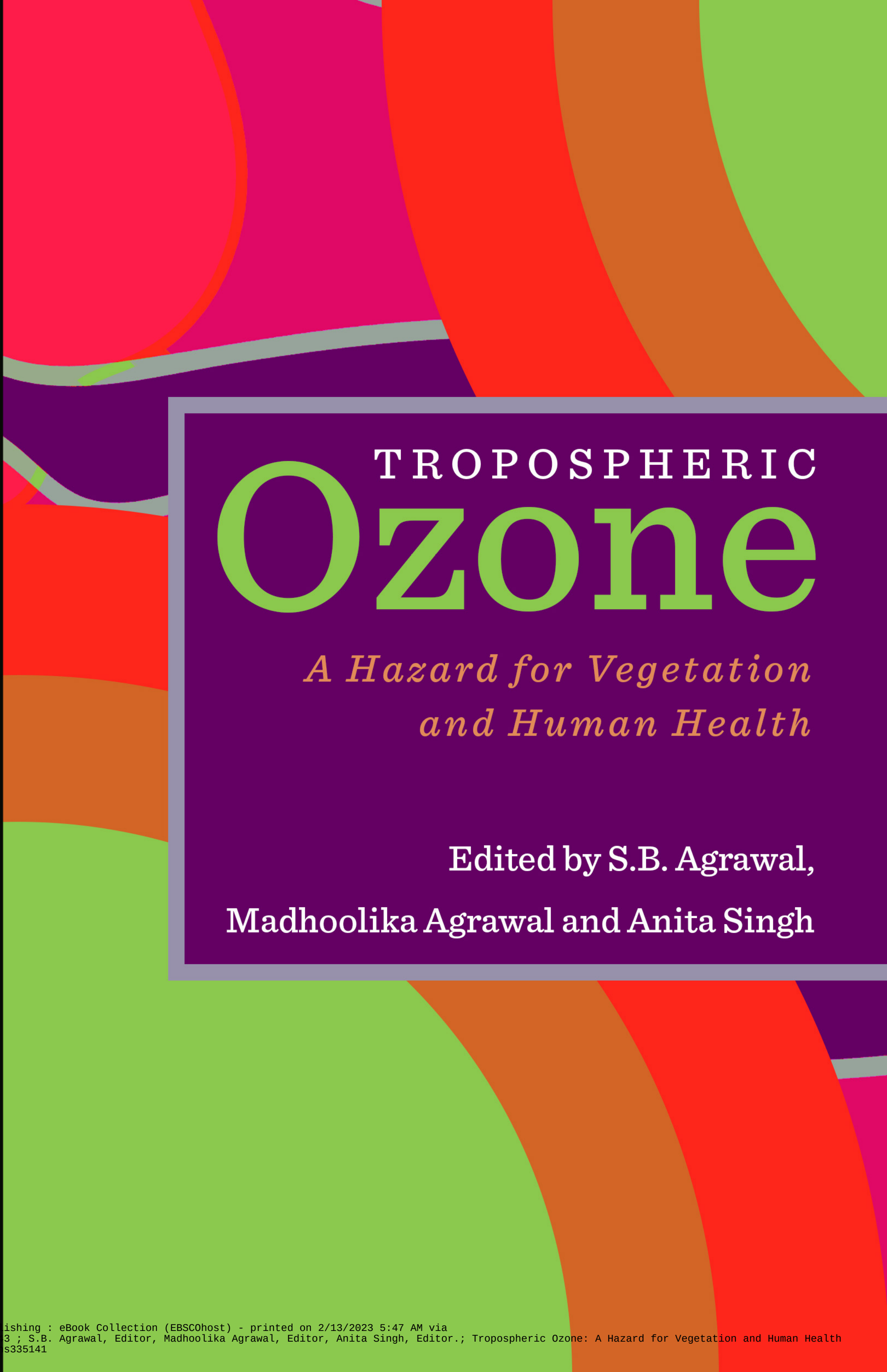

TROPOSPHERIC Ozone

A Hazard for Vegetation and Human Health

Edited by S.B. Agrawal,
Madhoolika Agrawal and Anita Singh

Tropospheric Ozone

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PREFACE

The tropospheric “ozone” (O_3), which is only 10% of the total concentration of O_3, has a large impact on plants and human health globally. Ozone is also the third most powerful greenhouse gas in the atmosphere. It has a potential impact on all the ecosystem services, including the supporting services (root growth), cultural services (aesthetic value) and economic services (crop yield). It causes visible injuries such as stippling and bronzing on sensitive species, reduces the growth and induces early senescence. Along with the vegetation, O_3 also adversely affects human health. Children are more sensitive to higher concentrations of O_3 as the lung development continues in the postnatal period.

In the above context, we have tried to compile the recent information provided by experts in the subject area in form of this edited book. This book presents an up-to-date report and a critical and well-discussed overview of the wide-spread impact of trophospheric O_3 on plants and humans, an emerging issue that needs an immediate attention of the entire world. Each chapter has covered detailed information on various important issues related to the tropospheric $O_{3.}$

The history of identification of O_3 in Los Angeles, USA at first and then in Europe as a result of photochemical reaction of hydrocarbons and NO_2 under sunlight was detailed in chapter I. The spatial scale of O_3 problem in Europe was correlated with decline in conifers. The chapter further explained the improvements in indices of risk assessment using different models. The history of O_3 also provides evidence of collaborative research work in strengthening the understanding of sources of O_3 problem. In the chapter II, “Tropospheric ozone: formation, distribution and trends over time”, the authors have discussed the O_3 precursors, the lifetime of tropospheric O_3 in the boundary layer and free troposphere, and the long–range transport from regional to hemispheric scale through trans–Atlantic, trans–Pacific and trans–Eurasian transport. Such long range transport affects remote areas including Arctic. However, the trend of O_3 concentration showed declining pattern in North America and Europe, but an increase in Asia. The chapter III