ENVIROMENTAL POLLUTION AND FUNCTION OF PLANT LEAVES

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Contents

1. Introduction
1.1. Global Change
1.2. Phytotoxic Air Pollutants
2. Ozone as Environmental Pollutant
2.1. Ozone Formation and Concentrations
2.2. Critical Ozone Doses for Plants
3. Plant Responses to Ozone
3.1. Ozone Uptake by Plants
3.2. Biochemical Responses
3.2.1. Antioxidative Systems Affording Protection from Ozone
3.2.2. Other Biochemical Defense Systems
3.2.3. Ozone-Induced Increase in Phenolic Compounds
3.3. Physiological Responses
3.3.1. Photosynthesis and Respiration
3.3.2. Stomatal Conductance
3.3.3. Growth and Carbon Allocation
3.4. Structural Responses and Visible Injuries
3.4.1. Structural Responses
3.4.2. Visible Injuries in Foliage
3.4.3. Role of Ethylene in Formation of Visible Injuries
3.4.4. Programmed Cell Death
3.5. Ozone and Forest Trees
3.5.1. Impact on Forest Ecosystems
3.5.2. Effects of Ambient Ozone on European Forests
3.5.3. Effects of Ambient Ozone on Forest Trees in North America
3.5.4. Sensitivity of Young Seedlings versus Mature Trees
3.5.5. Birch is a Sensitive Model Plant in Ozone Research
4. Combined Action of Air Pollutants and Other Environmental and Climatic Factors in Plants
4.1. Air Pollutant Interactions
4.1.1. Ozone and CO₂ Interactions
4.2. Environmental Interactions
4.2.1. Impact of Water Conditions on Plant Responses
4.2.2. Role of Nutrient Availability in Plant Responses
4.2.3. Impact of Temperature on Plant Responses
4.2.4. Impact of Light Regimes on Plant Responses
4.2.5. Interactions with Biotic Stress Factors
4.3. Disturbed Seasonality due to Global Change

Acknowledgments
Glossary
Bibliography
Biographical Sketch

Summary

Chemical composition of the atmosphere and environment has been changed because of increased world population, emission of gases from agriculture, combustion of fossil fuels, traffic, and industry. In addition to a rise in greenhouse gases such as CO$_2$ and ozone, the predicted global change includes an increase in the Earth’s temperature, changes in hydrological cycles, and an increase in ultraviolet radiation. Acidification is the most important threat for plant ecosystems in developing countries, while increasing ozone and nitrogen deposition cause severe problems in Europe and North America. Ozone is most widely spread phytotoxic pollutant in all industrialized regions of the Northern Hemisphere. Detrimental effects of ozone have been well documented for several plant species. The most important harmful ozone effects are loss of membrane integrity, reduction of photosynthesis, accelerated leaf senescence, and reduced yield.

To overcome ozone stress, plants need to reduce ozone uptake by stomatal closure and/or adapt to surrounding conditions by different detoxification mechanisms. Ozone toxicity can be ameliorated through the action of antioxidants leading to scavenging of reactive oxygen species, and through activation of ethylene, polyamine, and phenylpropanoid pathways resulting in synthesis of protective phenolic compounds. In high ozone concentrations the detoxification capacity of plants is exceeded, and real damage may appear as ultrastructural and visible foliar injuries, and ultimately programmed cell death. Forest trees have been chronically exposed to increased ozone levels for several decades, and thereby tropospheric ozone is estimated to be one of the major environmental risk factors to forest ecosystems in the twenty-first century, besides increasing CO$_2$ and nitrogen deposition. The combined action of air pollutants and other environmental and climatic factors is very complex, and it is difficult to predict how ecosystems will respond to increasing pollutants and global change. However, it is obvious that some species will be favored while others adversely affected in the changing environment.

1. Introduction

1.1. Global Change

World population continues to grow, resulting in increased emission of gases from agriculture, the combustion of fossil fuels, traffic, and industrial processes. Worldwide deforestation and destruction of natural habitats are accelerating rapidly to accommodate the need for space to support the population growth. As a result of these processes, the chemical composition of the atmosphere has been changed. The increase in concentration of greenhouse gases such as carbon dioxide (CO$_2$), carbon monoxide (CO), ozone (O$_3$), nitrous oxide (N$_2$O), methane (CH$_4$), and chlorofluorocarbons (CFCs) in the atmosphere is among the most serious threats to humankind. The biosphere acts as both a sink and a source for the greenhouse gases. Photosynthesis withdraws CO$_2$
from the atmosphere, resulting in the formation of carbon storages with a variable turnover rate. Water vapor and atmospheric gases are responsible for the temperature of the Earth’s surface and for the radiation and heat balances. Global warming would cause unforeseen environmental changes, which may be very different in various regions of the globe. Changes in hydrological cycles may cause droughts in some areas but floods in some other regions. Depletion in the stratospheric ozone layer shielding the Earth’s surface from the damaging ultraviolet radiation (UV-B) causes a risk not only for human health but also for vegetation (a threat for wood production) and sea biota.

