

## Effects of air pollutants on plants

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**Ozone is the most important air pollutant affecting plants in Switzerland and in many other regions of NW-Europe. Critical levels set by the UN/ECE to protect plants are exceeded over extended areas, but there is increasing evidence that selection for ozone tolerance can take place in response to intensive ozone stress. Effects of ozone, including visible injury, or long-term changes in growth, biomass allocation, yield, reproduction, competitiveness, and vitality, depend on the dose of ozone absorbed by the plant. In order to assess the ozone risk for vegetation, it is thus necessary to develop dynamic models for flux-effect relationships which take into account the modifying influence of environmental factors.**

Air pollutants, such as sulfur dioxide (SO<sub>2</sub>), nitrogen oxides (NO, NO<sub>2</sub>), or ozone (O<sub>3</sub>) are well known to be toxic to plants. Adverse effects can be observed when threshold concentrations are exceeded. These thresholds depend on species and plant-specific attributes (developmental stage, genetic make-up, etc.), and on the prevailing local environmental conditions. In recent years, it became evident that pollution climates in rural areas of Europe are dominated by ozone. Thus most of the effect-oriented pollution research was directed towards the investigation of mechanisms of ozone action and the possible range of plant responses to elevated ozone, or was designed to set-up a framework for spatial and temporal risk assessments. This latter activity has been stimulated by the need for a scientific underpinning of political decisions taken at the European level to reduce precursor emissions. The UN/ECE Convention on Long-Range Transboundary Air Pollution aims at establishing binding protocols for emission reductions based on effect-based criteria, such as critical levels for gaseous pollutants. Critical levels are referred to as those concentrations above which adverse effects may occur according to current knowledge.

The aim of this review is to highlight some important aspects with regard to the effect of excessive levels of ozone on plants, and to describe elements needed to assess the potential risk for crops and herbaceous vegetation.

### Flux of pollutants

As in the case of other gaseous pollutants, ozone uptake by leaves and needles is crucial for the effect of the gas on structural and functional components of plants. In other words, plants respond to the absorbed dose rather than to the external concentration in ambient air. Pollutant flux, i.e. the rate at which the pollutant is absorbed by plant surfaces, is determined by three transport components (Fig. 1):

- Atmospheric transport by turbulent diffusion.
- Molecular diffusion across the leaf boundary layer.
- Diffusion through the stomatal pore, i.e. stomatal uptake.

Thus the pollutant flux ( $F_s$ ) is a function of (i) air conductivity or atmospheric resistance ( $r_{am}$ ), (ii) diffusive resistance at the leaf-air boundary layer ( $r_{bg}$ ), and (iii) stomatal resistance ( $r_{st}$ ), and  $F_s$  can be expressed as

$$F_s = - r_t^{-1} (t) \{ [\chi]_{z1} (t) - [\chi]_{z2} (t) \}$$

where  $r_t$  is the total transport resistance,  $r_t = r_{am} + r_{bg} + r_{st}$ , and  $[\chi]_{z1}$  and  $[\chi]_{z2}$  are time-dependent concentration of the pollutant gas at the height  $z1$  or  $z2$ , respectively. By convention, the minus sign is needed to indicate that the flux is from the atmosphere towards the ground. The amount of the pollutant absorbed by the plant (pollutant absorbed dose = PAD) is a function of the flux integrated over the duration of the exposure ( $T$ ) [1].

$$PAD = \int_0^T F_s(t) dt.$$

The amount of the pollutant which penetrates the leaf and is effective inside the leaf, i.e. the effective flux (EF), can then be regarded as the balance between PAD and the defensive response of the plant ( $D(t)$ ) [2]. The latter depends on chemical detoxification and biochemical mechanisms of repair and/or compensation.

Under most circumstances, PAD will largely depend on stomatal uptake ( $r_{st}$ ) [3]. In open-top fumigation chambers (OTCs), which are commonly used to study effects of air pollutants on plants under field conditions,  $r_{am}$  and  $r_{bg}$  are virtually zero, and ozone uptake follows canopy conductance to water vapor very closely [4]. In order to estimate PAD, parameterized models for gas fluxes can be used [1]. They consider the influence of environmental parameters on the transfer resistance, for instance of light, wind velocity and

atmospheric stability, leaf-to-air vapor pressure deficit (vpd), temperature, and soil water potential.

