Stomatal ozone flux into the cultivar Nandu in the experimental year 2000.

As can be seen in Fig. 10, in the experimental year 2000, the $AF_{st}6$ into the well watered plants of Nandu under fumigated conditions was 5.4 times higher than the stomatal ozone flux of the treatment under fumigation and drought stress and 58 times higher compared with the drought stressed plants without fumigation.

The stomatal ozone flux of the well watered treatment without fumigation was 14.6 times higher than the drought stressed treatment without fumigation but 41% lower than the drought stressed treatment with fumigation and 73% lower than the well watered treatment with fumigation.

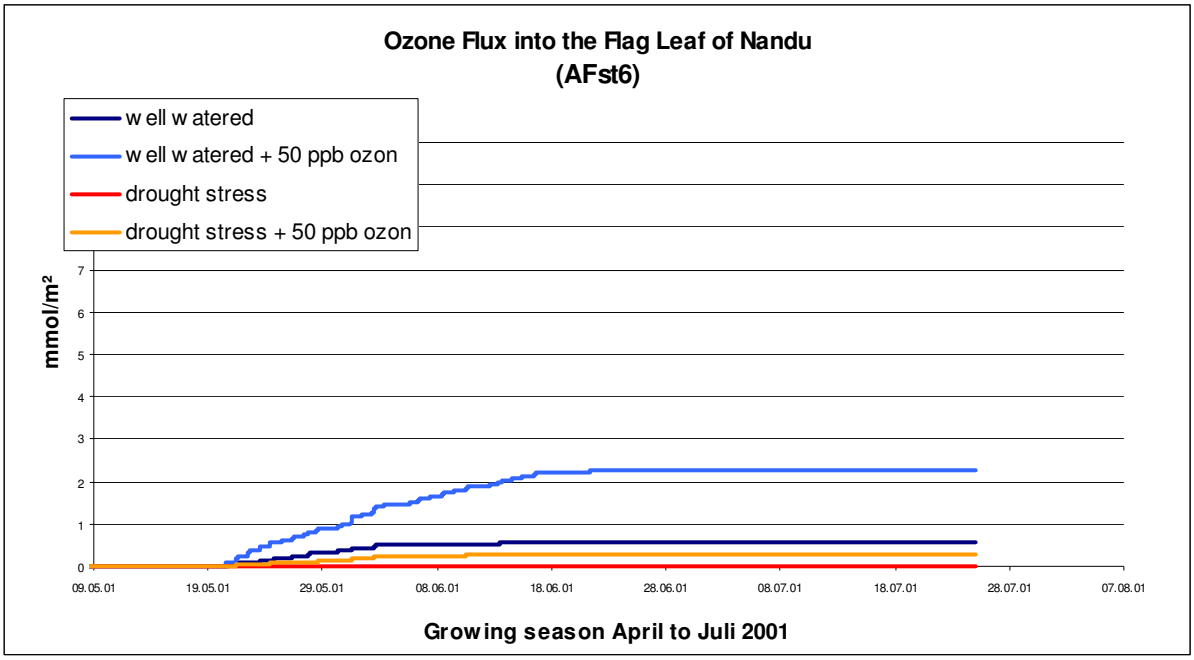


Fig. 11: Ozone flux into Nandu in the second experimental year 2001.

Fig. 11 shows that the $AF_{st}6$ into the well watered plants of Nandu under fumigated conditions was 7.4 times higher compared with the fumigated and drought stressed plants, and 187 times higher compared with the drought stressed plants without fumigation.

The stomatal ozone flux of the well watered treatment without fumigation was 45 times higher than the drought stressed treatment without fumigation but 52% lower than the drought stressed treatment with fumigation and 75% lower than the well watered treatment with fumigation.

In the experimental year 2000, the accumulated stomatal ozone flux into the cultivar Extradur was higher than $AF_{st}6$ into Nandu as well under well watered conditions without fumigation (2 times higher) and well watered conditions under fumigated conditions (1.3 times higher).

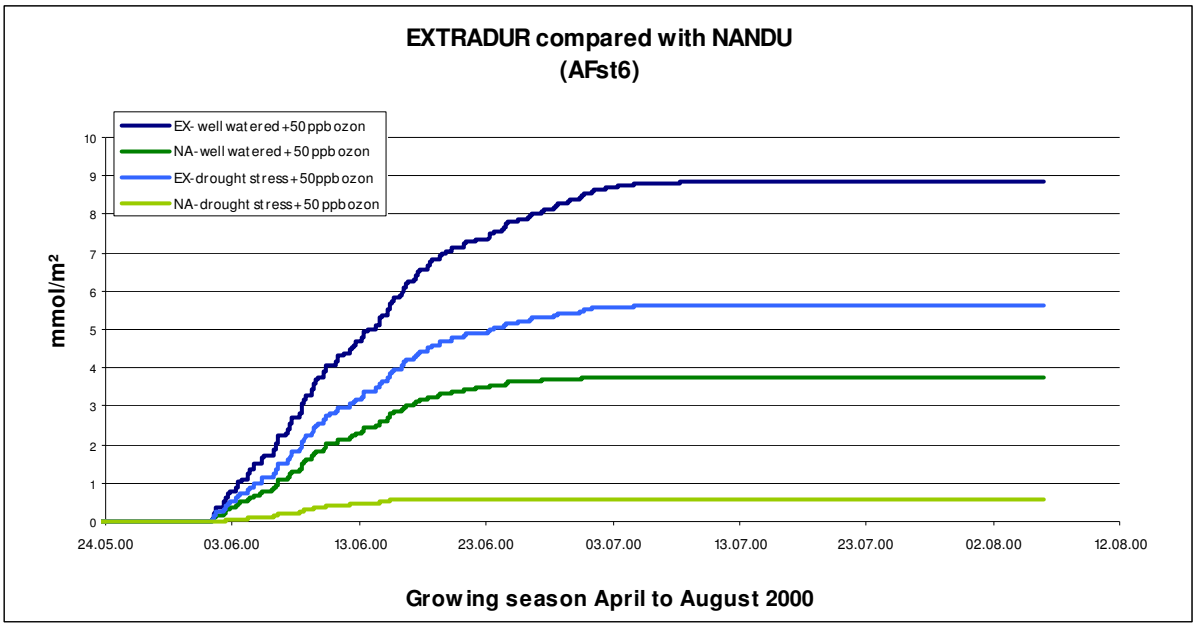


Fig. 12:Ozone fluxes of both cultivars Extradur and Nandu in the experimental year 2000.