reviews the spatial and temporal changes in O_3 levels under different emission and climate scenarios on different assumptions on climate, energy access policies, and land cover and land use changes. The results of different simulations performed using numerous global or regional chemistry models under the new RCPs scenarios for past, future and current trends of O_3 concentration were also discussed.

Bio-monitoring of O_3 pollution using plants is suggested to be a very low cost method of wider application to quantify spatio temporal changes in the living organisms in chapter IV. Ozone biomonitoring using tobacco Bel W3 is a widely accepted procedure throughout the world. Chapter V describes an adjustment in O_3 biomonitoring protocol, which suits to subtropical regions with dry winter and hot summer. Ozone uptake in leaf mostly takes place through stomata, but alternative routes for O_3 uptake and associated changes in the leaf structure of tropical plants have been discussed in chapter VI. The consequences of O_3 stress on series of interconnected physiological processes, modifying the responses of plants are discussed in chapter VII. Effects of O_3 on forest ecosystems are less explored. Chapter VIII reviews the information on O_3 impact on growth, carbon allocation, phenology and physiological functions of forest tree species. Species interactions affected by O_3 were also highlighted.

Ozone induced oxidative stress in plants and resulting responses of signaling pathways and antioxidative machinery revealed the mechanism of O_3 tolerance in chapter IX. Ozone induced changes at trancriptome, proteome and metabolome levels in plants were detailed in chapter X, which will improve the understanding of molecular mechanisms regulating the plant susceptibility to O_3. Chapter XI attempted to review the information on varying responses of crop plants under different agronomic practices such as nutrient amendments, water and weed management, use of antioxidants and other protectants. The review in chapter XII on influence of past, current and future O_3 concentrations on various ecological services including supportive, provisioning, regulating and cultural clearly emphasizes the need of such studies in future for the well being of mankind. Efficiency of ethylene diurea (EDU), a synthetic antiozonant compound and a chemical protectant was discussed in relation to new insights at molecular, nutritional and physiological levels in chapter XIII.