### Effects of ozone

Effects of PAD on plants can be divided into short-term effects (acute effects), and long-term effects (chronic effects). Short-term effects are caused by high PAD during limited periods of a few hours or days. They lead to rapid cell destruction and tissue death. In the case of ozone this can be observed visually as small necrotic flecks or “mottling” on the leaf surface. Plant species with low tolerance to short-term ozone exposure, such as white clover (*Trifolium repens*), can be used as active bio-monitors for the assessment of the spatial distribution of ozone episodes, but the timing and extent of observed symptom expression is modified by environmental conditions [5]. In the field, typical symptoms of ozone leaf injury have been observed in red and white clover, and recently also in knapweed (*Centaurea jacea*). In 1998, areas in which this was the case were the southern regions (observations were made near Cadenazzo, Ticino and Como/Italy) and the Geneva area (P. Bungener, personal communication). An example of a leaf of red clover (*Trifolium pratense*) with typical symptoms (necrosis) of ozone injury is shown in figure 2a. For *C. jacea* typical leaf decoloration due to ozone stress is shown in figure 2b. These epidemiological investigations also revealed large intra-specific differences in ozone sensitivity. The range of intra-specific variability in tolerance is important

with respect to the possibility of evolution of ozone tolerance in affected areas (see below).

Long-term effects depend on the cumulative uptake of ozone over extended periods of time (weeks to months). Primary effects may consist, for instance, in reduced growth or altered biomass allocation to different plant organs. Very often, they are accompanied by reduced longevity of leaves, i.e. by premature leaf senescence, as observed in wheat [6] and other crops. Typically, the symptom of premature senescence is leaf yellowing; however, this symptom is not specific for ozone, or other gaseous pollutants, and is thus not suitable as a visual indicator of air pollutant stress unless appropriate controls, for instance chemically protected plants or tolerant cultivars exposed in parallel, are available for comparison. Long-term effects may also be of secondary nature. In this case, no visual symptoms occur, but the plants are adversely affected in their resistance to other forms of stress. Most important are reduced resistance to pests and pathogens, drought, and cold.

Pollutant effects occur at different levels of biological organization [7]. Initially, effects of absorbed air pollutants occur at the sub-cellular level. In the case of ozone, the initial site of action is the plasma membrane. Ozone causes the formation of active oxygen species in the extra-cellular fluid. In turn, these reactive species may attack unsaturated components of the membrane, such as proteins. This may lead to the loss of membrane functions, and to metabolic and physiological disturbances, including reductions in net photosynthetic carbon assimilation. Effects at the cellular or organ level are the starting point for effects at higher trophic levels. These include, for instance, effects on the growth of

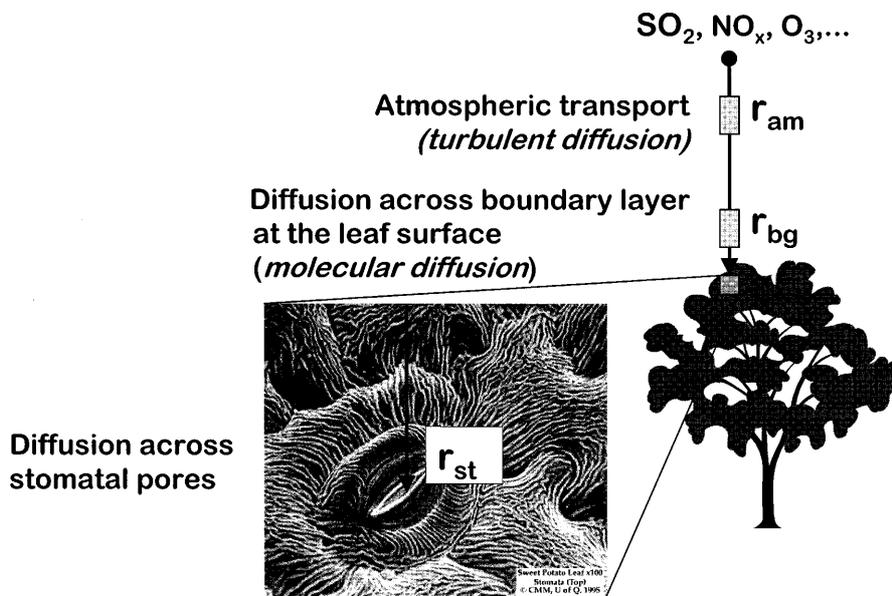


Figure 1. Conceptual diagram showing the main elements controlling ozone flux to plants.