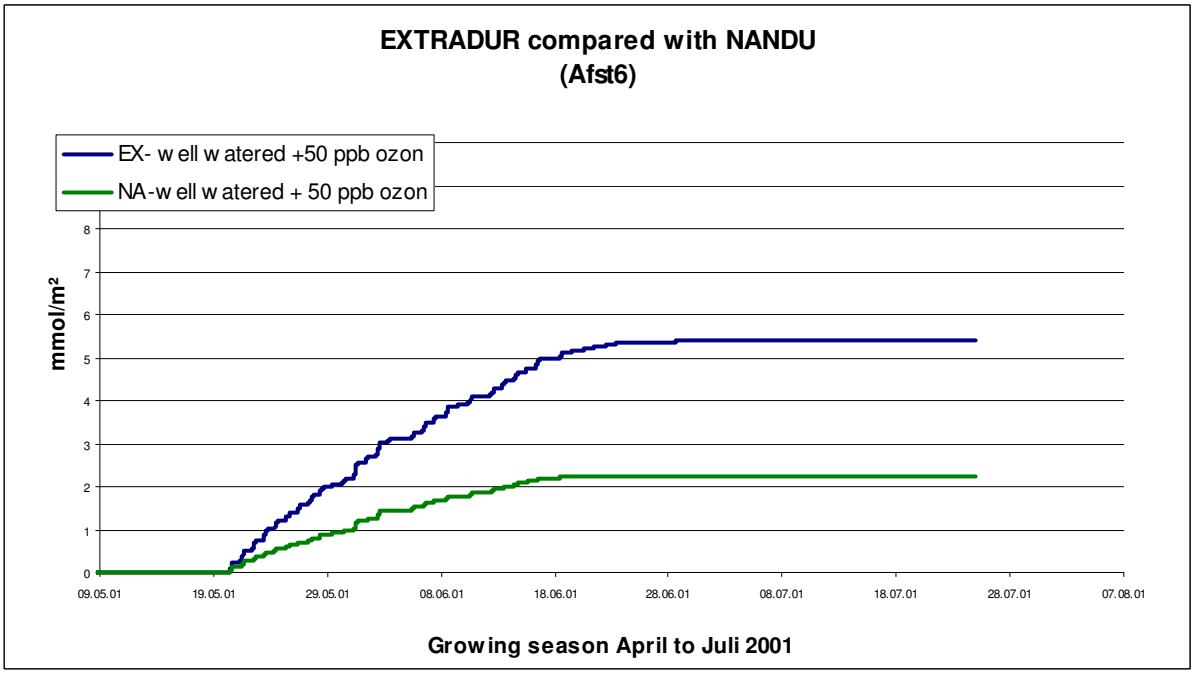


Fig. 13:A comparison of the cultivars Extradur and Nandu in the second experimental year 2001.

In the experimental year 2001, the $AF_{st}6$ into the well watered plants of Extradur was 1.4 times higher than the ozone flux into the well watered Nandu cultivars (Fig. 13). Both fluxes were calculated for the fumigated treatment.

4.2 Yield Reduction

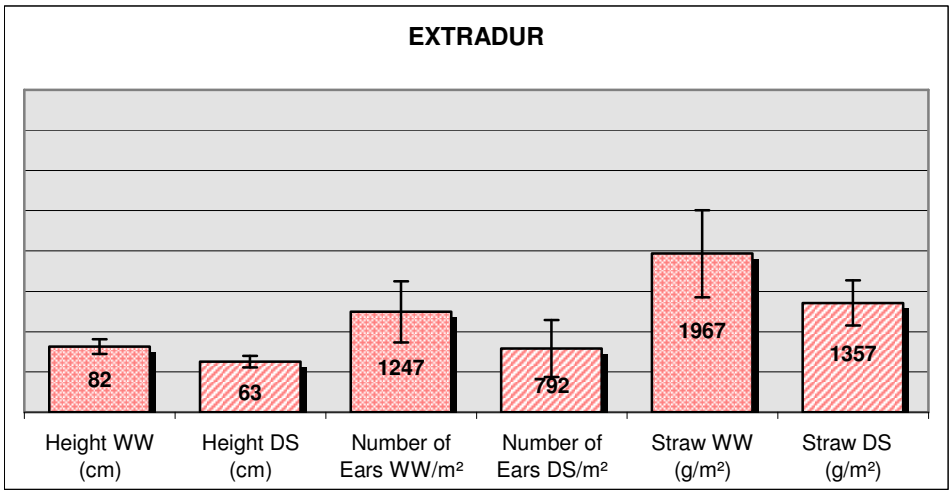


Fig. 14: Extradur well watered compared with Extradur under drought stress (under ambient conditions).

The yield parameters height, number of ears and straw dry mass of the cultivar Extradur had been compared under well watered as well as drought stressed conditions. The dry treatment was watered though 1x130 cm wicks of fibreglass (-40% soil water capacity), which caused drought stress. The well watered treatment was watered through 4x70 cm plus 1x130 cm wicks of fibreglass, a sufficient water supply over the vegetation period. All well watered treatments showed higher harvesting results. As can be seen in Fig. 14 the height of the well watered treatments was one third higher than the height of the drought stressed one, the number of ears of the well watered cultivars was 60% higher compared with the drought stressed plants and the dry mass of the well watered cultivars was 45% higher than those of the drought stressed ones.

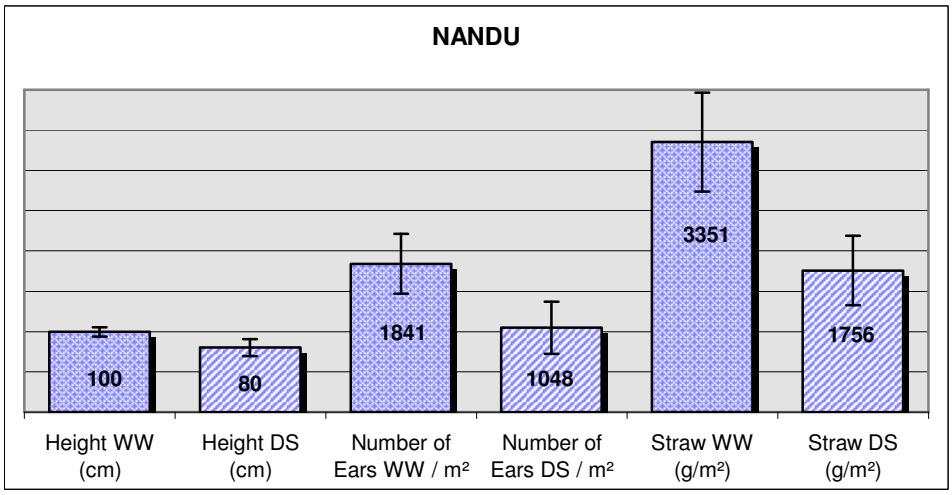


Fig.: 15: Nandu well watered compared with Nandu under drought stress and ambient conditions.

The yield parameters height, number of ears and straw dry mass of the cultivar Nandu have been compared under well watered as well as drought stressed conditions. The yield of all well watered treatments was higher compared with the drought stressed cultivars. Fig. 15 shows that the height of the well watered treatments was one fourth higher than the height of the drought stressed one, the number of ears of the well watered cultivars was 80% higher compared with the drought stressed plants and the straw dry mass of the well watered cultivars was 90% higher than those of the drought stressed ones.

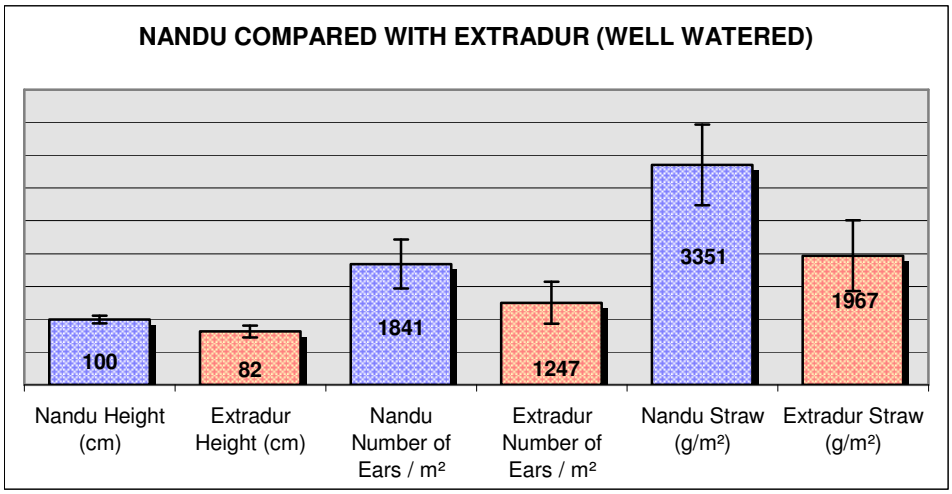


Fig. 16: Comparison of the well watered cultivar Nandu and the well watered cultivar Extradur under ambient conditions.