Isoprene, a VOC playing crucial role in formation and degradation of O_3 is suggested to be an important factor, which needs to be regulated under future climate change scenarios in chapter XIV. Productivity of crops directly reflects the influence of stress factors on food security. The chapters XV and XVI presented comprehensive reviews on impact of O_3 on crop yield in global and Indian perspectives, respectively. Ozone has also potential negative effects on quality of food grains/seeds. Influence of surface O_3 on human health in relation to cardiovascular, reproductive and neurological to respiratory disorders using epidemiological and exposure based studies, is reviewed in last two chapters, XVII and XVIII. Ozone has been found to contribute significantly in the global burden of diseases.

Overall, this edited volume compiles the recent available information on trends and extent of O_3 pollution in the world and its past, present and future influences on food security, human health and ecosystem services. The content of the book will help the academicians, scientists, policy makers and organisations involved in understanding and solving environmental issues in particular reference to O_3 pollution and its impact on the living world.

We highly appreciate all the authors for their quick response to our invitation and their timely submission of manuscripts, which made the edited volume possible even during the present period of COVID-19 pandemic. Our profound thanks also go to all the learned reviewers for making their critical reviews and providing constructive suggestions on different chapters. The help rendered by Professor Muhammad Iqbal and Dr Helen Edwards, Commissioning Editor, Cambridge Scholars Publishing, U.K. is gratefully acknowledged for bringing out this volume.

Finally, we would like to dedicate this book to our teacher Professor D.N. Rao, Ex Head, Department of Botany, Banaras Hindu University, India who initiated researches on O_3 stress in relation to plants in India.

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Anita Singh

CHAPTER ONE

HISTORY OF AIR QUALITY AND PLANTS: THE FUNDAMENTAL ROLE OF OZONE

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Abstract

Eighty years ago, smog in Los Angeles caused eye irritation and plant damage. The damages were different from those observed in smog episodes in the eastern USA and Europe. Haagen-Smit tested the action of ozone (O_3) and gasoline on crops and got symptoms similar to smog. Getting similar results with hydrocarbons and NO_2 under sunlight, he concluded that the smog resulted from the photochemical reaction of hydrocarbons and NO_2 from car exhausts and fuel combustion, O_3 being a secondary pollutant. Visible symptoms of O_3 damage on pines were identified in the mountains surrounding Los Angeles. In Europe, German scientists claimed that the conifers in Germany and France were declining. A co-operation started between the two countries, followed by European programs, allowing considerable knowledge about the physiology of crops and trees exposed to O_3. At the leaf level, decreased photosynthesis and increased respiration were the physiological symptoms of lower plants' growth. To improve the indices of risk assessment, the SUM0, and AOT40 metrics were abandoned in favor of PODs integrating the actual quantity of O_3 entering the leaf. The challenge remains to better include the detoxification capacity in the models. At similar PODs, C_4 plants show a faster decline in metabolic activities than C_3 plants under O_3, but they resist better in the field thanks to their lower stomatal conductance. The study of the behaviors of these two groups of plants under O_3 and associated stresses (drought, elevated CO_2, temperature) and the upscaling to ecosystems is needed.

Keywords: History; Air quality; Risk Assessment; Detoxification model

1.1 General Remarks

Histories have to be written from a perspective of the current time and place but cover a sequence of events that has a very different perspective (Zeitgeist[1]). Here we are trying to describe how urban air with highly oxidative components was discovered to injure plants and how scientific progress was made to understand how that injury occurred. We will focus mainly on the 20th century, but we will mention the ways that recent research was positioned primarily for improving the risk assessment index and the integration of experiments from cell to ecosystem.