Figure 2. Typical symptoms of leaf injury due to ozone observed in the area of Como (I) during the observation period in June 1998. (a) Necrotic leaf of red clover (*Trifolium pratense*), (b) Leaf decoloration in brown knapweed (*Centaurea jacea*).

plant organs and whole plants, on reproduction, and on the ability of the plant to compete with other plants at the community level. Ultimately, changes in plant communities may affect ecosystem processes, including biogeochemical cycles, energy balance, and biodiversity.

### Long-term effects of ozone on crops and meadows

Recent experimental work in Europe has focused at the investigation and quantification of effects of ozone on crops, particularly on wheat. As a result of this activity, it was possible to identify the key processes leading to reduced grain yield, the parameter which is of most interest from an economic point of view. Briefly, following initial injury at the sub-cellular level, photosynthesis declines with increasing

ozone stress, and at the same time rates of respiration increase due to the increased energy demand for repair processes. Decreased photosynthesis and increased respiration are associated with accelerated leaf senescence, and these effects lead to a reduction in the pool of assimilates available to sustain growth. Under these conditions the priority of allocation of assimilates to various organs is shifted in favour of the shoot at the expense of roots and seeds, which means that the total grain dry weight per plant at the time of harvest declines relative to the total plant biomass [8].

The reduction in grain yield of wheat with increasing ozone level has been observed in a number of experiments carried out in the framework of an European project, and also in the US National Crops Loss Assessment Network (NCLAN). When expressing ozone exposure by the cumulative exposure index AOT40 (i.e. accumulated hourly concentrations during daylight hours above a cut-off of 0.04 ppm), a highly significant linear relationship could be obtained between the change in grain yield relative to the control treatment and ozone exposure (Fig. 3). In most experiments the control treatment had an AOT40 of  $\approx 0$  ppm h. The regression line in figure 3 shows a significant deviation from 0% yield reduction when the AOT40 exceeds 3 ppm: h. Based on this finding, the UN/ECE adopted the value of 3 ppm: h, calculated for a period of three months, as the critical level to protect crops in Europe [9].

In Switzerland, as well as in many other European countries, the largest portion of land used for agriculture is covered by grasslands. Therefore, grasslands are important receptors for air pollutants, and adverse effects are of ecological and economic importance. Grasslands represent mixtures of species differing in ozone tolerance. Because these species compete for common resources, such as light, water and nutrients, the effect of the pollutant cannot be predicted on the basis of the responses of individual species to the stress. In fact, it could be possible that the dry matter yield (in  $\text{kg m}^{-2}$ ) of a relatively tolerant species may increase when grown in a mixture together with a relatively sensitive species. This is due to increased resource availability (e.g. light) resulting from the decline of the more sensitive species. This theoretical prediction was confirmed in an experiment with sown and frequently cut pasture [10]. In this experiment, the cumulative yield of clover (*Trifolium repens* and *Trifolium pratense*) from a total of 7 harvests during two years of exposure declined, but increased in the case of the grasses (total of different grass species) (Fig. 4). This result for a relatively simple plant community, consisting only of a small number of species with a pronounced difference in ozone tolerance, indicated that long-term effect of ozone stress may alter the floristic composition of meadows. Experiments are now underway to test this hypothesis with more complex species mixtures, such as those typical of extensively managed, permanent grasslands.

