

Chapter 12 Effects of ozone on vegetation: critical levels

Introduction

The potential for ozone to damage vegetation has been known for over 30 years, but it is only over the last decade that its impacts have become of concern in Europe. It is now clearly established that ozone, at the ambient concentrations found in Europe, can cause a range of effects including visible leaf injury, growth and yield reductions, and altered sensitivity to biotic and abiotic stresses (1,2). Furthermore, because ozone is a secondary pollutant with a regional distribution, these effects may occur over large areas of rural Europe. Research in recent years has advanced our understanding of the mechanisms underlying ozone effects on agricultural crops, and to a lesser extent on trees and native plant species. It is now possible to determine biologically meaningful yet simple indices to characterize ozone exposure, and to identify the critical levels of exposure above which, by definition, adverse direct effects on receptors, such as plants, may occur. Since air quality guidelines should represent the upper level of a pollutant that would not pose any hazard to the receptor of interest, i.e. the lowest-observed-adverse-effect level (3), they can be set on the same basis as the critical levels (2), and by using new information on critical levels it is possible to update the existing guideline values for preventing adverse effects from ozone on terrestrial vegetation. The present update is largely based on the most recent scientific progress made in Europe.

Data for other photochemical oxidants, such as peroxyacetyl nitrate (PAN) and hydrogen peroxide (H_2O_2), are insufficient to update the existing guidelines or to introduce additional values. According to the existing guidelines, PAN concentrations should stay below $300 \mu\text{g}/\text{m}^3$ (approximately 60 ppb) for 1 hour and $80 \mu\text{g}/\text{m}^3$ (approximately 16 ppb) for 8 hours. Measurements in rural areas of Europe generally reveal concentrations clearly below these values. For instance, in a forest in Germany summertime maximum PAN levels of 4.6 ppb were recorded (4), and maximum concentrations reported for Swiss sites were also <5 ppb (5). In the United Kingdom, concentrations are typically 0.1 ppb in winter and 0.5 ppb in summer, and are largest during photochemical episodes when concentrations of PAN are typically 1% of those of ozone (6). Monthly mean H_2O_2 gas-phase concentrations in the United Kingdom lie in the range 0.1–0.6 ppb, with maximum hourly means reaching 3 ppb (6). Similarly, a recent study in Switzerland suggested typical H_2O_2 concentrations in the range 1–3 ppb (7). The effects of gaseous H_2O_2 have been little studied. However, fumigation of cherry trees with 20 ppb H_2O_2 for 8 weeks was reported to increase soluble protein concentration and antioxidant levels (8), while fumigation of wheat with about 5 ppb H_2O_2 over six weeks had no significant effect on growth or gas exchange (9). H_2O_2 is highly soluble; liquid-phase concentrations typically average about $1 \mu\text{mol}/\text{l}$, with maxima in the range 30–50 $\mu\text{mol}/\text{l}$ (6). Exposure to liquid-phase concentrations in the range 20–100 $\mu\text{mol}/\text{l}$ over several weeks has been shown to cause anatomical and physiological effects on a range of species (10,11). While there are insufficient data to set a guideline for this pollutant, and those experiments that have been reported are at long-term mean concentrations in excess of those commonly found in Europe, the possible impacts of this pollutant should not be ignored and need further investigation.

Characterization of ozone exposure

Most of the ozone in the atmosphere is located in the stratosphere, where ultraviolet light triggers the production of ozone by dissociation of oxygen. Ozone levels in the troposphere are much lower but are still sufficiently high to threaten agricultural crops, trees and native plants. The level of ozone in the troposphere is controlled by a complex set of photochemical reactions, which produce or destroy ozone, and by the vertical and horizontal transport of air.

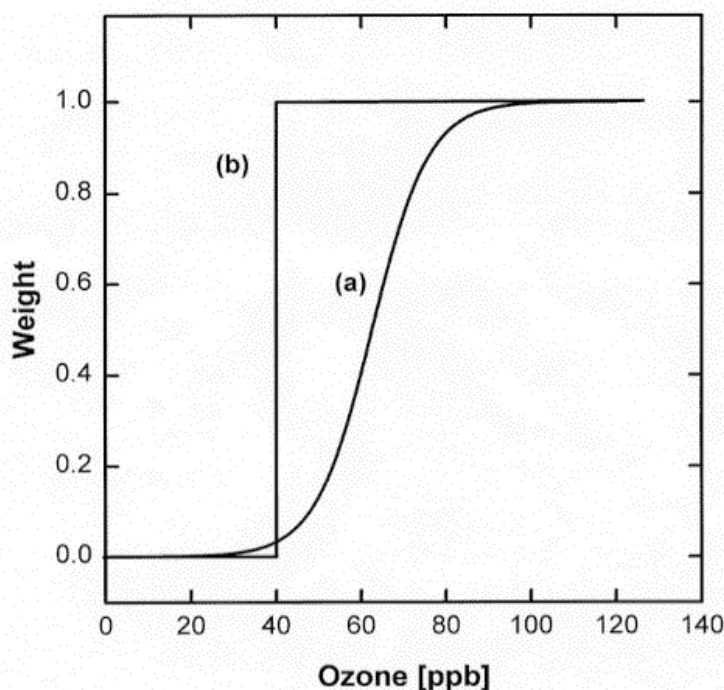
Owing to the variability of sources and sinks, ground-level ozone concentrations fluctuate in space and time (12). The diurnal pattern varies with altitude; at low elevation sites, ozone declines during the night. Provided that thermal stratification of the atmosphere leads to stable nocturnal layers with virtually no ozone exchange between them, ozone concentrations near the ground can decrease to zero. In urban air, this decline can be attributed to the destruction of ozone by reaction with nitric oxide and the absence of nitrogen dioxide photolysis, whereas at rural sites nitric oxide concentrations are small and dry deposition dominates the removal of ozone. During the day, the surface air is well coupled to the bulk of the mixing layer by turbulent transfer, and ozone concentrations near the ground increase owing to the downward transport of ozone from higher levels. In the absence of the stability-related processes, diurnal variations of ozone are less pronounced, i.e. over rough surfaces such as forests, or in the presence of strong winds or overcast skies. Highest boundary layer concentrations normally occur during the afternoon, when photochemical ozone production is most active. At higher elevations, the diurnal variation is dampened because of the absence of thermal stratification of the atmosphere, and ozone levels remain high during the night. Hence, sites with different local features experiencing the same regional ozone distribution may have different exposure levels, and thus different effects.