By the century's end, we believe that science had a general picture of the mechanisms, although as the remainder of this book shows, more details are emerging. Much of the progress depended upon the technology available and the understanding of biological processes. We also wish to emphasize the role of various countries and people in this process. Overall, the investigations really began in the US, especially in a polluted area, such as Southern California, and then spread to the rest of the US and Europe. While researchers in Japan played a role in some areas, the remainder of the world became involved much later due to a lack of recognized areas of urban pollutants and the political will to fund such studies.

Why is such a discussion needed now? The Earth has nearly 10 billion people to support, and the ecological problems seem to be building exponentially. Yet some of the important policymakers are denying there are any issues. Policy as usual, or even retrograde changes, appears to be the rule. While the rise in the Earth's atmospheric CO_2 level is important, we feel that it is time to take stock of the problem of pollution by other molecules concerning that rise and to emphasize the need for research responding to this challenge, for instance, by developing fruitful cooperation.

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[1] the general intellectual, moral, and cultural climate of an era.

This chapter is an example of how many countries and their scientists came together to define a problem and then indicate ways of solving the problem. That problem was the building up of toxicants in urban air regions; O_3 was the major component. Those toxicants ultimately force an alteration of the full plant ecology, whose understanding is only now being assembled (see Jolivet et al. 2016, Cailleret et al. 2018, Grulke and Heath 2020).

1.2 First warning signs of air pollution problems in the world

The Industrial Revolution during the 18th and 19th centuries changed everything, including the air that gives Earth its life. The burning of coal in factories for energy and homes for domestic heat led to high levels of urban air pollution.[2] During foggy episodes in winter, under certain atmospheric conditions, known as air inversion, the polluted fog could become trapped beneath a warm air layer leading to days of dense haze. These events compiled in an excellent review (Heidorn 1979), caused high death rates from respiratory diseases, especially among the old and the very young. In London, during December 1873, 650 people died due to the noxious fog that lasted 3 days. The 1875 Public Health Act by the United Kingdom contained a smoke abatement section to reduce smoke pollution in urban areas; that section was revealed to be largely insufficient. In the second half of the 19th century, France also strengthened its industrialization, and cities and industrial regions discovered the harmful effects of factories and mines. The impact of this pollution on buildings and vegetation became obvious. A French novelist in the mid-1800s, in a novel on the mining work, wrote that "the foliage of trees remains covered with fine, shiny coal dust" (Berthet 1866, cited in Cooper-Richet 2019, The Conversation).

At the beginning of the twentieth century, big cities in Europe were still impacted by heavy fog. This appears in the paintings of the River Thames in London by Claude Monet in 1903. The foggy aspect was due to haze and smoke. To describe this combination of smoke and fog, the term "smog" was coined in 1905 by Dr. Henry Antoine Des Voeux in a paper presented at a Public Health Congress meeting in London. The smog contained black soot and sulfur dioxide, resulting from the heavy use of

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[2] Early pollutant episodes were mainly particulates, which are very small particles of what was burnt since the visibility was greatly reduced. No doubt, there were other compounds, but such detection technology was not present.

coal[3] to heat homes and to run factories. Unfortunately, the problem of air pollution persisted. About thirty years later, in December 1930, in the Meuse Valley of Belgium, an episode of industrial air pollution combined with temperature inversion lasting 3 days caused several hundred cases of illness and killed 60 people.

In December 1952, London was hit by a disastrous episode of smog lasting 5 days. A temperature inversion again occurred, leading to the cold foggy air becoming trapped over the city by a high-pressure weather system. The smog, composed of a heavy fog combined with sulfurous fumes and nitrogen oxides from coal fires, vehicle exhaust, and power plants, caused the premature death of 4,000 people, mainly the elderly, young children, and people with respiratory problems. This smog episode was the worst air pollution crisis in Europe, leading a few years later, to the British Parliament passing the Clean Air Act of 1956, which restricted the burning of coal in urban areas and offered grants to convert from coal to alternative heating systems. A few years earlier, in October 1948, the same phenomenon was observed in the USA's industrial towns. At Donora, a town southeast of Pittsburgh in Pennsylvania, a similar air inversion led to one of the United States' worst air pollution events. The smog, composed of a mixture of hydrogen fluoride, sulfur and nitrogen dioxides, was trapped for five days by the inversion layer, killing 20 people and sickening 7,000 more. The events at Donora led to the appearance of a clean air movement in the United States, ultimately leading to the Clean Air Act amendment in 1967 (also called the Air Quality Control Act) and the Clean Air Act of 1970.