Both cultivars have been compared under well watered conditions. The main finding was a significant higher productivity of the cultivar Nandu. As can be seen in Fig. 16 for all yield parameters of Nandu higher values had been observed compared with Extradur. The height of the Nandu wheat stems was 20% higher than those of Extradur. In particularly straw dry mass was two third higher for the cultivar Nandu compared with Extradur. The number of ears of the cultivar Extradur was half the amount of the cultivar Nandu. The most significant difference was observed for the yield parameter number of ears.

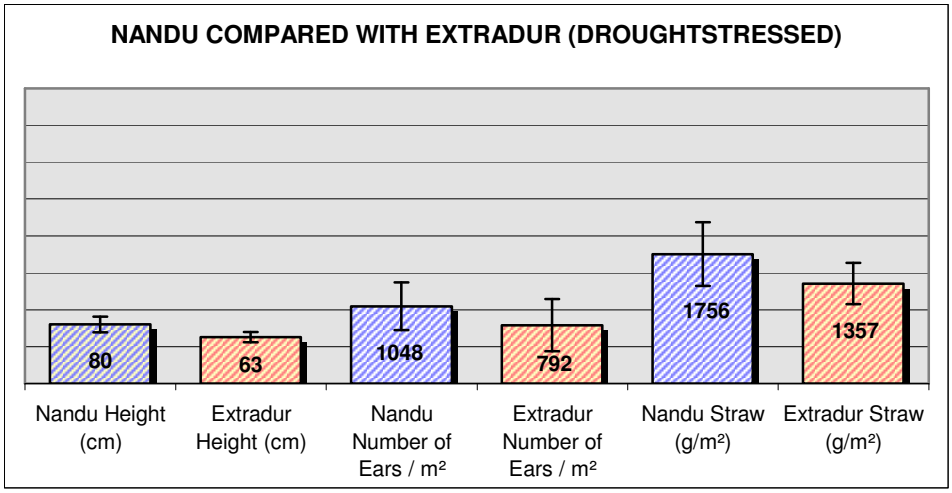


Fig. 17: Comparison of the cultivars Nandu and Extradur under drought stress and ambient conditions.

Fig. 17 shows a comparison of both cultivars under drought stress that resulted in a slightly higher yield of all parameters for Nandu. The yield differences under drought stress were reduced for all parameters, particularly for the yield parameter straw dry mass (23%). A smaller reduction was observed for the other parameters (height 27% and number of ears 36%).

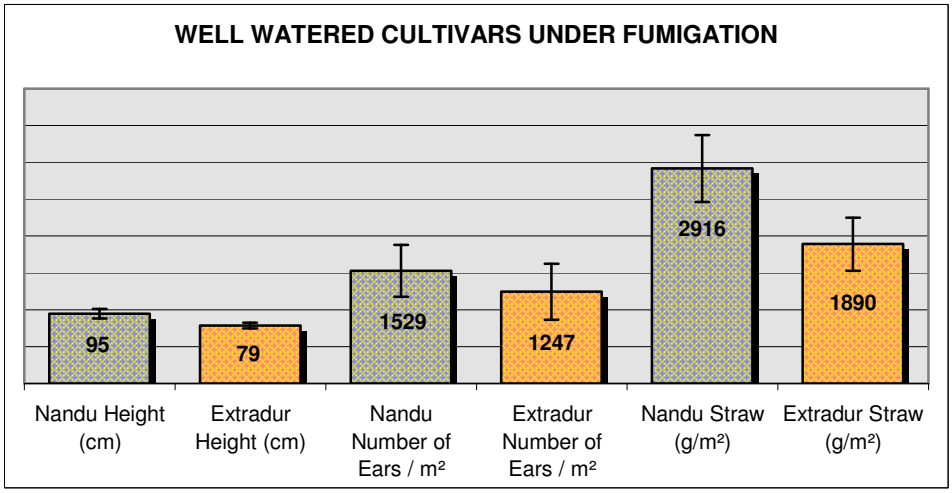


Fig. 18: Comparison of both well watered cultivars under ozone fumigation.

As can be seen in Fig. 18 the cultivar Nandu yields higher at elevated ozone. For all yield parameters a higher yield was observed for Nandu under well watered ozone fumigated conditions. Height was 20% higher for Nandu compared with Extradur, number of ears one fourth higher and particularly straw dry mass 55% higher for Nandu compared with the cultivar Extradur. But yield differences between the two cultivars decreased under ozone fumigation, particularly for height and straw (a reduction of straw dry mass by 55% under fumigation compared with 90% by ambient air without fumigation)

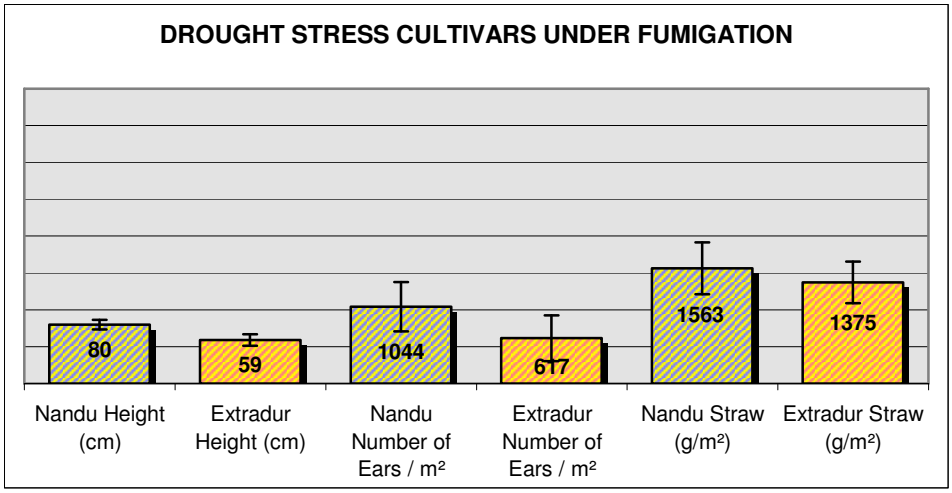


Fig. 19: Comparison of the cultivars Nandu and Extradur under ozone fumigation and drought stress.

The ozone and drought stressed sensitive cultivar Nandu yields slightly higher under ozone fumigated conditions. Fig. 19 shows that straw dry mass was 14% higher for Nandu compared with Extradur, the height of the stems was one third higher for Nandu compared with Extradur and particularly the number of ears was two third higher for Nandu compared with the cultivar Extradur. The most significant reduction of differences could be observed for the yield parameter straw by ozone fumigation and drought stress: Only 14% compared with 90% under well watered ambient conditions, one third under drought stressed ambient conditions and 55% under well watered and fumigated conditions.