To relate ozone exposure to effects, it is necessary to summarize concentrations averaged over 1-hour intervals in a biologically meaningful way, which can serve as a surrogate for dose (13). In principle, the exposure index must be based on the concept of effective dose (14), i.e. it must capture the characteristics of exposure that most directly relate to the amount of ozone that is absorbed by vegetation. Uptake of ozone could be estimated by multiplying the concentration near the leaf surface by the leaf conductance for ozone, and the absorbed dose would then be the integral of the rate of uptake (flux) over time (15). This concept could be expanded to take into account the conductivity of the atmosphere (16). In situations with sufficient air mixing (high air conductivity), the diurnal pattern of ozone flux is determined by leaf conductance and ozone concentration. This is the case in open-top exposure chambers. Owing to the lack of leaf conductance data, the use of radiation as a surrogate for leaf conductance has been suggested in agricultural crops (17), and the most simple approach is to use ozone concentrations measured during daylight hours (e.g. $>50 \text{ W/m}^2$ global radiation) to characterize exposure. For species with substantial leaf conductance at night, however, no such discrimination should be made. Other factors, e.g. air humidity, soil water availability and temperature, are also known to influence leaf conductance, but to date these factors have not been used to characterize ozone uptake or dose in long-term experiments.

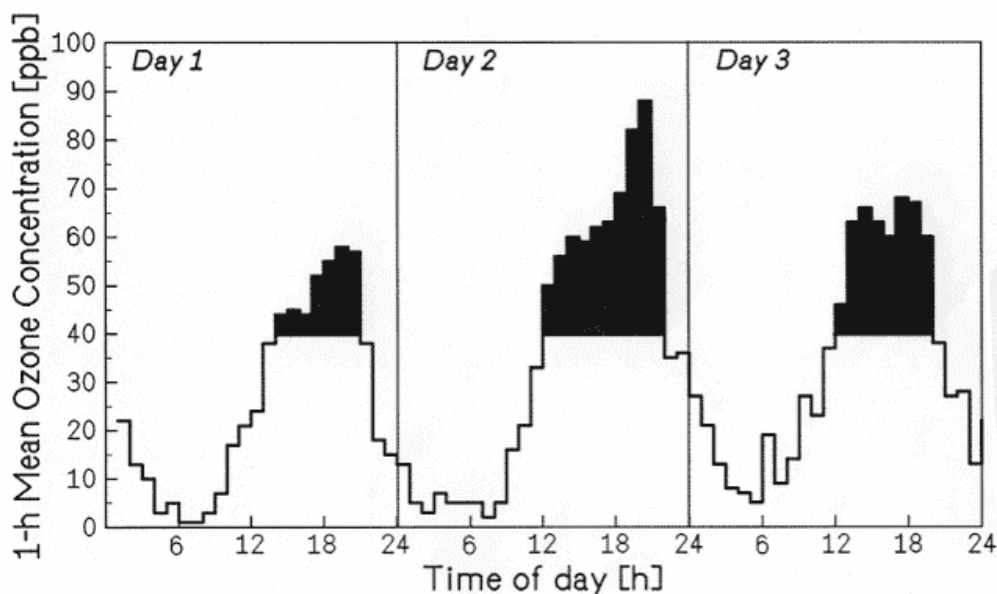
Long-term exposure to ozone can lead to growth and yield reduction. Hence the most suitable exposure indices to be related to long-term effects are cumulative, i.e. they integrate exposure over time. Previous air quality guidelines for long-term effects have been based on mean concentrations over a given period of time, e.g. the arithmetic mean over the growing season of the daily mean concentrations during a specific 7-hour period (usually 09.00–16.00 hours). The use of a mean concentration over a given period of time implicitly gives equal weight to all

concentrations. However, experimental exposure–response studies with ozone suggest that this is not appropriate, and that it is the intermittent exposure to higher concentrations that is most important in causing long-term effects (18,19). This can be explained physiologically by the capacity of the plant to detoxify ozone and other oxidants; it is only when the concentration or flux of ozone exceeds this capacity that adverse effects result.

Fig. 1. Illustration of ozone concentration weighting using (a) continuous weighting with a sigmoidal function that gives increasing weight to increasing concentrations, or (b) discontinuous weighting with a cut-off concentration (“threshold”) that gives equal weight to all concentrations above (weight = 1) or below (weight = 0) the cut-off point



This phenomenon can be incorporated into the exposure index by using procedures for continuous or discontinuous weighting of concentrations (20). Fig. 1 illustrates the two possibilities: (a) the use of a continuous sigmoidal weighting function that assigns increasing weight from 0 to 1 to increasing concentrations between 0 ppb and, for instance, 100 ppb; or (b) the use of a discontinuous weighting procedure that assigns the weight of 0 to all concentrations below the threshold and a weight of 1 to all concentrations above the threshold. There are sound biological reasons for believing that the former procedure, using sigmoidal weighting, in theory gives a better representation of exposure. However, given the limited exposure–response data and the difficulties of accurately mapping or modelling complex exposure indices, the latter procedure, which depends on the definition of a threshold concentration, is more practical. In order to calculate the cumulative exposure index, the positive differences between the actual hourly mean concentration and the threshold concentration are then summed for the exposure period of interest. The calculation of the cumulative exposure index using this method is illustrated in Fig. 2, in which the shaded area represents the cumulative exposure.