Smith (1872) authored the first scientific report on air pollution, primarily on acid rain, discovered in the 1850s as a problem resulting from coal-powered factories. The release of sulfur and nitrogen compounds into the atmosphere negatively impacts plants. The deleterious effects of air pollution were observed on the growth of grasses in industrial regions (Crowther and Ruston 1912) and the blackening on conifer needles by soot caught on them (Rhine 1924). Up to the end of the Second World War, the main air phytotoxicants implicated in plant injury were sulfur and nitrogen dioxides, fluorides, and halogens (Thomas 1951).

This chapter's remainder is concerned with the formation and effects upon plants of 20th-century pollutants, in general, created by modern machinery – the automobile with its internal combustion regime. These pollutants are generated by atmospheric effects and sunlight upon the

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[3] Coal comes in different forms: often, the coal used here had great qualities of sulfur compounds, which were converted into SO_2 by heating.

organic materials released by the exhaust – O_3 and organic oxidants. This particular type of plant damage is due to the release into the atmosphere of unsaturated hydrocarbons and nitrogen oxides from car exhaust and industrial fuel combustion, photochemically producing O_3 as a secondary pollutant (Haagen-Smit et al. 1952). In Europe, thirty years later, O_3 was incriminated in a general decline of forest trees (Blank 1985, Guderian 1985); this secondary but major pollutant will be largely treated in this chapter.

1.3 A Tale of a City

In southern California, the Los Angeles basin is surrounded by high mountains of nearly 4000 m (12,000 ft) pushed up by the collision of two continental plates of the Earth. The basin is formed by a plain that is connected to the Pacific Ocean. On that plain, a series of valleys formed by smaller hills gives different growing conditions for plants and urban settings. The basin area is nearly 100 km x 100 km (60 x 60 miles) within the mountain ranges (Fig. 1.1).

Historically, this basin was home to many Indian tribes, which had low population densities and relied upon natural ecology for food and housing. In the 17th century, Spanish/Mexican peoples arrived from the south through Baja California, spreading into valleys, but the main population density was along the coast where transportation was more accessible. In the 1840s, a US population,[4] moving westward from the Eastern seaboard and the Midwest, arrived in California. This initially small population of Eastern Americans explosively increased due to the discovery of gold in 1848, the Republic of California's formation by Americans displacing the Spanish/Mexican population, and the completion of the transcontinental railroad in 1863.

More and more Americans moved to the coastal regions due to the great climate and open lands. In Los Angeles, the real boom started during the first few decades of the 20th century. Houses were built, and an urban light rail was started.

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[4] The original Americans were native Americans called Indians by the early explorers. The Europeans of the Eastern Atlantic Coastline, whose colonialization started in the early 1600s, had largely displaced the native Americans by the mid-19th century. Of course, this population of the "Original Thirteen Colonies" had groups of Africans, mostly slaves, and Hispanics.