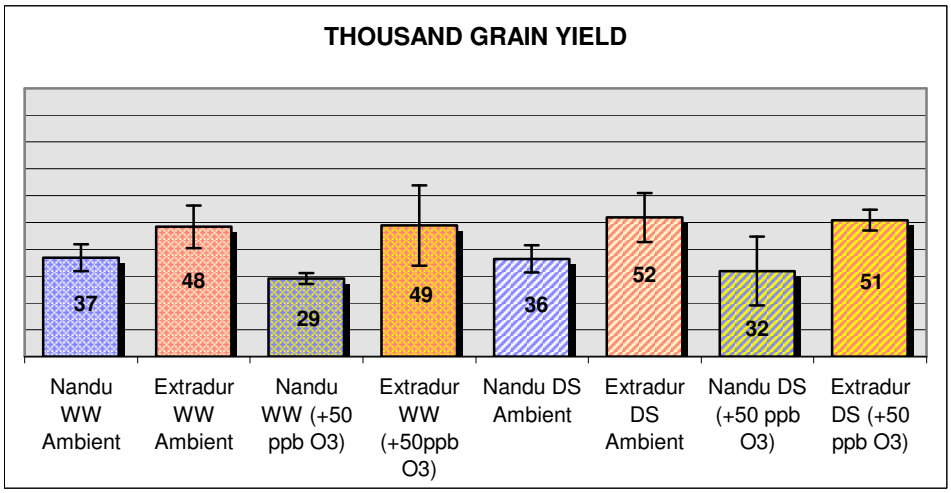


Fig. 20: Comparison of the thousand grain yield of both cultivars.

As can be seen in Fig. 20 the thousand grain yield of Extradur was under all treatment conditions higher than the thousand grain yield of Nandu. The thousand grain yield of Nandu was one third lower compared with Extradur under well watered and ambient conditions, and two third lower under well watered but ozone fumigated conditions.

Under drought stressed, ambient conditions the thousand grain yield of Nandu was one third lower compared with Extradur, and under ozone fumigated drought stressed conditions 37% . The thousand grain yield of the well watered plants of the cultivar Nandu was 22% reduced by ozone fumigation, and under drought stressed conditions, thousand grain yield was 10% reduced by ozone. No influences of ozone fumigation and drought stress could be observed for the less ozone sensitive cultivar Extradur.

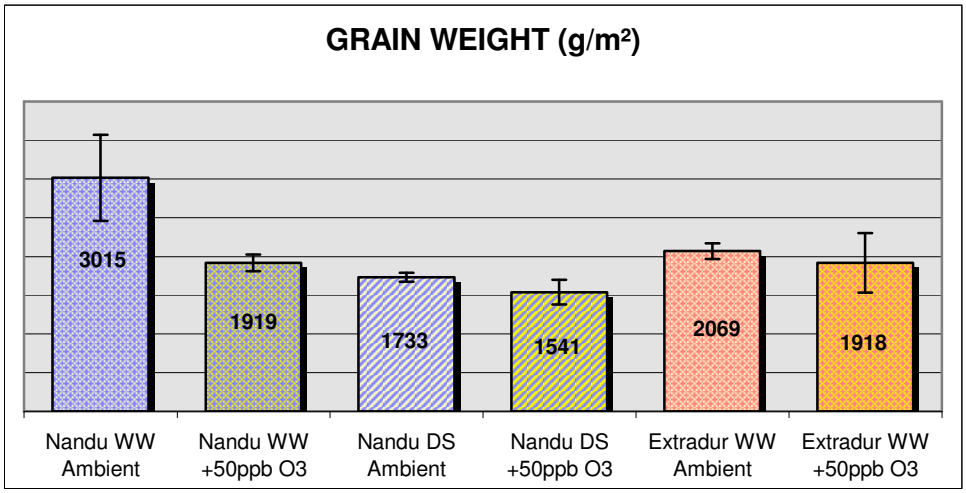


Fig 21: Comparison of the grain weight of Nandu and Extradur under different treatments.

The grain weight of both cultivars had been compared under well watered as well as drought stressed ambient and fumigated conditions. Fig. 21 shows that ozone reduced the grain weight of the well watered Nandu cultivars by 36% compared with the ambient treatment. Under drought stressed conditions ozone reduced the grain weight of Nandu by 11%. The grain weight of the drought stressed Nandu cultivars was 40% lower compared with the well watered cultivars. Under ozone fumigated and drought stressed conditions the grain weight of Nandu was 20% reduced compared with cultivars under ambient conditions. Ozone reduced the grain weight of the cultivar Extradur by 7% compared with the ambient treatment.

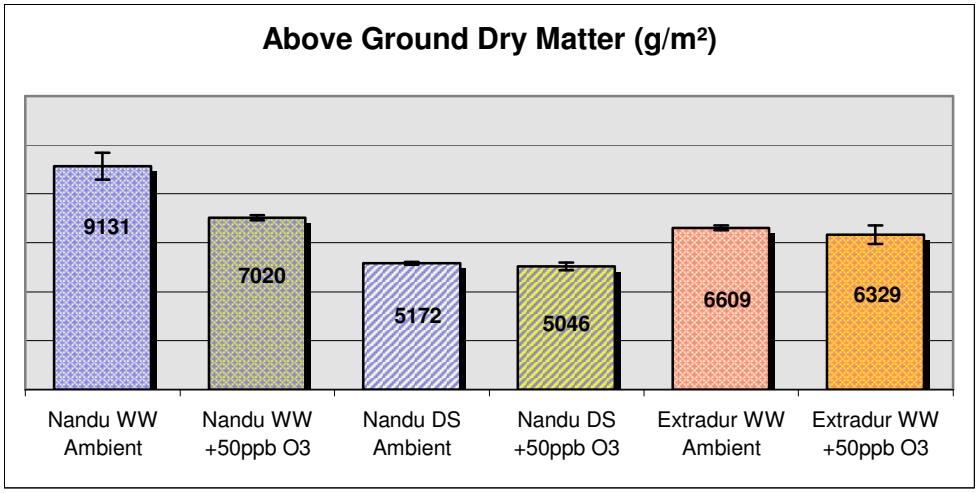


Fig. 22: Comparison of the above ground dry matter of both cultivars under different treatments.

The above ground dry matter of both cultivars had been compared under well watered as well as drought stressed ambient and fumigated conditions. As can be seen in Fig. 22 ozone reduced the above ground dry matter of the well watered Nandu cultivars by 23% compared with the ambient treatment. Under drought stressed conditions ozone reduced the above ground dry matter of Nandu by 2%. The above ground dry matter of the drought stressed Nandu cultivars was 40% lower compared with the well watered cultivars. Under ozone fumigated and drought stressed conditions the above ground dry matter of Nandu was 20% reduced compared with cultivars under ambient conditions. Ozone reduced the above ground dry matter of the cultivar Extradur by 4% compared with the ambient treatment.

4.3 Statistical Analysis with SPSS

Height of tillers, number of ears, straw dry mass, thousand grain weight, above ground dry matter and grain weight/m^2 of the two cultivars Nandu and Extradur were analysed under two different ozone fumigation levels as well as under two different water supply levels. The tests were carried out with 3-factorial ANOVA. Normal distribution of the random samples was tested with the Kolmogorow-Smirnow-test and homogeneity of variances after Levene. All tests were carried out with SPSS.

The sensitivity of both cultivars towards ozone as well as drought stress is significant ($p=<0.001$).

Height of tillers:

Interactions between drought stress and ozone reduced the final height of the wheat plants marginally significant ($p=0.07$).

Number of ears/pot:

Marginally significant interactions between cultivar and drought stress could be observed (p=0.061).

Straw dry mass:

The variance of cultivars towards decreasing water capacity was mostly affected for the straw dry mass (p=0.001). Significant interactions could be observed between cultivars and drought stress (p=0.001). Also significant interactions could be observed between ozone and cultivar (p=0.039).

Thousand grain weight:

No significant effects of ozone and drought stress could be observed but cultivar difference was significant (p=0.001).

Grain weight/m^2:

Ozone affected the grain weight/m2 significantly (p=0.0002). No significant interactions between drought and ozone could be observed.

Above ground dry matter:

Significant effects of ozone could be observed (p=0.0007) as well as significant interactions (p=0.02) between drought and ozone.