Most continental environments (soils and freshwaters) are naturally acidic (pH < 7) owing to the oxidation and respiration reactions in biotic and abiotic systems. During the twentieth century, the acidity of many regions of the world continuously increased as a consequence of energy and food production. Since the early 1970s, the phenomena and impacts of acid rain (mainly NO₂ and SO₂ deposition) on vegetation have been studied intensively in Europe, North America, and Asia. Regional concentrations of pollutants such as SO₂ have decreased by emission reduction in Europe and North America, but on the other hand, there are explosively increasing emissions from recently industrialized countries in South America, South-East Asia, China, and India. Large cities in developing countries, which contain numerous small industries, domestic fires, and increasing traffic, also are big source of acidifying air pollutants.

Therefore, air pollution remains a continuously increasing problem throughout the world, and the problems in industrialized countries differ from those in developing countries. International cooperation is needed to control the environmental pollution and to study the effects of locally and regionally increasing concentrations of toxic, acidifying, and eutrophying air pollutants on agricultural and natural ecosystems.

1.2. Phytotoxic Air Pollutants

The most important anthropogenic phytotoxic gaseous air pollutants include ozone (O₃), nitrogen dioxide (NO₂), ammonia (NH₃), sulfur dioxide (SO₂), hydrogen sulfide (H₂S), hydrogen chloride (HCl), hydrogen fluoride (HF), chlorine (Cl₂), ethylene (C₂H₄), and peroxycetyl nitrate (PAN). These gases can be categorized according to their biochemical/physiological activity in plant cells as given in Table 1.

<table>
<thead>
<tr>
<th>Metabolic activity</th>
<th>Air Pollutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidizing</td>
<td>ozone, PAN</td>
</tr>
<tr>
<td>Acidifying</td>
<td>SO₂, NO₂, HF, HCl</td>
</tr>
<tr>
<td>Mutagenic</td>
<td>SO₂</td>
</tr>
<tr>
<td>Reducing</td>
<td>SO₂, NO₂, H₂S</td>
</tr>
<tr>
<td>N-eutrophying</td>
<td>NH₃, NH₄⁺, NO₂, NO</td>
</tr>
<tr>
<td>Disturbing hormonal balance</td>
<td>ozone, PAN, SO₂, HF, C₂H₄</td>
</tr>
</tbody>
</table>

Table 1. The main metabolic activities of phytotoxic air pollutants in plants
Ozone and PAN-compounds are major photochemical oxidants, having serious adverse effects on vegetation. In addition, it has recently been reported that hydrogen peroxide (H₂O₂) and organic peroxides are produced by the reaction of ozone with volatile emissions by plants. Numerous experiments have been carried out to clarify the biochemical and physiological mechanisms of toxicity of ozone in crop plants and forest trees, whereas the effects of other photochemical oxidants have not been studied extensively.

The oxidation products of atmospheric nitrogen (NOₓ) are chemically reactive gaseous pollutants with deleterious effects on biological systems. The most extensively studied phytotoxic nitrogen compound is NO₂, because it is a precursor molecule for most other NOₓ components and also for ozone. Accordingly, this article will deal mainly with the matter of the phytotoxicity of ozone, which is globally one of the most important atmospheric pollutants for plant ecosystems today. In addition, most ozone-induced physiological and biochemical responses in plants resemble those caused by other environmental pollutants, such as NOₓ and SO₂, and thereby ozone is an excellent example of the impacts of environmental pollution.

2. Ozone as Environmental Pollutant

2.1. Ozone Formation and Concentrations

Unlike in the stratosphere, tropospheric ozone is formed through a complex set of chemical reactions, including photochemical oxidation of volatile organic compounds (VOCs) in the presence of nitrogen oxides. In the main reaction of ozone production, NO₂ is photolyzed by sunlight (λ< 430 nm) to NO and atomic oxygen (O), which reacts with molecular oxygen (O₂) forming ozone (O₃). In further reactions between ozone and nitric oxide, nitrogen dioxide is reformed (NO + O₃ → NO₂ + O₂), and a part is deposited to land and vegetation surfaces.