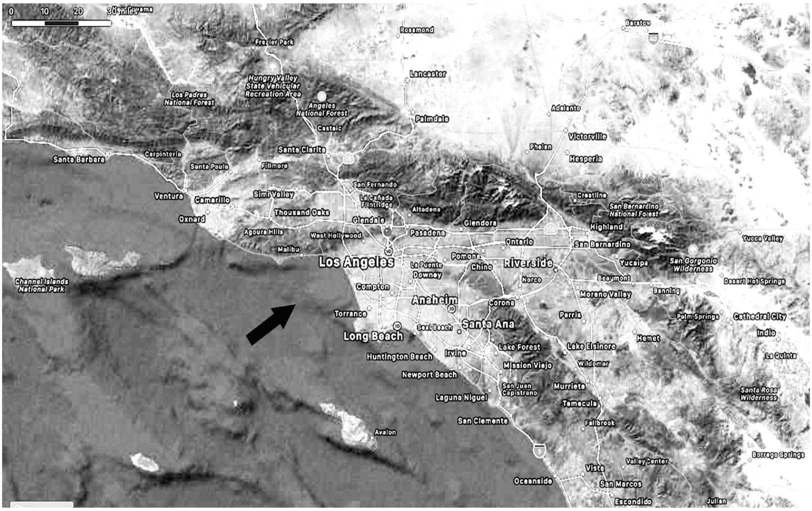

Fig. 1.1. Los Angeles Basin. The Pacific Ocean is on the left, a grey area. Mountains are indicated by the darkened areas. The on-shore sea breeze from the Pacific Ocean is indicated as a black arrow. The scale is about 450 km (270 miles) across the figure horizontally. North is up. *Modified map using a Google map initially*

> *By 1910 ...Los Angeles was the hub of extensive Pacific electric, 'a 1,100-mile interurban system whose big red cars skirted mile after mile of sandy shorelines, swept past endless acres of orange groves and climbed into the foothills of the San Gabriel Mountains'. Interestingly, a network of streetcar systems, rather than the car, created the sprawling nature of Los Angeles and its suburbs. All its main highways had a streetcar line running down them, and therefore it was the distance to the streetcar stop that was the limiting factor in local development. The Pacific electric inner-urban system was the brainchild of developer Henry E. Huntington, who built it as a loss leader financed by profits from his housing project. He saw the excellent transportation was as essential as ensuring the houses had water and electricity and therefore was not concerned that he lost money on providing it. This extensive urban spread gave Los Angeles a reputation, which has lasted to this day, as the very urban sprawl model. (Christian Wolmar 2012).*

For the population in the Los Angeles basin, another push came during and after World War II, due to the many experiences of military personnel while on the West Coast, especially in the ports of San Diego and Long Beach/Los Angeles. The climate was perfect, and the Pacific Ocean provided near-perfect beaches. As long-distance transportation provided by trolleys was eliminated due to unfair competition by petroleum producers and car manufacturers, the automobile's rise led to the final problem. Now everyman had a nice house and car, leading to the filling-in of the land of the Los Angeles basin.

By the late 1940s, downtown Los Angeles was beginning to be known as the USA's smog capital (see Fig. 1.2). By 1945 all the parts of the system to produce oxidative toxicants within the atmosphere of that valley were present: a large and growing population, transportation by vehicles using internal combustion engines, a climate of bright sunshine and warm air, and periods where the atmosphere did not clear or blow through the region. Furthermore, the Los Angeles basin was capped by an inversion layer due to cooler air above kept in by the high mountain ranges. A pressure cooker filled with a large number of strange molecules from partially combusted petroleum was present. That atmosphere generated many heretofore unknown oxidative carbon and nitrogen compounds that increased as the sun rose and moved into the inland valleys as the on-shore breezes swept inland during the afternoon to clear as the inversion layer broke at night, allowing the warm polluted air to escape.

Fig. 1.2. A photo from above of Hollywood on 26 July 1943, looking eastward towards downtown Los Angeles. At that time, the largest building downtown was the city hall at 32 stories. The road shown is the beginnings of the Hollywood Freeway (now Interstate 10). If the photo were in color, the layer of smog would be brown. Photo from WIRED, Source: http://www.wired.com/thisdayintech/2010/07/0726la-first-big-smog/, non-copyrighted

In 1947, California authorized Air Pollution Control Districts in every county to respond to the "Black Wednesday" in 1943 during World War II. Smog in Los Angeles blinded drivers, and residents thought it was a gas attack (Fig. 1.2). In 1967, the then-Governor Ronald Reagan understood that air quality regulations debated in the US Congress would be much less than those California had already passed. He asked for a waiver to allow California to set higher standards than those in the rest of the US. Every President save the present one, renewed that waiver for California. That President's "statement" is being fought in the courts (Jonathan Taplin, "California is so not 'over'." Los Angeles Times, Sunday, December 1, 2019, 18).