ANOVA-(Height-Cultivar,Drought, O_3)					
Source	**Square Sum of Type III**	**df**	**Mean Squares**	**F**	**Significance**
Corrected Model	15665.375	7	2237.910714	42.7809658	8.18955E-27
Intercept	611237.7788	1	611237.7788	11684.71215	4.4076E-102
Cultivar	5194.471154	1	5194.471154	99.29998162	1.7688E-16
Drought	8991.240385	1	8991.240385	171.8808284	4.08705E-23
Ozone	1253.086538	1	1253.086538	23.95459837	3.97591E-06
Cultivar * Drought	45.77884615	1	45.77884615	0.8751302	0.351886223
Cultivar * Ozone	5.086538462	1	5.086538462	0.097236689	0.755847486
Drought * Ozone	175.2403846	1	175.2403846	3.349978555	0.070308745
Cultivar * Drought * Ozone	0.471153846	1	0.471153846	0.009006801	0.92458876
Error	5021.846154	96	52.31089744		
Total	631925	104			
Corrected Cumulative Variation	20687.22115	103			
R-Square = .757 (corrected R-Square = .740)					

ANOVA-Number of ears-Cultivar, Drought, O_3					
Source	**Square Sum of Type III**	**df**	**Mean Squares**	**F**	**Significance**
Corrected Model	28268.31914	7	4038.33131	11.7170323	1.1509E-10
Intercept	256759.0685	1	256759.068	744.97461	9.4767E-47
Cultivar	11930.08829	1	11930.0883	34.6146016	5.9966E-08
Drought	9170.526038	1	9170.52604	26.6078589	1.3594E-06
Ozone	2616.261168	1	2616.26117	7.59096127	0.00703175
Cultivar * Drought	1235.591858	1	1235.59186	3.58501286	0.06134626
Cultivar * Ozone	631.8043881	1	631.804388	1.83315133	0.17896796
Drought * Ozone	723.1604547	1	723.160455	2.09821675	0.15076368
Cultivar * Drought * Ozone	1828.416601	1	1828.4166	5.30506655	0.02344457
Error	32742.20513	95	344.654791		
Total	319211	103			
Corrected Cumulative Variation	61010.52427	102			
R-Square = .463 (corrected R-Square = .424)					

ANOVA- Straw dry mass-Cultivar, Drought, O_3					
Source	**Square Sum of Type III**	**df**	**Mean Squares**	**F**	**Significance**
Corrected Model	359.9607847	7	51.4229692	40.7574206	4.5811E-26
Intercept	10535.97759	1	10535.9776	8350.7288	3.8025E-95
Cultivar	113.159227	1	113.159227	89.6890685	2.0434E-15
Drought	208.5349021	1	208.534902	165.283041	1.3623E-22
Ozone	15.54542616	1	15.5454262	12.3211764	0.00068385
Cultivar * Drought	14.92686708	1	14.9268671	11.8309115	0.00086358
Cultivar * Ozone	5.521436835	1	5.52143684	4.37624522	0.03908242
Drought * Ozone	2.085708492	1	2.08570849	1.65311532	0.20162821
Cultivar * Drought * Ozone	0.187216951	1	0.18721695	0.14838661	0.70093414
Error	121.1216258	96	1.2616836		
Total	11017.06	104			
Corrected Cumulative Variation	481.0824105	103			
R-Square = .748 (corrected R-Square = .730)					

ANOVA-1000 grain weight-Cultivar, Drought, O_3					
Source	**Square Sum of Type III**	**df**	**Mean Squares**	**F**	**Significance**
Corrected Model	0.000485899	7	6.9414E-05	13.3423328	2.0319E-05
Intercept	0.012940932	1	0.01294093	2487.42275	4.3731E-18
Cultivar	0.00044777	1	0.00044777	86.0674042	1.3283E-07
Drought	6.29502E-08	1	6.295E-08	0.01209988	0.913868
Ozone	7.73166E-06	1	7.7317E-06	1.48612929	0.24164251
Cultivar * Drought	8.10963E-06	1	8.1096E-06	1.55878072	0.23097976
Cultivar * Ozone	1.11264E-05	1	1.1126E-05	2.13865108	0.16426355
Drought * Ozone	1.57137E-06	1	1.5714E-06	0.30203778	0.59069698
Cultivar * Drought * Ozone	1.45383E-06	1	1.4538E-06	0.27944627	0.60479868
Error	7.80382E-05	15	5.2025E-06		
Total	0.013522919	23			
Corrected Cumulative Variation	0.000563937	22			
R-Square = .862 (corrected R-Square = .797)					

ANOVA- Grain weight/m^2, Drought, O_3					
Source	**Square Sum of Type III**	**df**	**Mean Squares**	**F**	**Significance**
Corrected Model	5,27134442	3	1,75711481	21,1133959	6,4468E-10
Intercept	1585,42097	1	1585,42097	19050,3322	5,0241E-89
Drought	3,81105876	1	3,81105876	45,7934747	2,963E-09
Ozone	1,22822487	1	1,22822487	14,7582832	0,00026069
Drought * Ozone	0,17870074	1	0,17870074	2,1472584	0,14717819
Error	5,99203778	72	0,08322275		
Total	1609,30384	76			
Corrected Cumulative Variation	11,2633822	75			
R-Square = ,468 (corrected R-Quadrat = ,446)					
R-Square = ,600 (corrected R-Square = ,583)					

ANOVA- Above ground dry matter, Drought, O_3					
Source	**Square Sum of Type III**	**df**	**Mean Squares**	**F**	**Significance**
Corrected Model	689395,818	3	229798,606	37,4505625	1,0763E-14
Intercept	8549932,88	1	8549932,88	1393,39312	7,4477E-49
Drought	574393,839	1	574393,839	93,6096729	1,1698E-14
Ozone	76470,2585	1	76470,2585	12,4624524	0,00072827
Drought * Ozone	32930,8432	1	32930,8432	5,36678017	0,02337292
Error	441795,758	72	6136,05219		
Total	9940389,8	76			
Corrected Cumulative Variation	1131191,58	75			
R-Square = ,529 (corrected R-Square = ,510)					
R-Square = ,609 (corrected R-Square = ,593)					

5 DISCUSSION

The main findings from the introduction of the flux maps were that areas experiencing the highest ozone exposures were frequently not the same as those regions calculated as having the highest ozone fluxes (Emberson et al., 2000).

This study demonstrates an increased stomatal ozone uptake under severe, elevated ozone concentrations (fumigation +50ppb). Stomatal closure was reduced, which increased the uptake of ozone into the leaf. Higher yield loss consequently was observed for the cultivars, which were exposed to elevated ozone fumigation. But yield reduction by severe drought stress was more pronounced than those induced by ozone (Khan and Soja, 2003).

For the cultivar Extradur the findings show an increase (2.6 times) of ozone uptake under ozone fumigation. Ozone uptake of the well watered cultivar was only one third compared with the drought stressed plants. The direct effect of ozone on stomata was a main role in the impairment of photosynthesis (Plazek et al., 2000, Guidi eta l., 2001, Calatayud et al., 2004, Feng, 2007). O_3 also inhibits the synthesis of photosynthetic pigments, decreases the electron transport rate between the photo systems I and II (Calatayud, et al., 2004, Feng, 2007).

Longer-term exposure to O_3 causes stomatal responses to become sluggish (McAinsh et al., 2002, Harmens, 2005). This results in slower, less complete stomatal closure in response to drought, VPD and photosynthetic photon flux density (Paoletti, Paoletti and Grulke, 2005, Harmens, 2005).