Tropospheric ozone concentrations at mid latitudes in the Northern Hemisphere approximately doubled during the twentieth century. The present annual increase in atmospheric ozone level in the Northern Hemisphere is estimated to be 0.5% to 2% per year. The rise in ozone concentrations has been correlated with the presence of nitrogen oxides, volatile hydrocarbons, high solar radiation, and temperature, thereby linking ozone formation with anthropogenic as well as biogenic emissions. Current ambient background ozone concentrations are generally above 25 nL L⁻¹, whereas highest peak concentrations in industrialized countries are well above 100 nL L⁻¹. Such peak episodes occur when ozone precursors accumulate under warm, dry, and sunny weather conditions, especially with stagnant air conditions. In addition to high-ozone episodes in summer, there are springtime maximum concentrations originating from the stratospheric flux of ozone into the troposphere, low deposition of ozone to land and plant surfaces, and changed atmospheric photochemistry because of the wintertime accumulation of air pollutants. Because the annual maximum concentrations coincide with the growing season, plants are exposed to chronic ozone stress with variable physiological and biochemical responses. Diurnally, maximum concentrations are measured in the late afternoon, and lowest concentrations at night. Because ozone travels long distances (over several hundred kilometers), elevated ozone concentrations
are common in rural areas, where effects on crops, natural vegetation, and trees may occur.

2.2. Critical Ozone Doses for Plants

There is a large variation in prevailing ozone concentrations over Europe and the United States, highly dependent upon climatic conditions. Because ambient ozone concentrations have continuously increased over the past decades, current tropospheric ozone concentrations may represent a risk for several plant species in areas where critical levels are exceeded. To protect vegetation against the adverse effects of ozone in Europe, concepts of critical loads/levels have been designed after analysis of available experimental data on crop plants, natural vegetation, and European forest trees. The current European critical levels for ozone are expressed as the cumulative ozone exposures using the index AOT40 (that is, the sum of hourly ozone concentrations above a cut-off level of 40 nL L\(^{-1}\) during daylight hours when global radiation exceeds 50 W m\(^{-2}\)) over a six-month growing season. For agricultural crops and natural vegetation (annual plants), critical ozone exposure has been defined as an AOT40 value of 3 nL L\(^{-1}\) h (= 3000 nL L\(^{-1}\) h), whereas an AOT40 of 7 μL L\(^{-1}\) h was considered to be the most appropriate for semi-natural perennials, and an AOT40 value of 10 μL L\(^{-1}\) h was defined for forest trees. These critical exposures correspond to the ozone exposure associated with a significant reduction in the growth of selected model plants (like wheat for crops and beech for forest trees). Because current European critical levels are based on a relatively small number of open-top chamber experiments with a very limited number of plant species, there has been active research to develop improved standards to protect vegetation, and current AOT40 values (described above) have been used only for modeling and mapping exceedance and recognizing geographical areas at risk (referred to as Level I assessment).

For most plant species and areas, a realistic estimate on the actual ozone impact is not feasible using the available Level I data, and therefore an advanced Level II approach, which can be used on continental, national, and local scales, is being developed. In this Level II approach, attention is given to the influence of environmental modification factors (that is, soil moisture) on ozone exposure and plant response, differential sensitivity within and between different plant species, phenology, interactions with other pollutants, characterization and calculation of the biological response, and economic assessment of the losses of economic yield or forest use values. The Level II model will be based on the ozone flux into the canopy, absorbed through stomata into the plant leaves or needles, rather than ozone exposure in the atmosphere. Ozone flux, characterizing the internal dose of ozone received by plants, cannot be measured directly, and therefore models are being designed at several levels of complexity and detail. The latest flux-oriented approach for conifers and deciduous trees suggests that adverse effects on sensitive plant species are possible below 25 nL L\(^{-1}\), which is near the preindustrial background ozone concentrations.