An initial screening experiment revealed a large variability in the specific biomass response to ozone, ranging from strongly negative effects on above-ground biomass to

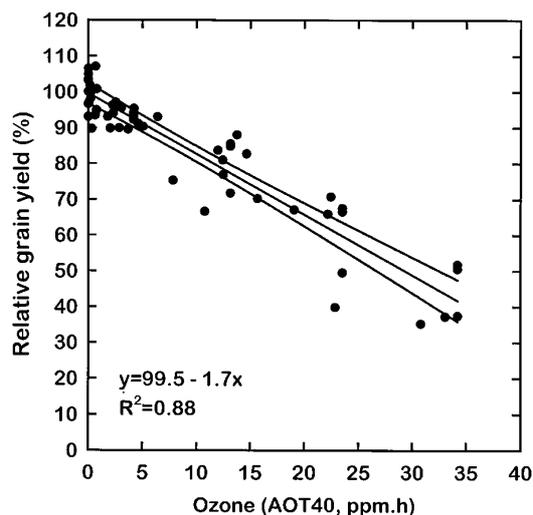


Figure 3. Relationship (with 99% confidence interval) between the relative grain yield of wheat and accumulated ozone exposure (AOT40). Data from different European and US experiments are pooled (from [9]).

stimulation of growth in some species [11]. To some extent, this inter-specific variability in ozone tolerance could be linked to the plant strategy for survival *sensu* Grime [12]. According to the three-strategy model, primary strategies conform to three distinct types: the competitors (C) exploiting conditions of low stress and low disturbance, the stress-tolerators (S), associated with high stress and low disturbance, and the ruderals (R), characteristics of low stress and high disturbance. Using the C-S-R system, species classified as generally stress tolerant are least affected by ozone, whereas ruderal and competitor species are more sensitive [13]. In addition to the variable response of plant growth it could be observed that specific changes in biomass allocation may occur [13]. Some species tend to respond to ozone stress by an increase in the relative biomass allocation to leaves, while others tend to increase relative biomass allocation to reproductive structures (Fig. 5). These shifts may have important consequences for the outcome of competition under ozone stress, and these preliminary results suggest that extrapolation of effects observed at the single species level to plant communities may be extremely difficult. It is only by studying responses of intact grassland communities that realistic long-term effects of pollutants on the floristic diversity and species dominance of grassland ecosystem can be observed.

Long-term exposure of plant populations may lead to the selection of tolerant genotypes, if the intra-specific variability in ozone tolerance is sufficiently large. There is increasing evidence that such an evolutionary response to ozone stress occurs on a large scale. For instance, *Plantago major* plants grown from seeds collected at sites differing in ozone exposure across Europe tend to be more ozone tolerant when

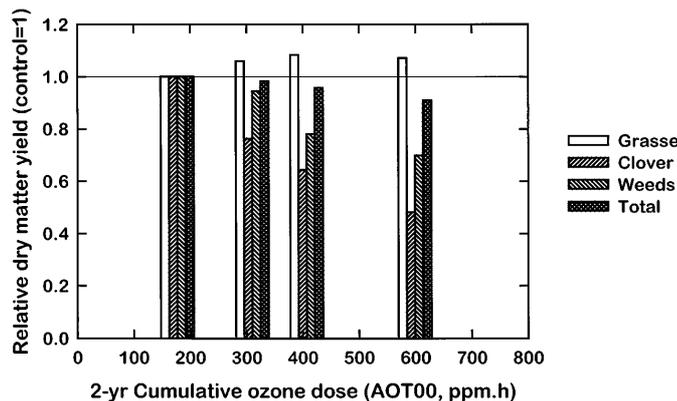


Figure 4. Effect of increasing ozone levels on the 2-yr cumulative total dry matter yield of managed pasture, and on the fractional yield of grasses, clovers, and weeds (after [10]). Ozone levels are given by the index AOT00, i.e the sum of hourly ozone concentrations >0 ppm.

the collection site was exposed to high ozone levels during the previous year, as compared to plants from sites with lower ozone levels [14]. In Switzerland, a test with *Trifolium campestre* showed the same tendency: plants collected from populations in the Ticino, an area typically exposed to high levels of ozone, were more resistant to ozone in terms of visible leaf injury, as compared to plants collected on the Central Plateau with less ozone pollution [14]. In 1998, a survey of leaf injury in red clover carried out in the southern region of the Ticino (Switzerland/Italy) revealed very little injury in spite of high levels of ozone (unpublished observation). Again, this may indicate the predominance of tolerant genotypes in this region, although further investigations must be carried out to critically evaluate this possibility. Other species were more strongly injured in the same region, such as knapweed, and hence may be less subjected to selective processes. Because of the importance of evolutionary processes, associated with the potential loss of genetic fitness of plant populations, the possibility of long-term shifts in population genetics will be of high priority in further studies.