Fig. 2. Example of a typical 3-day time course of hourly mean ozone concentrations

Hourly concentrations above a threshold concentration (shaded area) are summed over the exposure period of interest to calculate the cumulative exposure index. In this example, 40 ppb is chosen as the cut-off concentration, and the respective index is referred to as AOT40.

This concept was adopted at the United Nations Economic Commission for Europe workshop at Egham in 1992, when a threshold concentration of 40 ppb was tentatively suggested (21). This exposure index has been called the AOT40, i.e. accumulated ozone exposure above a threshold concentration of 40 ppb, expressed in units of ppb/hour or ppm/hour. Statistical analysis of yield data from European open-top chamber experiments has demonstrated that the use of this threshold generally provides better linear fits to exposure–response data than the use of higher thresholds (22). A linear exposure–response relationship provides a sounder statistical basis for defining critical levels corresponding to a specific effect than do other types of exposure–response relationship. The use of 40 ppb as the threshold has been favoured over lower threshold concentrations because, in Europe, it broadly corresponds to the boundary between mean concentrations in areas with low and high frequencies of photochemical episodes. However, the choice of this threshold does not imply that concentrations below 40 ppb have no effect. Hence, the threshold concentration does not present a threshold for effects, but rather a cut-off concentration. Because of the increase in the proportion of background ozone with increasing altitude, the use of a cut-off concentration of 40 ppb may not be appropriate for higher elevations.

The index would be calculated with concentrations during daylight hours only (i.e. hours with a potential global radiation equal to or greater than 50 W/m^2) because only small rates of ozone deposition have been measured over agricultural crops and forests during night time. However, it should be noted that in well mixed fumigation chambers, substantial ozone in trees can occur (see below). Based on a typical exposure duration, the AOT40 is calculated for crops over 3 months (e.g. May–July) and for forest trees over 6 months (April–September).

Data from monitoring sites across Europe suggest that AOT40 values are highly variable. Lowest values of AOT40 (daylight hours, May–July), around $<3 \text{ ppm/hour}$, typically occur in

northern Scandinavia ($>62^{\circ}$ N) and values in continental Europe are in the range 5–25 ppm/hour (23).

Methods for studying uptake and effects

It is essential to have data from experiments in which plant response has been examined over a range of ozone concentrations, i.e. exposure–response relationships, in order to establish critical levels (24). Furthermore, these relationships should have been derived in conditions close to those found in the field.

In the case of ozone, it is not usually possible to use field observations because the sharp spatial gradients found for some other pollutants do not exist. In the case of visible injury, however, exposure–response relationships have been derived from field observations on the basis of temporal variation in ozone concentration (25–27). In such cases, in contrast to controlled fumigation with ozone, the relationships incorporate the effect of climatic factors, such as temperature and humidity, in modifying ozone uptake and hence plant response.

Nevertheless, experimental studies using open-top chambers provide the best available source of data for other plant responses. Typically, cylindrical open-top chambers are placed over field plots of soil-grown plants and supplied with filtered air (to provide exposure to below-ambient concentrations), non-filtered air, or non-filtered air with ozone added (to provide exposures to elevated ozone concentrations). Owing to rapid air exchange, such chambers provide climatic conditions that are similar, but not identical, to those outside (28), and thus some reservations about extrapolation to field conditions remain. In particular, recent data (29,30) suggest that, because of forced turbulence, the ozone flux in such chambers is normally higher than that outside, even though the chamber does not modify the ozone concentration. This would suggest that open-top chamber data would tend to overestimate the adverse effects of a given ozone concentration.

While open-top chambers are well suited to studies of annual crops, there are major difficulties in interpreting their results when applied to trees. This is because of size limitations, which mean they can only be used with young trees, while experimental duration can only be a fraction of a tree's lifetime. According to a study with northern red oak, experiments with seedlings may underestimate the ozone sensitivity of larger and more physiologically mature trees (31). Thus the derivation of air quality guidelines for mature forests from such data is problematical. Similar problems may arise for many other types of natural vegetation. As an alternative to open-top chambers, chambers can be used in which individual branches of a mature tree are exposed to the pollutant (32). In these branch chambers, physiological and growth effects of ozone can be studied, but effects at the whole-tree level remain unknown.

Exposure–response data can also be derived from closed chamber studies in glasshouses or controlled environment facilities. Although more care is needed in this case in interpreting its significance for field conditions, such facilities do provide the possibility of testing how environmental factors may modify the observed exposure–response relationship. An alternative to open-top chambers are open-air fumigation systems, which avoid the disturbing influence of chamber structures, allow natural infestations of pests and diseases, and are suitable for exposure of larger field plots (33). However, these systems cannot be used to expose plants to levels below those in ambient air, and active fumigation with elevated ozone is not possible at low wind speeds because of the lack of air mixing and transport across the experimental plot.

Finally, chemical antioxidants, such as ethylene-diurea (EDU) may offer a useful source of data on field responses, since they avoid the use of experimental enclosures (34,35). Plants treated with EDU can be used as controls and can be compared with untreated plants, thus providing an estimate of ozone effects. However, derivation of air quality guideline values from such studies is difficult because the extent of plant protection from ozone provided is uncertain.