Stomatal responses to O_3 are reduced under drought stress. Higher ozone concentrations within the fumigated chambers as well as stomatal responses under drought stressed conditions might have resulted in a higher ozone flux into the plants (5 times higher) under drought stress and ozone fumigation.

Drought stress and high temperatures are among the most important environmental factors that limit net photosynthesis rate (Hassan et al., 1998, Yordanov et al. 1999, 2000, Hassan, 2005). Drought stress is accompanied by decreased stomatal conductance; the stomatal ozone flux into the leaf is reduced, thereby decreasing the effects of ozone in the leaf and for the cultivar (Soja and Soja, 1995).

Drought stress reduced ozone uptake to one third for well watered plants. This seems to be due to the directly limiting influence on g_s. SMD is the key driver limiting flux under current central European conditions (Harmens, 2005). The control of stomatal aperture by environmental signals depends on coordinated alterations to guard cell turgor (ionic

fluxes and sugars), cytoskeleton organization, membrane transport and gene expression (Hetherington, 2003). Differences in water supply can select for a wide range of plant responses. Wheat yields are positively correlated with maximum stomatal conductance (Hetherington, 2003).

The more productive cultivar Nandu, a spring wheat species that is generally cultivated at our latitudes under humid conditions, with higher yields for all examined yield parameters, except thousand grain yield, was significantly more ozone sensitive compared with the less productive cultivar Extradur, a summer wheat species that is mainly grown in the Mediterranean area and therefore more adapted to drought than Nandu (Reichenauer, 1998, Herbinger, 2002). Pell et al, (1994), Sellden and Pleijel, 1995 and Franzaring et al., 2000 and Danielsson, 2003 suggest that plants with a high potential growth rates ought to be more sensitive to ozone exposure compared to plants with low growth rate also within the species.

In the experimental year 2001 the influence of fumigation was more obvious than in 2000. The vegetation period in 2001 was cooler, showed more rainfall and reduced ambient ozone concentration. In the second experimental year the ozone uptake was halved compared with 2000. This difference was reduced under ozone fumigation.

Ambient ozone concentrations were lower in the second year. The slow reactions of stomata did not allow the cultivars to adapt to increased fumigation, which might have lead to higher ozone fluxes into the plant.

The cultivar Nandu was more sensitive to ozone as well as drought stress. Ozone fumigation increased the stomatal ozone flux into the plant 3.8 times under well watered conditions and 9.2 times under drought stress. Also for Nandu it was obvious that drought stress reduced stomatal conductance because of the partial closure of the stomata, leading to a reduced flux.

The $AF_{st}Y$ approach represents a physiologically more relevant representation of the variation that occurs in ozone sensitivity under fluctuating climatic conditions (Pleijel et al., 1998, Soja et al., 2000, Pleijel, 2006).

Yield loss was observed for both cultivars by elevated ozone fumigation and severe drought stress. Drought stress effects were mostly recognized for the overall crop productivity, straw dry mass was strongest affected by increased ozone uptake as well as by drought stress (Khan and Soja, 2003). But also a reduced height of the wheat, a reduction of the number of ears and consequently a reduction of the grain weight and above ground dry matter were observed for ozone stress and drought stress (Fuhrer et al., 1989a, Soja and Soja, 1995, Kobayashi, 1995, Khan and Soja, 2003).

All yield parameters were stronger affected by drought stress than by ozone. Ozone reduced straw dry mass by 27% and 46% (Extradur and Nandu) under well watered

conditions and by 47% for Extradur and 42% for Nandu by drought stress. Yield loss was higher for the ozone and drought sensitive cultivar Nandu. The reactions to elevated ozone were mostly pronounced without drought stress. The higher productivity of Nandu decreased in comparison to Extradur and yield differences between the two cultivars decreased because of drought stress and elevated ozone, particularly the yield parameter straw dry mass was mostly affected by stress. A reduction of straw dry mass by 55% was observed under fumigation compared with 89% by ambient air without fumigation, 30% by drought stress and only 14% by drought stress and elevated ozone. The grain weight of Nandu was significantly affected by ozone (36% under well watered conditions) and drought stress (40%), as well as the above ground dry matter (by ozone 23% and by drought 40%). Ozone reduced the grain weight of Extradur by 7% and the above ground dry matter by 4%. The cultivar Nandu was obviously more affected by drought and ozone than the less sensitive cultivar Extradur.

The higher ozone tolerance of Extradur was discussed earlier by Reichenauer, 1998 and Herbinger, 2002, who found a significant lower leaf water potential of Extradur under imposed drought exposure and a better ability of photosynthetically active mesophyll cells to cope with photooxidative stress. Reichenauer (1998) investigated that a higher ozone tolerance of Extradur was not caused by decreased ozone uptake via the stomata as Extradur had the greatest leaf conductivity under control and ozone-treated plants. These findings correspond with the findings in this study. Extradur showed higher stomatal conductivity and consequently higher ozone flux into the flag leaf compared with the more sensitive cultivar Nandu.

For the less sensitive cultivar Extradur the yield parameter thousand grain weight was not affected by drought stress as well as ozone. Ozone effects on thousand grain yield were observed by Finnan et al. 1996, Amundson et al. 1987, Pleijel et al. 1991 and Khan and Soja, 2003. Ozone-induced grain yield were especially pronounced under high water supply. Moderate drought stress did not result in ozone-induced reduction of thousand grain yield (Khan and Soja, 2003). As uptake of ozone is largely controlled by stomata (Kerstiens and Lendzian, 1989), closure of stomata is caused by drought. For the ozone sensitive cultivar Nandu marginal effects of ozone as well as drought stress on the thousand grain yield could be observed. Drought stress reduced the thousand grain yield to a greater extent (one third) than ozone (22%), which was also observed by Finnan et al. (1996), Amundson et al. 1987, Pleijel et al. 1991 and Khan and Soja, 2003. But the thousand grain yield was stronger affected by ozone under high water supply (by 22%) and showed only a decrease of 10% under drought stress. This corresponds with the findings of Khan and Soja, 2003.

The present study did not observe significant interactions between water supply and ozone stress on thousand grain yield. This corresponds with the results of Fangmeier et

al. (1994a), who found that thousand grain weight was not affected by water stress. The reason for these findings might be the choice of the water treatment (Khan and Soja, 2003). Only distinctive higher or lower water supply resulted in either increased or lowered yield reductions due to ozone (Khan and Soja, 2003), and in the medium range no interactions could be observed (Fangmeier et al. 1994a). Water supply in this study might have been in this medium range, where no significant interactions could be observed for thousand grain yield.

The yield results of straw dry mass, number of ears and height of wheat in this study show that water stress is a very potent modifier of ozone injury in wheat. An exact quantification of the influence of different levels of drought stress on ozone injury becomes more important for a realistic assessment of the risk to crop yield in future ozone scenarios, as drought stress may more frequent occur in Central Europe in future climate change scenarios.

6 CONCLUSION

There is evidence of increase in global background ozone concentration (Ashmore, 2005), which leads to direct and indirect effects of other changes in the global atmosphere and cause modified plant responses to ozone.

Stomatal flux models provide a basis for future regional policy assessments. In a future climate complex interactions have to be considered. Risk assessment has to include complex effects of ozone, interactions with water availability and species interactions, which leads to a full understanding of the capacity of species to adapt to climate change and reduced water availability.

7 SUMMARY:

The modifying effects of elevated ozone and severe drought stress under fluctuating micro-climatic conditions on the stomatal ozone flux were investigated in 2000 and 2001. Stomatal ozone flux into two wheat cultivars was observed and the resulting yield loss through ozone and drought stress was calculated. The experiments were conducted in East Austria. Plants were grown in Open Top Chambers ventilated with ambient air and elevated ozone (+50ppb).