The present air quality standard to protect vegetation in the United States is based on measured concentrations (that is, exposure) rather than on plant uptake rates (dose). The current standard, set at a level of 80 nL L\(^{-1}\), is defined as the average on the annual fourth highest daily maximum eight-hour ozone concentration over a three-year period.
The importance of high ozone concentrations has, however, been questioned because of temporal phase difference between the maximum stomatal conductance (usually in the mid-to-late morning) and the maximum ozone concentrations (usually in the late afternoon). It has been suggested that plants can be more sensitive to ozone during the night as a result of lower plant detoxifying capacity, and therefore a component indicating plant defensive impacts as well as feedback mechanisms in photosynthesis should be included in the new standards.

3. Plant Responses to Ozone

Ozone experiments have identified several biochemical and physiological effects on plants. At the biochemical level, ozone oxidizes sulfhydryl and fatty acid double bonds, increases membrane permeability, disrupts membrane-bound photosynthetic systems, and lowers foliar sugar and polysaccharide levels. At the physiological level, net photosynthesis, stomatal conductance and water use efficiency are reduced, dark respiration is affected, carbon allocation is changed (resulting in shoot/root imbalance), leaf senescence is accelerated, foliar visible injuries and leaching are increased, floral yield is decreased, and fruit set is delayed. Ozone-induced reduction in growth rates and yield occurs through impaired net photosynthesis and regeneration. Ozone impacts on plants have also been observed as altered morphology. For example, changes in wood quality have appeared as reduced wood density and tracheid length, resulting in reductions in wood strength, pulp yield, and quality.

3.1. Ozone Uptake by Plants

Once ozone has reached the surface of the vegetation by diffusion and turbulent transport through the boundary layer, it can either react with cuticular components or diffuse through the open stomata into the leaf. Ozone uptake through waxy cuticles is about $10^4$ times lower compared to open stomata, and thus negligible. Therefore, the main route of entry of ozone into the plant is via stomata at the plant leaves. Once it has entered the leaf, ozone is dissolved in the aqueous phase of the cell wall matrix (called apoplastic fluid). The solubility of ozone in aqueous media is about tenfold higher than that of O$_2$. Therefore ozone does not bear a risk because it might accumulate to phytotoxic concentrations inside living cells, but mainly because it reacts rapidly with organic materials and gives rise to reactive oxygen species (ROS) such as hydrogen peroxide (H$_2$O$_2$), hydroxy (OH$^-$), super oxide (O$_2^-$), and peroxyl ($^1$O$_2$) radicals, which cause oxidative destruction of lipids and proteins. For example, $^1$O$_2$ is an activated, highly reactive state of ground-state dioxygen, which oxidizes preferentially electron-rich compounds (olefins, dienes) such as methionine, histidine, and tryptophan. In plant chloroplasts, $^1$O$_2$ leads to chlorophyll degradation resulting in a reduction in photosynthesis. H$_2$O$_2$ and O$_2^-$ are more stable and thereby less dangerous than $^1$O$_2$ or OH$^-$, and are detoxified by cellular antioxidative systems. Increased formation of ROS in the apoplast may trigger many intercellular reactions, such as hypersensitive response (HR) leading to premature cell death in acute ozone stress, and systemic acquired resistance (SAR), which is the signaling system in plant–pathogen interactions. There is also increasing evidence that activated oxygen species are involved in ozone-mediated foliar injury, observed for example as loss of soluble proteins, premature senescence, and necrosis.
For plants, there are two main pathways to be protected from ozone: first, exclusion of ozone by stomatal closure, and second, detoxification of ozone and its secondary products (ROS) using cellular protective systems. Although stomatal closure might be a useful way to reduce or prevent ozone uptake during high ozone concentrations, such a mechanism can only provide a limited protection from chronic long-term ozone stress, since CO₂ uptake for photosynthesis would also be restricted. The recent experiments indicate that the relationship between ozone responses, stomatal conductance, and ozone tolerance is rather complex. For example, in the British population of Plantago major, the high ozone tolerance of some populations could not be explained by the function of stomata, but appeared also to be the result of biochemical protection. In accordance, in bean (Phaseolus vulgaris), some of the tolerant varieties were resistant by limiting ozone flux by lower stomatal conductance, while the others appeared to ameliorate ozone toxicity through the action of antioxidants.