Ozone deposition and uptake by needles and leaves

Understanding of the fate of ozone in canopies and leaves is still rather limited, restricting the possibility of establishing a basis for exposure–response relationships. One of the greatest sources of uncertainty is the link between ozone transport from the troposphere through the stomata and intercellular spaces to target sites inside the leaf.

Ozone deposition comprises several processes that can be described at different scales of resolution (36). One scale focuses on atmospheric processes above the plant canopy, which are governed by wind turbulence and the roughness of the terrestrial landscape, including altitude and type of vegetation. The second scale, very much used in ozone dose–response studies, concerns the individual leaf; ozone is deposited to vegetation canopies through uptake by leaves, mainly through the stomata. The third and finest scale of resolution is driven by reactions inside the leaf. In forests, sinks other than the stomata may also play a role in ozone deposition, such as cuticles, bark, litter, soil and canopy air space, where ozone can be scavenged by biogenic hydrocarbons or oxides of nitrogen emitted from organic decomposition in the soil or by the foliage (37).

The extent of gas exchange via the stomatal pores, i.e. the ozone flux, depends on the total pore area per unit leaf area, i.e. pore density (number of stomata per mm^2 of leaf surface times area per pore). In most plants the pore area comprises 0.5–1.5% of the leaf surface (38). The degree of opening of the pores, and thus the stomatal diffusion resistance, depends on the environment and on the interior state of the plant. The most important external factors are light, temperature, humidity, water supply, wind speed and altitude, while the internal factors include the partial pressure of carbon dioxide in the intercellular system, the content of water and ions in the tissues, and plant growth regulators (gibberellic acid and cytokinin promote opening and abscissic acid promotes closing).

Ozone uptake by crops in highly turbulent situations is directly related to leaf (stomatal) conductance and follows the diurnal pattern of radiation (39). Under less turbulent situations, however, and especially over low-stature crops (e.g. grassland), the canopy may be decoupled from the atmosphere, and ozone deposition depends primarily on atmospheric transport with little control exerted by the stomata.

Because of their structure, forest canopies are strongly coupled to the atmosphere under most conditions, and the flux or deposition of ozone strictly depends on leaf conductance (40). Looking at the uptake, i.e. the absorbed dose, the specific leaf area as a measure of the leafiness of the tree on a dry weight basis (i.e. the area of assimilatory leaf material per unit dry weight) becomes the main determining factor. Measurements at different altitudes using branch cuvettes on spruce trees revealed that the pattern of ozone deposition velocity differs between high- and low-elevation sites (41,42). Average midday conductance increases with increasing altitude. Finally, there is evidence from transpiration measurements that some stomatal uptake of ozone may also occur at night in certain coniferous species (43). However, because of the lack of a substantial rate of ozone deposition to forests at night in most circumstances, for trees the

calculation of an accumulated exposure (e.g. AOT40) should only consider the daylight hours (as for crops).

Inter- and intra-specific differences

It is well established that there are differences between species in their sensitivity to ozone. However, many of the published lists of sensitive species are based on visible injury induced by acute ozone exposures; while these are relevant to instances of visible injury in the field, they may not be related to relative sensitivity based on effects on growth or physiology of longer-term exposures. It is not currently possible to provide comprehensive lists of relative sensitivity of species to these longer-term exposures.

There are also substantial inter-specific differences in ozone response. In the case of crop cultivars this arises as a by-product of breeding for other characteristics, but this is not the case for non-cultivated species, in which large differences in ozone response have also been found between ecotypes. This may partly be the result of evolution of tolerance in populations exposed to high ozone levels in the field; for example, in the United Kingdom, the most resistant ecotypes of *Plantago major* were found at sites with the highest ozone exposures (44).

The reasons for these inter- and intra-specific differences in ozone sensitivity are not fully understood. However, it is clear that stomatal conductance, which determines ozone uptake, is often related to sensitivity (45). This was confirmed recently by comparing clones of white clover differing in ozone sensitivity (46), and by comparing different ecotypes of various species (47). Other authors (48,49) have reported that faster-growing species, which tend to have higher stomatal conductance, are more sensitive to ozone. Stomatal responses to ozone may also be important, with stomatal conductance decreasing in response to ozone exposure in more resistant species or genotypes (50). It seems possible that stomatal conductance could be used as a surrogate for classifying the potential sensitivity of different species, although other factors, such as antioxidant levels or the evolution of stress ethylene, can also be related to ozone sensitivity (see below).

Effects of ozone

Effects of ozone may occur at various levels of organization, i.e. from the cellular level through the level of individual organs and plants to the level of plant communities and ecosystems. The best documented ozone-induced ecosystem effect is the degradation of forests in southern California (51). Today, there is also evidence, mainly from controlled experiments in Europe and from field and laboratory studies in Canada and the United States, showing that ozone affects the health and production of forests in these parts of the world. The magnitude of the effect of ozone on forests, however, is still not quantified. Effects on crops are much better understood. Results from crop loss networks in the United States and Europe have provided exposure–response relationships for a range of crop species, although because of differences in experimental conditions results from the two networks cannot always be directly compared.