Based on the Emberson Model (2000, Modelling stomatal ozone flux across Europe) g_{sto} as a function of leaf phenology and the environmental variables VPD, temperature, light, SMD was calculated using climate data, measured on the site. The drought stressed treatment was watered through 1x130 cm wick of fibreglass according to the treatment protocol of the IC protocol, which caused significant drought stress. The well watered treatment was watered through 4x70 cm plus 1x130 cm wicks. The best correlations between effect and accumulated stomatal flux were obtained (Danielsson, 2003, Pleijel, 2002) when using the stomatal flux based critical level of $AF_{st}6$ (accumulated stomatal flux of ozone above a flux rate threshold of 6 nmol ozone m_{-2} projected sunlit leaf area, based on hourly values of ozone flux), which was used as threshold level for calculating stomatal ozone flux in this study. The calculation of ozone uptake was based on repeated measurements of leaf conductance.

It was confirmed that stomatal ozone flux into wheat was strongly influenced by ozone concentration and drought stress. When compared with ambient air conditions elevated ozone fumigation caused higher ozone uptake into the plant. Drought stress effects had more impact on the overall crop productivity, straw dry mass was strongest affected by increased ozone uptake and by drought stress.

The quantification of the influence of drought stress on ozone injury becomes more important for a realistic assessment of the risk to crop yield loss in future ozone scenarios, as drought stress may more frequently occur in Central Europe in future climate change scenarios.

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9 APPENDIX

9.1 Tables

9.2 Figures

9.3 Flux calculations

					Astart	-200,00		Aend	700,00

Year-day	Date - time	Ozone concentration AA	Ozone concentration NF	Ozone concentration + 50 ppb	Irradiance (PPFD)	Air temperature, OTC	Air RH, Met	Saturated vapour pressure, Air T°C (met)
		(ppb)	(ppb)	(ppb)	(umol m^{-2} s^{-1})	(°C)	%	(Pa)
103,00	12.04.2000 00:00	34,6	31,1	31,1	0,0	9,60	90,00	1200,1
103,04	12.04.2000 01:00	34,0	30,6	30,6	0,0	9,40	93,00	1184,0
103,08	12.04.2000 02:00	32,2	29,0	29,0	0,0	9,70	93,00	1208,2
103,13	12.04.2000 03:00	31,7	28,5	28,5	0,0	10,10	87,00	1241,0
103,17	12.04.2000 04:00	35,2	31,7	31,7	0,0	9,80	88,00	1216,3
103,21	12.04.2000 05:00	33,8	30,4	30,4	0,0	10,10	85,00	1241,0
103,25	12.04.2000 06:00	33,3	30,0	30,0	24,8	10,00	88,00	1232,7
103,29	12.04.2000 07:00	33,9	30,5	30,5	175,1	10,70	88,00	1291,7
103,33	12.04.2000 08:00	34,1	30,7	30,7	593,9	12,60	81,00	1464,5
103,38	12.04.2000 09:00	38,4	34,5	84,5	955,7	13,70	71,00	1573,6
103,42	12.04.2000 10:00	42,6	38,3	88,3	1282,6	16,10	57,00	1836,7
103,46	12.04.2000 11:00	43,9	39,5	89,5	1149,8	15,60	55,00	1778,9
103,50	12.04.2000 12:00	45,2	40,7	90,7	1003,9	16,20	55,00	1848,4
103,54	12.04.2000 13:00	44,7	40,2	90,2	642,0	16,20	52,00	1848,4
103,58	12.04.2000 14:00	48,5	43,7	93,7	496,1	16,80	48,00	1920,4
103,63	12.04.2000 15:00	50,8	45,7	95,7	382,3	17,70	43,00	2032,9
103,67	12.04.2000 16:00	39,4	35,4	85,4	253,9	10,90	64,00	1309,0
103,71	12.04.2000 17:00	47,8	43,0	93,0	194,1	9,10	77,00	1160,4
103,75	12.04.2000 18:00	47,3	42,5	42,5	45,2	8,60	78,00	1121,8
103,79	12.04.2000 19:00	46,7	42,1	42,1	7,3	8,20	79,00	1091,8
103,83	12.04.2000 20:00	45,2	40,7	40,7	0,0	8,90	70,00	1144,8
103,88	12.04.2000 21:00	44,7	40,2	40,2	0,0	8,50	73,00	1114,2
103,92	12.04.2000 22:00	41,4	37,3	37,3	0,0	8,50	72,00	1114,2
103,96	12.04.2000 23:00	34,2	30,8	30,8	0,0	7,80	82,00	1062,5
104,00	13.04.2000 00:00	34,2	30,8	30,8	0,0	7,50	87,00	1040,9

					Max	**0,9865**	**1**	**1**	**1**	**1**
Vapour pressure, RH (met) + Air T°C, (met)	**VPD**	**Soil Volumetric water content**	**Soil water potential**	**Effective temperature sum**	**g_{max}**					
(kPa)	(kPa)	(m^3/m^3)	MPa	(°C days)	(mmol H_2O $m^{-2\ PLA}$ s^{-1}	**f_{phen}**	**f_{light}**	**f_{temp}**	**f_{VPD}**	**f_{SWP}**
1,08	0,12	0,000	0,000	-1030,954	450	0,01	0,000	0,01	1,00	1,00
1,10	0,08	0,000	0,000	-1030,954	450	0,01	0,000	0,01	1,00	1,00
1,12	0,08	0,000	0,000	-1030,954	450	0,01	0,000	0,01	1,00	1,00
1,08	0,16	0,000	0,000	-1030,954	450	0,01	0,000	0,01	1,00	1,00
1,07	0,15	0,000	0,000	-1030,954	450	0,01	0,000	0,01	1,00	1,00
1,05	0,19	0,000	0,000	-1030,954	450	0,01	0,000	0,01	1,00	1,00
1,08	0,15	0,000	0,000	-1030,954	450	0,01	0,229	0,01	1,00	1,00
1,14	0,16	0,000	0,000	-1030,954	450	0,01	0,841	0,01	1,00	1,00
1,19	0,28	0,000	0,000	-1030,954	450	0,01	0,998	0,08	1,00	1,00
1,12	0,46	0,000	0,000	-1030,954	450	0,01	1,000	0,23	1,00	1,00
1,05	0,79	0,000	0,000	-1030,954	450	0,01	1,000	0,50	1,00	1,00
0,98	0,80	0,000	0,000	-1030,954	450	0,01	1,000	0,45	1,00	1,00
1,02	0,83	0,000	0,000	-1030,954	450	0,01	1,000	0,51	1,00	1,00
0,96	0,89	0,000	0,000	-1030,954	450	0,01	0,999	0,51	1,00	1,00
0,92	1,00	0,000	0,000	-1030,954	450	0,01	0,995	0,57	1,00	1,00
0,87	1,16	0,000	0,000	-1030,954	450	0,01	0,982	0,65	1,00	1,00
0,84	0,47	0,000	0,000	-1030,954	450	0,01	0,930	0,01	1,00	1,00
0,89	0,27	0,000	0,000	-1030,954	450	0,01	0,870	0,01	1,00	1,00
0,88	0,25	0,000	0,000	-1030,954	450	0,01	0,378	0,01	1,00	1,00
0,86	0,23	0,000	0,000	-1030,954	450	0,01	0,074	0,01	1,00	1,00
0,80	0,34	0,000	0,000	-1030,954	450	0,01	0,000	0,01	1,00	1,00
0,81	0,30	0,000	0,000	-1030,954	450	0,01	0,000	0,01	1,00	1,00
0,80	0,31	0,000	0,000	-1030,954	450	0,01	0,000	0,01	1,00	1,00
0,87	0,19	0,000	0,000	-1030,954	450	0,01	0,000	0,01	1,00	1,00
0,91	0,14	0,000	0,000	-1019,504	450	0,01	0,000	0,01	1,00	1,00