### Risk assessments

In order to evaluate the risk of ozone pollution for plants across larger areas, for instance on a national or Pan-European scale, and over extended periods of time, it is necessary to use a practical framework and suitable tools. According to the UN/ECE, such framework may consist of two levels.

1. The Level I approach which is based on three single critical levels for all crop species (AOT40 of 3 ppm: h,

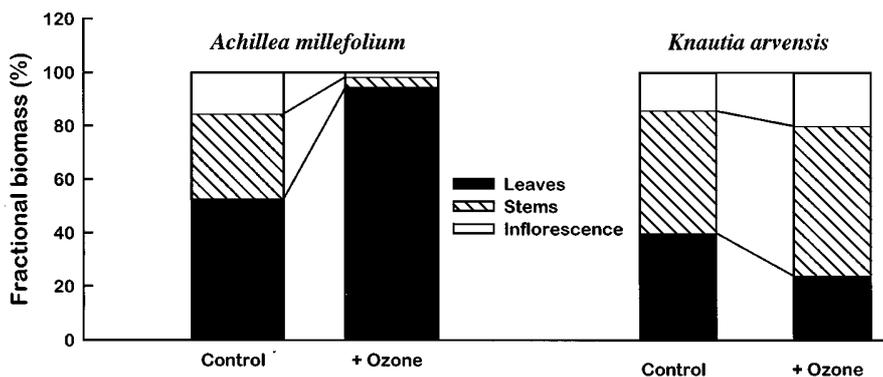


Figure 5. Change in fractional biomass in leaves, stems, and inflorescence in two species exposed to ozone, relative to the control (after [13]).

calculated for 3 months), for herbaceous species mixtures (same as for crops), or forest trees (AOT40 of 10 ppm: h, calculated for 6 months) [9]. The potential risk can then be expressed as the difference between the measured or modeled AOT40 in each grid square and the critical level for the receptor of interest. The spatial distribution of the risk can be displayed as maps of critical level exceedance [16].

2. A Level II approach which is based (i) on species-specific critical levels adjusted to local site conditions by taking into account scaling factors to correct for the modifying influence of important environmental factors, or (ii) on PAD estimated with the help of dynamic models for ozone flux.

Using the Level I approach, exceedance maps have been produced for several European countries, including Switzerland, and for Europe [17]. These maps indicate that the critical levels for all three receptors are exceeded in large parts of NW- and S-Europe. Maps for Switzerland indicate a relatively homogeneous distribution of exceedance across the Central Plateau, and largest exceedances in the southern part (Ticino) and in the Geneva area [18].

The Level II approach is currently being developed. Scalars which could be used for vapour pressure deficit (vpd), wind speed and soil moisture content are shown in figure 6. The vpd scalar leads to a decrease in the ozone risk when approximately 1 kPa is exceeded. This is important because highest ozone levels typically occur in combination with vpd of > 1 kPa. The soil moisture scalar suggests that ozone effects are diminished under dry soil conditions. A reduction in ozone risk also occurs when wind speed declines; this accounts for the decline in atmospheric turbulent diffusion with decreasing wind speed. The approach based on modeled PAD is in a preliminary stage, but models for ozone flux to specific vegetation types, such as wheat canopies, exist. Figure 7 shows an example of a flux pattern for a period of several days. The plot indicates that the day-to-day variation in ozone level and in ozone flux differ, which in this case can be explained by differences in atmospheric condition (turbulence, vpd). Estimated fluxes can be integrated over time (see above), but further research is