After passing through the stomatal pore, ozone can react with organic molecules (e.g. ethylene, isoprene) in the intercellular air space or with components of the extracellular fluid. In both cases, secondary oxidants (e.g. primary ozonides, hydroxyhydroperoxides) may be formed, which in turn could react with the protein component of the cell membrane (52). This reaction is prevented to some extent by the presence of radical scavengers, such as ascorbic acid and polyamines (53). Formaldehyde, formate and acetate accumulate in damaged tissue, possibly as a result of the reaction between ozone and ethylene or between ozone and the phenylpropanoid

residues of lignin (54). There is evidence that ethylene formation determines the sensitivity of plants to ozone (55,56). High levels of ozone cause target cells to collapse, leading to local visible tissue destruction. The effect on the plasma membrane can cause changes in membrane functions that may affect the internal concentrations of ions (e.g. Ca^{2+}) (57). This changes the osmotic potential of the cytoplasm, which in turn can reduce photosynthetic processes in the chloroplasts. Reduction in carbon dioxide fixation by the enzyme ribulosebisphosphate carboxylase is a typical symptom found in leaves exposed to ozone over longer periods of time (58). Further inhibition of carbon dioxide assimilation results from direct or indirect inhibition of stomatal opening that reduces uptake (59). Stimulated dark respiration often occurs together with reduced photosynthesis (60), probably due to increased respiration associated with maintenance and repair (61). The combined effects of reduced assimilation and increased respiratory loss of carbon dioxide consist of an overall reduction of assimilate production and export from the source leaves. In the leaves of crop species exposed long term, the onset of senescence is advanced, and accelerated catalysis leads to the rapid loss of protein and chlorophyll (62). As a result of the reduction in leaf duration, the period with positive net assimilation of carbon dioxide is diminished, and the overall production of assimilates declines. Under conditions of reduced assimilate supply through photosynthesis, allocation of carbon to different organs may be altered, leading to altered growth responses of these organs. Typically, higher priority is given to the shoot relative to roots and/or other storage organs (e.g. seeds). This results in reduced root:shoot weight ratios or in a reduction of the ratio between seed yield and total biomass production. In agricultural crops, this results in reduced grain or seed yield (63). Reduced assimilate supply may also restrict the plant's ability to tolerate additional stresses, such as stress due to drought or low temperatures.

Today's knowledge about the mechanisms underlying ozone effects at the biochemical and cellular levels originates mainly from studies of species other than trees (64). But, as in the case of crops, it has been established that, in general, net photosynthesis is negatively affected in tree species following short-term ozone exposure (65). The same seems true on a longer-term basis (one season or longer) (66–68). However, both short episodes (69) and one season of exposure have been shown to cause persistent stimulation of net photosynthesis of the current year's needles in Norway spruce seedlings (70,71) and in Ponderosa pine (72). This demonstrates that different age classes react differently, and suggests that carry-over and long-term effects must be examined very carefully.

Altered carbon allocation and growth are always the ultimate response of trees to stress. Tree growth has been shown to be affected by ozone in several controlled fumigation studies, but most of these have only covered young seedlings exposed for one or two seasons (73,74).

The most important impact of ozone on plant communities may not be through an impact on growth or productivity, or through visible injury, but through shifts in species composition, loss of biodiversity, and changes in genetic composition. Several studies of mixtures of herbaceous species have demonstrated a shift in the relative proportions of the species in response to ozone, although this is not always accompanied by effects on the total growth of the mixture (75–77). This is to be expected where species are actively competing, since any reduction in the performance of one species will provide opportunities for other, less sensitive species, although it is also possible that direct allelopathic effects are involved (78). In frequently cut, managed pasture, clover growth was repressed by long-term ozone exposure, whereas the growth of the relatively resistant grass species was enhanced, possibly because of improved resource availability (e.g. light).

The longer-term effects of ozone on species composition are uncertain, although field studies in the San Bernadino Mountains of California have shown that sensitive tree species have been eliminated and replaced by other, less sensitive, tree or shrub species (79). There have been relatively few studies of growth responses of individual plants of native herbaceous species, but these do indicate that some European species are very sensitive to ozone. There is also limited evidence of evolution of resistance to ozone in field populations (44); this could have potentially detrimental effects on the population if it were accompanied by a significant loss of genetic variation, although there is no concrete evidence of this.

Reproductive success is crucial to the survival of populations of many annual plants. There is some evidence of complete loss of flower production at relatively low ozone concentrations (80), while pollen may also be sensitive to ozone (81).

Interaction with other stresses

Under field conditions, ozone is rarely the only stress factor for crops and trees. The reverse is probably true in most situations: the effects of ozone may be masked by other, stronger stresses, and the impact of ozone is known to be modified by a range of other factors. The most important of these are soil water stress, and atmospheric vapour pressure deficit, which can cause reductions in stomatal conductance and hence in ozone uptake (82), although this will depend on the stomatal responses to air humidity of individual species. This is an important factor, as high ozone levels often occur in conditions causing water stress. A number of other climatic and edaphic factors may influence plant response to ozone, but the evidence relating to these is limited.

The combination of ozone and drought often produces plant responses that are due primarily to the effect of drought. Ozone stress has been reported to be reduced or prevented by drought (72,83). Nevertheless, increased susceptibility to drought as a result of ozone exposure has also been suggested (84), and an impaired water balance in decreasing humidity has been reported for Norway spruce following ozone fumigation (85). There is also evidence that leaves that are sun-acclimatized react more strongly to ozone in full light than under shade conditions, while the reverse is true for shade-acclimatized leaves (86). Less studied are situations with sequential stresses, which is a phenomenon very abundant in nature, and one in which ozone may have the potential to increase sensitivity to climate stress. Experiments with Norway spruce, red spruce and Sitka spruce have highlighted the possibility that ozone exposure in the summer may result in a delayed cold hardening and later frost injury (87–90).

The presence of other pollutants may also influence the impact of ozone, although relatively few studies have been carried out at concentrations close to suggested critical levels. The most recent detailed European studies involving ozone, sulfur dioxide and nitrogen dioxide produced variable results, although there was a tendency to observe synergistic interactions between ozone and sulfur dioxide when they were applied simultaneously, and antagonistic interactions between ozone and nitrogen dioxide when they were applied sequentially (91). However, there is evidence from earlier studies of strong synergistic interactions, in particular between ozone and sulfur dioxide (92). Antagonistic interactions have been found between ozone and ammonia (93).