0,910041717	**409,5187728**	**0,009988263**			**0,009163792**	**375,715465**
relative g	**Stomatal conductance (g_{sto} O_3)**	**Stomatal conductance (g_{sto} O_3)**	**Leaf boundary layer resistance (rb)**	**Leaf surface resistance (rc)**	**g_{total}**	**g_{total}**
fraction	(mmol O_3 m^{-2} PLA s^{-1})	(m/s) P	s/m	s/m	m/s	(mmol m^{-2} PLA s^{-1})
0	0,00	0,0000	8,66	2500,00	0	0
0	0,00	0,0000	8,66	2500,00	0	0
0	0,00	0,0000	8,66	2500,00	0	0
0	0,00	0,0000	8,66	2500,00	0	0
0	0,00	0,0000	8,66	2500,00	0	0
0	0,00	0,0000	8,66	2500,00	0	0
0,002292568	1,03	0,0000	8,66	2352,04	2,507E-05	1,027870614
0,008409336	3,78	0,0001	8,66	2031,29	9,19057E-05	3,768134909
0,009980415	4,49	0,0001	8,66	1962,55	0,00010906	4,471454315
0,009999562	4,50	0,0001	8,66	1961,74	0,000109269	4,480024105
0,009999986	4,50	0,0001	8,66	1961,72	0,000109274	4,480213988
0,009999943	4,50	0,0001	8,66	1961,72	0,000109273	4,480194758
0,009999736	4,50	0,0001	8,66	1961,73	0,000109271	4,480101983
0,009988185	4,49	0,0001	8,66	1962,22	0,000109145	4,474932111
0,009945325	4,48	0,0001	8,66	1964,03	0,000108677	4,455747771
0,009819396	4,42	0,0001	8,66	1969,38	0,000107302	4,399380936
0,009304586	4,19	0,0001	8,66	1991,54	0,000101681	4,168934008
0,008696719	3,91	0,0001	8,66	2018,36	9,5044E-05	3,896802273
0,003780113	1,70	0,0000	8,66	2265,06	4,1331E-05	1,69457136
0,00073678	0,33	0,0000	8,66	2450,46	8,05813E-06	0,330383147
0	0,00	0,0000	8,66	2500,00	0	0
0	0,00	0,0000	8,66	2500,00	0	0
0	0,00	0,0000	8,66	2500,00	0	0
0	0,00	0,0000	8,66	2500,00	0	0
0	0,00	0,0000	8,66	2500,00	0	0

18,43807571	36,29364215	12,43807571	30,29364215		15275144,9	15,2751449
F_{st} NF	F_{st} NF + 50 ppb	F_{st}6 NF	F_{st}6 NF + 50 ppb	NF Stomatal ozone flux (AF_{st})	NF Acumulated Stomatal ozone flux (AF_{st})	NF Acumulated Stomatal ozone flux (AF_{st})
(nmol m^{-2} PLA s^{-1})	(nmol m^{-2} PLA s^{-1})	(nmol m^{-2} PLA s^{-1})	(nmol m^{-2} PLA s^{-1})	(nmol m^{-2})	(nmol m^{-2})	(mmol m^{-2})
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0,030842286	0,030842286	0	0	111,0322283	111,0322283	0,000111032
0,11489797	0,11489797	0	0	413,6326908	524,6649191	0,000524665
0,137067961	0,137067961	0	0	493,4446581	1018,109577	0,00101811
0,154628032	0,378629237	0	0	556,6609152	1574,770492	0,00157477
0,171731082	0,395741782	0	0	618,2318966	2193,002389	0,002193002
0,177093138	0,401102876	0	0	637,5352982	2830,537687	0,002830538
0,18229087	0,406295969	0	0	656,2471305	3486,784818	0,003486785
0,179865421	0,403612027	0	0	647,5155166	4134,300334	0,0041343
0,194533492	0,417320881	0	0	700,320571	4834,620905	0,004834621
0,201100102	0,421069149	0	0	723,960367	5558,581272	0,005558581
0,147642798	0,356089498	0	0	531,5140724	6090,095345	0,006090095
0,167640434	0,362480547	0	0	603,5055616	6693,600906	0,006693601
0,072092149	0,072092149	0	0	259,5317378	6953,132644	0,006953133
0,013894924	0,013894924	0	0	50,0217264	7003,15437	0,007003154
0	0	0	0	0	7003,15437	0,007003154
0	0	0	0	0	7003,15437	0,007003154
0	0	0	0	0	7003,15437	0,007003154
0	0	0	0	0	7003,15437	0,007003154
0	0	0	0	0	7003,15437	0,007003154

	32667764,13	32,66776413		4578394,803	4,578394803	
NF +50ppb Stomatal ozone flux (AF_{st})	**NF +50ppbAcumulated Stomatal ozone flux (AF_{st})**	**NF +50ppb Acumulated Stomatal ozone flux (AF_{st})**	**NF Stomatal ozone flux ($AF_{st}6$)**	**NF Acumulated Stomatal ozone flux ($AF_{st}6$)**	**NF Acumulated Stomatal ozone flux ($AF_{st}6$)**	**NF +50ppb Stomatal ozone flux ($AF_{st}6$)**
(nmol m^{-2})	(nmol m^{-2})	(mmol m^{-2})	(nmol m^{-2})	(nmol m^{-2})	(mmol m^{-2})	(nmol m^{-2})
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
111,0322283	111,0322283	0,000111032	0	0	0	0
413,6326908	524,6649191	0,000524665	0	0	0	0
493,4446581	1018,109577	0,00101811	0	0	0	0
1363,065254	2381,174831	0,002381175	0	0	0	0
1424,670415	3805,845246	0,003805845	0	0	0	0
1443,970355	5249,8156	0,005249816	0	0	0	0
1462,665487	6712,481088	0,006712481	0	0	0	0
1453,003297	8165,484384	0,008165484	0	0	0	0
1502,35517	9667,839554	0,00966784	0	0	0	0
1515,848935	11183,68849	0,011183688	0	0	0	0
1281,922194	12465,61068	0,012465611	0	0	0	0
1304,929971	13770,54065	0,013770541	0	0	0	0
259,5317378	14030,07239	0,014030072	0	0	0	0
50,0217264	14080,09412	0,014080094	0	0	0	0
0	14080,09412	0,014080094	0	0	0	0
0	14080,09412	0,014080094	0	0	0	0
0	14080,09412	0,014080094	0	0	0	0
0	14080,09412	0,014080094	0	0	0	0
0	14080,09412	0,014080094	0	0	0	0

18631794,44	18,63179444
NF +50ppbAcumulated Stomatal ozone flux ($AF_{st}6$)	**NF +50ppb Acumulated Stomatal ozone flux ($AF_{st}6$)**
(nmol m^{-2})	(mmol m^{-2})
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0

$AF_{st}0$ of NF treatment in growing season 2000 (mmol m^{-2}):	**15,27514**
$AF_{st}0$ of NF+50 ppb treatment in growing season 2000 (mmol m^{-2}):	**32,66776**
$AF_{st}6$ of NF treatment in growing season 2000 (mmol m^{-2}):	**4,578395**
$AF_{st}6$ of NF+50 ppb treatment in growing season 2000 (mmol m^{-2}):	**18,63179**