3.2. Biochemical Responses

3.2.1. Antioxidative Systems Affording Protection from Ozone

Because reactive oxygen species (ROS) are also formed during the normal cellular metabolism (covering functions such as photosynthesis, fatty acid metabolism, and respiration), plant leaves are provided with a number of continuously forming and regenerating metabolites with an antioxidative function such as ascorbate, glutathione, α-tocopherol, polyamines, and phenolics. These compounds can either scavenge ROS directly or serve as substrates for defense enzymes like superoxide dismutases, catalases, and peroxidases. Intracellular antioxidative systems are relatively well documented, but much less is known about apoplastic antioxidative systems, although these defense mechanisms might be extremely important for ozone detoxification. Several experiments have shown that ascorbate (= vitamin C) is a regular constituent of the apoplastic space in many herbaceous and tree species, and accumulates in millimolar concentrations in plant cells. In addition, the apoplastic space contains significant concentrations of phenolics and considerable peroxidase activities. Together with ascorbate, these compounds catalyze the detoxification of ROS. However, if the defense capacity of the cells is overcome, the cell integrity can be altered and result in disturbances in photosynthetic systems, reductions in concentration and activities of several essential enzymes (such as Rubisco) and changes in pigment, lipid, and nutrient concentrations. Membranes appear to be the primary sites of ozone attack. At low ozone concentrations, protein membrane components are oxidized, while at higher concentrations lipids undergo peroxidation, resulting in deleterious changes in membrane permeability.

3.2.2. Other Biochemical Defense Systems

It has been found that the inherent capability of plants to synthesize stress ethylene in response to ozone exposure can be determining a factor in plant sensitivity to ozone. For example, in the ozone sensitive Bel W3 tobacco, ethylene synthesis increased considerably after ozone exposure, while ethylene synthesis remained low in the insensitive Bel B tobacco. Increases both in the ethylene evolution and in the concentration of the ethylene precursor ACC took place rapidly, one to two hours after
the onset of the ozone fumigation, and were correlated with the appearance of visible foliar injury in the sensitive plant. For further information about ethylene, see Section 3.4.3.

In addition to stress ethylene formation, polyamine pathways, related to ethylene pathways, are activated during ozone fumigations. In several herbaceous crop plants, there was a strong positive correlation between ozone exposure and the induction of arginine decarboxylase (ADC, the key enzyme in plant stress polyamine synthesis) activity, resulting in increased spermidine content in barley and increased putrescine content in ozone-resistant tobacco and potato. These accumulations in polyamine compounds were connected with improved protection against ozone damage in leaves. Polyamines are known to play an indirect role in preventing ozone-caused lipid peroxidation by chelating metal ions that catalyze peroxidation. In addition, another possible mechanism for preventing ozone damage is the suppression of ethylene synthesis by polyamines. Thereby, polyamines may act as important stabilizing agents of membranes and scavengers of oxygen radicals.

Ozone stress has been shown to increase the activities of PAL (phenyl alanine ammonium lyase), CHS (chalcone synthase), and CAD (cinnamyl alcohol dehydrogenase) enzymes controlling respectively the phenylpropanoid, flavonoid, and lignin biosynthesis pathways. These pathways lead to the synthesis of important protective phenolic compounds including flavonoids (protection against UV radiation), furanocoumarins (acting as phytoalexins), and lignin (protection against pathogen attack and ozone).

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Biographical Sketch

**Elina Johanna Oksanen** was born in 1964 in Vehmersalmi, eastern Finland, the daughter of Jarmo Räsänen and Aune Laakkonen, who were landowners and farmers in the northern part of the province of Savo, in a region rich in beautiful scenery. Elina was the second offspring in the family. Her older brother Tapio became a well-known forest researcher in Helsinki and her younger sister Anna became an editor. Elina’s interest in nature and her fondness for plant and bird life appeared early during the summers she spent in the countryside. After attending schools in her native village and the city of Kuopio, she entered the University of Helsinki as a biology student. She completed plant physiological studies in Helsinki in 1992, and continued with ozone stress studies at the University of Kuopio. She completed her dissertation on the effects of ozone on European white birch in Kuopio in 1996, and thereafter continued to refine her expertise in plant stress science, collaborating closely with the Swiss Federal Institute for Forest, Snow, and Landscape Research (Birmensdorf, Switzerland), Michigan Technological University (USA) and Tokyo University of Agriculture and Technology (Japan). Since 1998 she has been working as a project leader in a long-term project designed to identify and study the physiological and morphological impacts of elevating ozone and CO2 on deciduous forest trees. In 2001 she gained the senior researcher post of the Academy of Finland, which allowed her to continue with large-scale open-field experiments with greenhouse gases and forest ecosystems in Finland, the United States, and Japan.

In addition to her scientific career, Elina found time to raise three children (Vilma b.1988), Sanni (b. 1990) and Antton (b. 1999).