Ozone can also cause a range of chemical, physiological and morphological changes to leaves, which alter plant sensitivity to other stresses. There is direct evidence of such effects for tolerance of cold stress, attack by herbivorous insects and attack by fungal pathogens. Insect and fungal attack can be induced by relatively low ozone exposures; for example, ambient air

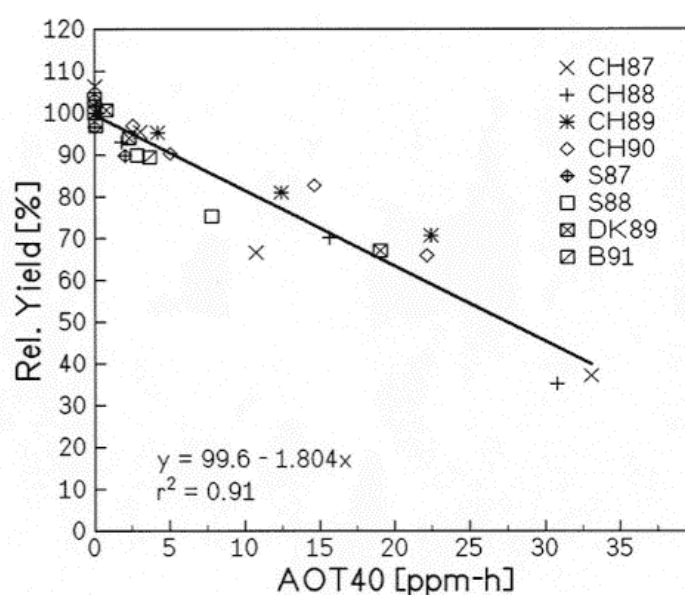
pollution has been shown to increase the performance of insect herbivores on field bean (94) and beech (95), while increased infestation of fungal pathogens on wheat was observed after one month's exposure to 30 ppb ozone (96). Thus, such interactions may be of significance in terms of setting air quality guidelines.

Exposure–response relationships and critical levels

Crops

To determine critical levels, the relationships between the pollutant exposure and the effect of interest need to be quantified. Such relationships have a degree of uncertainty, however, and the data necessary for deriving them are often scarce. A consensus has been reached as to how exposure should be expressed, and the type and acceptable level of effect to be used as criteria for defining the critical level. On the basis of results from recent experiments with a limited number of agricultural crop species, the cumulative exposure index using a threshold of 40 ppb (AOT40) has been accepted as the best available exposure index, using hourly concentrations during daylight hours over a 3-month period (see above). In these experiments, the main parameter used to determine the direct effect of ozone was grain or seed yield. This parameter was therefore accepted for use in setting the critical level (2). The exposure–response relationship between AOT40 and grain yield of spring wheat is shown in Fig. 3. The data shown can be fitted by a linear model. In this case, no threshold level of exposure exists, and a critical level corresponds to any acceptable level of yield reduction. Hence, the critical level could be defined as the AOT40 corresponding to a yield reduction of 5% or 10%. The respective AOT40 values are 3 ppm/hour and 6 ppm/hour (rounded to the nearest ppm/hour) (2,3). Statistical analysis has shown that the least significant deviation from a 100% yield that can be estimated with 99% confidence is 4–5% (97). From a practical standpoint, a change in yield of 5% seems to be small and in most instances not detectable; it is therefore recommended that the risk is evaluated by calculating exceedance of the critical level over a five-year period.

Fig. 3. The relationship between relative grain yield of spring wheat and ozone exposure expressed as AOT40 for 3 months (with 99% confidence interval of the regression)



Data are from eight European open-top chamber experiments. CH: Switzerland; S: Sweden; DK: Denmark; B: Belgium (22).

It must be emphasized that exposure–response studies usually apply to open-top field chambers, and extrapolation to the open field may be difficult. Hence, critical levels derived from such studies should be applied only to conditions resembling those found in open-top chambers, such as where there is no limit to the water supply. Furthermore, the presence of significant concentrations of other pollutants (sulfur dioxide, nitrogen oxides) may affect exposure–response relationships and hence critical levels.

Other exposure–response relationships exist for European crop species. For instance, field experiments in England and France supplied data for green beans (98), and critical levels calculated from these data are similar to those for wheat (22). Exposure–response relationships for barley and oats suggest higher ozone tolerance of these crops as compared to wheat (99). However, there are many species that have not been investigated experimentally in Europe, especially those of the Mediterranean region. Exposure–response data have been obtained for a larger range of crop species in the United States, in particular through the National Crop Loss Assessment Network (NCLAN) programme (100), such as data for three cultivars of winter wheat tested at Argonne National Laboratory. Although these data fit well with the European data for spring wheat, the cultivars generally used in the United States may differ in ozone sensitivity from those in Europe, while climatic differences between the two continents and differences in crop management may also alter sensitivity. Furthermore, the experimental exposure protocols adopted in the NCLAN studies are not necessarily appropriate for ambient ozone exposure patterns in Europe. For these reasons, the use of NCLAN exposure–response data to derive critical levels for crops in Europe is not appropriate.