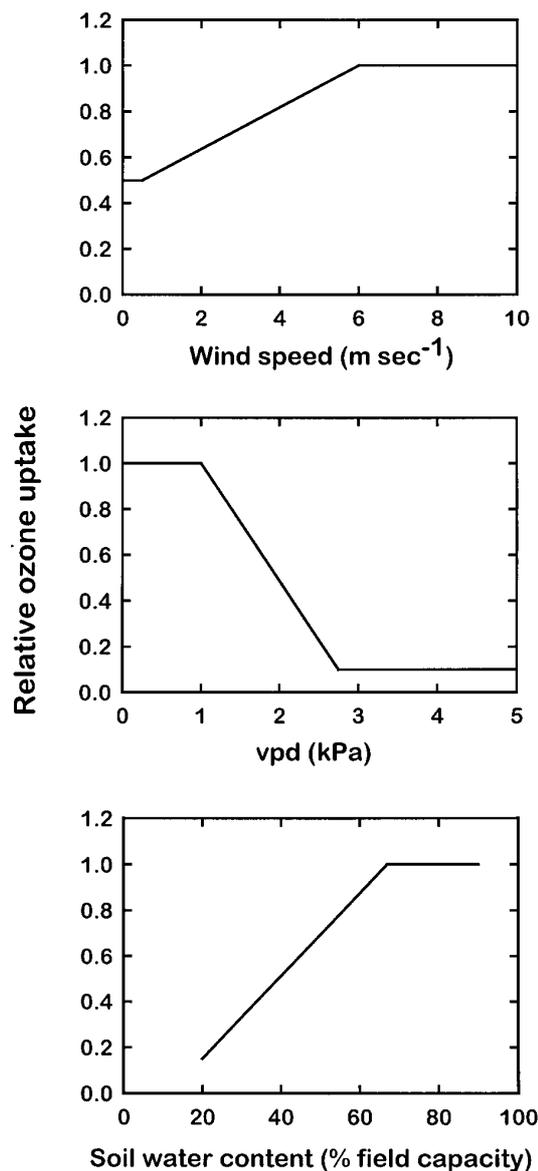


Figure 6. Scaling factors for the influence of wind speed, vapour pressure deficit (vpd), and soil moisture deficit on ozone uptake by crops.

necessary to validate the relationship between the absorbed dose (PAD) and measurable plant responses.

### Conclusions

During the growing season, ozone is the most important air pollutant with a large potential to adversely affect plants in Switzerland and in many other regions of NW- and S-Europe. Critical levels set by the UN/ECE to protect plants are exceeded over extended areas. The effect of ozone on crops, forest trees and herbaceous vegetation, including visible injury and long-term changes in growth, biomass allocation, yield, reproduction, competitiveness and vitality, depends to a large extent on the absorbed dose of ozone. Thus, at a given ozone concentration in ambient air, adverse effects are most likely to occur when environmental conditions and plant-specific parameters are most suitable for large ozone fluxes, i.e. during daylight hours under high wind speed and low vpd, and without limiting soil moisture condition. In order to assess the ozone risk for vegetation it is thus necessary to develop dynamic models for flux-effect relationships. However, it must be taken into account that in the long run, ozone tolerance may evolve in plant communities exposed to ozone.

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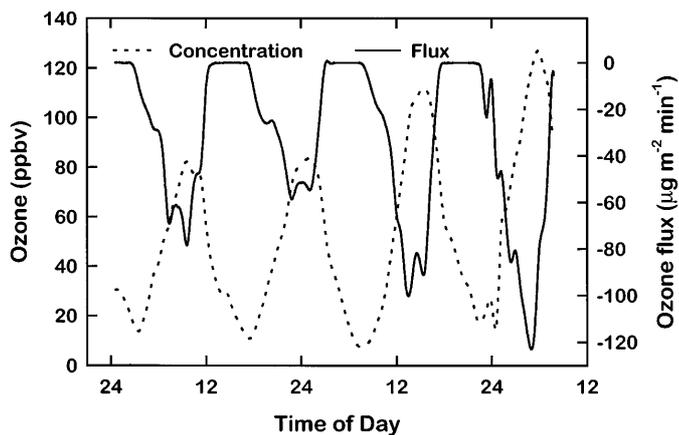


Figure 7. Diurnal pattern of ozone concentration and modelled ozone flux over wheat.