During ozone episodes, visible injury has been observed on leaves of a range of crop species (Box 1). Because of the short period of exposure necessary for injury development, and the lack of consistent relationships between visible injury and long-term effects on growth and yield, a different critical level for short-term injury is needed. In the case of species that are sold for their foliage, these effects may be of economic significance. A comparison of injury assessments and ozone exposure characteristics carried out for selected ozone episodes at various locations, combined with multifactorial data analysis using artificial neural networks, suggested that injury typically occurred after 5-day periods with elevated ozone. For the 5 days preceding the appearance of injury, an AOT40 of 500 ppb/hour was necessary when the mean vapour pressure deficit between 9.30 and 16.30 hours exceeded 1.5 kPa (i.e. dry air conditions), whereas an AOT40 of only 200 ppb/hour was necessary when the deficit was below 1.5 kPa (humid air conditions) (101). Hence, the critical level to protect crops from short-term ozone effects that depends on this deficit can be set at either 0.5 ppm/hour or 0.2 ppm/hour for 5 days using daylight hours.

Box 1. Crops that have developed ozone injury when grown commercially in Europe

Alfalfa	Potato
Artichoke	Soybean
Bean	Spinach
Clover	Tobacco
Cotton	Tomato
Courgette	Watermelon
Grapevine	Wheat
Maize	

Source: Fuhrer & Ackermann (2).

Tree species

Exposure–response studies of trees require decisions in advance as to the selection of season, time-scale, plant age, shoot age or needle/leaf age. It also appears necessary to decide on the type of “receptor-site” in the tree (an enzyme, the photosynthetic apparatus, the stomata, ultrastructure, carbon allocation, growth) since the various sites differ strongly in sensitivity, and their response also depends on exposure pattern, plant phenology and age.

In Europe, two exposure–response studies of potted tree seedlings exposed to ozone in field fumigation chambers have been published: one on the effect on biomass in birch (102), the other on photosynthesis in Norway spruce (68). In Denmark, exposure–response data have been obtained for Norway spruce exposed to ozone in branch chambers (32). Data obtained from studies with beech, oak, Norway spruce, Scots pine and silver fir show that ozone in combination with sulfur dioxide and nitrogen dioxide can cause biomass reductions (103). The effect of non-filtered versus filtered air has been tested on seedlings of beech and Norway spruce at different altitudes in Switzerland with prevailing ozone air pollution. Beech reacted to ambient ozone levels with a 10% biomass reduction within three years (104). However, there are considerable difficulties in scaling-up from seedlings to mature trees. Consequently, the available exposure–response data from studies with seedlings may not reflect the response to ozone of older, mature trees or forest stands, and the effects of long-term exposure on trees with a long life-cycle are unknown. Thus at this stage the use of data for seedlings in defining provisional critical levels for forest trees is unavoidable.

As in the case of crops, it has been agreed that exposure should be characterized by the index AOT40. For trees this should be calculated for daylight hours over a 6-month period covering the period of highest sensitivity of the tree (2). Under mid-European conditions, the 6-month period would start at the beginning of April, although for some Mediterranean species the period April–October is not appropriate.

Table 1 lists European studies for which ozone exposure has been recalculated to obtain the AOT40 corresponding to a 10% change in biomass production. Table 1 also includes studies of parameters other than biomass. For protection of European forests, a critical level of an AOT40 value of 10 ppm/hour using all hours of the day was proposed earlier. Only data for plant biomass obtained in the field or in open-top chambers were considered; other parameters, such as chloroplast size or photosynthetic rate, were not regarded as suitable criteria. Also, results from studies in which other pollutants (e.g. sulfur dioxide, nitrogen dioxide) were added at concentrations close to their critical level are difficult to interpret. Last but not least, considerable uncertainties still exist with respect to both the response of mature trees and the long-term effects of ozone exposure. Re-analysis of the data for beech, representing the most sensitive receptor, yielded a significant regression of biomass decrease versus AOT40 for daylight hours. For this analysis, the data from experiments of different durations were normalized to one growing season. The parameters obtained after normalization provided an estimated critical level of 10 ppm/hour, which was associated with a 10% decrease in biomass. Hence, an AOT40 of 10 ppm/hour for daylight hours, accumulated over a 6-month growing season, can be used as the critical level for the protection of forest trees (105).

Table 1. Exposure–response studies with trees used to roughly recalculate exposure expressed AOT40 corresponding to specific effects

Species	Experimental method	Parameter	Magnitude of effect (%)	AOT40 recalculated (ppm/hour)	Exposure period (seasons)	Reference
Birch	Open-top chambers	Biomass	10	9 ^a –13 ^b	1	102
Beech ^c	Open-top chambers	Biomass	10	6 ^d	2	103
Oak ^c	Open-top chambers	Biomass	10	6 ^d	2	103
Beech	Open-top chambers	Biomass	10	7 ^e	3	104
Norway spruce ^c	Open-top chambers	Biomass	10	12 ^d	2	103
Scots pine ^c	Open-top chambers	Biomass	10	12 ^d	2	103
Silver fir ^c	Open-top chambers	Biomass	10	12 ^d	2	103
Norway spruce	Open-top chambers	Photosynthesis (C + 2) ^f	10	10 ^g	3	68
Norway spruce	Branch chambers	Pigments	Significant changes	30 ^g	2	32
Norway spruce	Open-top chambers	Chloroplasts ^h (C + 1) ⁱ	30% decrease	5 ^g	1	106

^aThe calculation is based on a 1-year experiment with a reference ozone concentration of 0 ppm.

^bThe calculation is based on biomass reduction relative to the biomass at 20 ppb ozone (reference concentration), which is determined by interpolation between 0 ppb and 50 ppb, assuming a linear exposure–response relationship.

^cPollutant mixture.

^dCalculated on the basis of a 2-year open-top chamber experiment with charcoal-filtered air as reference.

^eCalculated on the basis of a 3-year open-top chamber experiment with charcoal-filtered air as reference.

^fC + 2: 2-year old needles exposed for three seasons.

^gDaylight hours, seasonal value.

^hDecrease.

ⁱC + 1: 1-year old needles exposed for two seasons.

Species mixtures

There are very few European experiments that provide exposure–response data for ozone effects on species mixtures. The most useful data come from open-top chamber studies of natural or artificial grassland communities. Studies in Switzerland (77) and the United Kingdom (76) provide evidence of significant shifts in species composition in such communities in response to ozone. Although the data show more scatter than similar plots for crop yield, they provide strong evidence of a linear response to ozone exposure expressed as AOT40. Both studies involved grass/forb mixtures, with the forb component, which in both cases included clovers, decreasing with increasing ozone exposure. For the United Kingdom study, the slope of the line was greater when the artificial community was regularly cut to simulate grazing, possibly

because of greater ozone penetration into the canopy.

Since the exposure–response data available for herbaceous plant communities are very limited and tend to relate to relatively short-term experiments on artificial mixtures, it is not possible to derive critical level values directly. However, there is evidence of significant changes in species composition at values of AOT40, similar to the critical levels set for agricultural crops. As for crops, it is possible to calculate from the exposure–response relationship the ozone exposure corresponding to a given effect. Selecting as the criterion a reduction of 10% in the proportion by biomass of the sensitive species, the equivalent ozone exposure was 6.3 ppm/hour for the Swiss data and 5.7 ppm/hour for the United Kingdom data on cut swards. Subsequent United Kingdom experiments using calcareous grassland rather than acidic grassland species gave a value of 7.8 ppm/hour (107). All these values are very similar, and also are comparable to the value of 6 ppm/hour for a 10% loss in yield in wheat. A more recent analysis of data from experiments on species of seminatural plant communities indicated that reductions in shoot biomass or seed production of individual plants may occur when the AOT40 value exceeds approximately 3 ppm/hour (108). However, the cumulative effect of these ozone exposures on species composition should they continue for several years is unknown. On the basis of the limited data available, it is suggested that the critical level for such communities should be no higher than that set for crops corresponding to a 5% yield loss.

Evaluation of ecological risks

As described above, exposure to elevated ozone concentrations causes effects on individual crop and tree species, and on species mixtures, leading to losses in economic values, quality traits, and ecological and genetic resources. Negative effects on crop yield cause significant economic losses in agriculture, and visible injury may reduce the market value of crops sold for their foliage. Long-term effects on trees may impair the function of forest ecosystems, i.e. their role with respect to the water and energy balance, to soil protection from erosion, etc. Current understanding is that many plant responses show a linear relationship with AOT40, and a change in yield, biomass or species composition of 5% or higher has been chosen as the basis for setting critical levels. It is therefore implicit in this approach that smaller changes in biomass, etc. will occur at exposures below the critical level; in some situations these smaller changes might lead to adverse economic or ecological effects. Because of the variability in ozone exposure from year to year, the AOT40 values calculated for receptor-specific annual periods should be averaged over 5 years when evaluating the ecological risk by calculating the exceedance of critical levels. Furthermore, biochemical and physiological changes that may occur at exposures below the critical level could in some (though by no means all) circumstances have adverse effects through an alteration in sensitivity to other biotic or abiotic stresses.

The data used to derive critical levels are almost entirely drawn from experiments in open-top chambers in central and northern Europe, using plants that are adequately supplied with water and nutrients. There are uncertainties in using these data to define air quality guidelines for vegetation throughout Europe. Among the most important of these uncertainties are the following.

- The open-top chamber technique tends to overestimate the effects.
- Many species have not been investigated experimentally in Europe, especially those of the Mediterranean region.

- The critical level is likely to be higher when water supply is limited, because ozone flux is reduced. This is important in many areas of Europe, especially as periods of water stress often coincide with periods of high ozone concentration.
- The data for trees are more variable than those for annual crops, and there is uncertainty in extrapolating from experiments of limited duration with young pot-grown trees to long-term effects on forest ecosystems.
- For changes in the composition of species mixtures, the experiments are also of limited duration, and there is uncertainty about the long-term effects of ozone.

In spite of these uncertainties, it is preferred to recommend guidelines based on critical levels for which a sound scientific basis exists, rather than incorporate uncertainty factors. These critical levels fulfil the primary aim of air quality guidelines in providing the best available scientific basis for the protection of vegetation from significant effects, and a solid information base for risk assessments. Current measurements in rural areas of Europe indicate that ambient ozone levels substantially exceed the critical levels for crops and trees over large areas. This shows that a high risk for ozone damage to vegetation exists in Europe.

Guidelines

The following guideline values for ozone are recommended. To protect agricultural crops from long-term effects and yield losses of 5% or 10%, the accumulated ozone dose above a cut-off level of 40 ppb (AOT40) for daylight hours (i.e. >50 W/m² potential global radiation) over 3 months (e.g. May–July) should not exceed 3 ppm/hour or 6 ppm/hour, respectively. To protect natural and seminatural vegetation from changes in species composition and reductions in shoot growth and seed production of individual plants, the corresponding AOT40 should not exceed 3 ppm/hour. To protect forest tree species from long-term effects and growth reductions, the AOT40 over 6 months (e.g. April–October) should not exceed 10 ppm/hour.

For evaluating ecological risk by calculating the exceedance of critical levels, the AOT40 values calculated for receptor-specific annual time periods should be averaged over 5 years.

These guideline values may not protect sensitive species from short-term acute effects. The critical level to protect agricultural crops from short-term visible leaf injury would be an AOT40 of 0.5 ppm/hour for a 5-day period (daylight hours only) when the mean vapour pressure deficit exceeds 1.5 kPa, and 0.2 ppm/hour when the vapour pressure deficit is less than 1.5 kPa.

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