1.4 A Problem is Observed

Those inland valleys were perfect for growing a wide variety of vegetable and fruit crops: such as leafy spinach and lettuce, citrus fruit, and grapes. That agricultural production leads to the observation that leafy crops seem to suffer pathological changes – necrotic and chlorotic patches – and a collapse of the mesophyll regions, its air spaces filled with water (areas of water-logging). In the 1920s, UCLA was formed as the second campus of the land-grant college in California (Berkeley was the first in 1868). However, UCLA required a site for studies of how crops grew in warmer inland valleys along the coast. The UC Agricultural Research Center at Riverside was established, where the visible injury to plants was first described by John T. Middleton (1956). That Center later became the University of California at Riverside, where further research (carried out in the Statewide Air Pollution Research Center) was summarized in the Annual Review of Plant Physiology (Middleton 1961).

On the other hand, Prof. Haagen-Smit from Caltech (California Institute of Technology) was able to demonstrate that the cause of these injuries was O_3 contained in smog (Haagen-Smit et al.1952), and was the first to link the symptoms of injury to a photochemical process and the gas, O_3. After a few hours of fumigation, the development of damage symptoms was similar to that noticed on plants exposed to smog. In these experiments, the O_3 concentration was adjusted to 0.2 ppm, which corresponds to rubber cracking during severe smog conditions. However, this successful experiment, creating a synthetic smog, was incomplete since only O_3 was used as the pollutant. Knowing that the organic compounds could be oxidized by air in the presence of light and nitrogen dioxide, Haagen-Smit developed experiments with unsaturated hydrocarbons, nitrogen dioxide, and sunlight (Table 1.1).

The fundamental reaction of oxidant smog is between molecular oxygen in the atmosphere and the oxide of nitrogen (NO_2) released by the combustion of gasoline, as given below:

$$NO_2 + O_2 + h\nu \rightarrow NO + O_3 \qquad [1\text{-}1]$$

The presence of NO_2 in the atmosphere is easily seen as a brown tinge, especially at sunset. This equilibrium reaction is why much of the early pollution control efforts focused upon eliminating the oxides of nitrogen (from the fuel before combustion and in the tailpipe by catalytic converters).

By submitting the plants to a mixture of unsaturated hydrocarbons and NO_2 with sunlight, Haagen-Smit got symptoms of injury similar to those produced by “Los Angeles smog” (Table 1.1). By contrast, no effect was observed by using SO_2 in combination with hydrocarbons under sunlight (Table 1.1). Haagen-Smit finally concluded that the photochemical dissociation of nitrogen oxides, forming atomic oxygen and O_3, would be, in the presence of organic material such as hydrocarbons, responsible for plants’ visible symptom damage. All these investigations, published in two landmark papers (Haagen-Smit 1952, Haagen-Smit et al. 1952) led to the conclusion that the major source of the typical smog, responsible for damages to plants and human health, is the release into the atmosphere of hydrocarbons and nitrogen oxides from car exhausts and industrial fuel combustion producing the photochemical smog with O_3 as a secondary pollutant.

> *In the last decade, a new group of air-borne phytotoxicants was described and identified as smog components. Since ‘smog’ refers to smoke and fog, neither of which are responsible for vegetation damage, the polluted air mass containing the damaging incitants is herein called ‘photochemical’ or ‘community’ air pollution. The toxic components in community air pollution are typically the oxidation products of hydrocarbons and result either from the dark reaction of* O_3 *and olefins or the photolytic reaction of nitrogen oxides and hydrocarbons in the presence of sunlight. (Middleton 1961, 431).*

Later, chemists found that the atmosphere is complex, and once combustion products of gasoline are released into it, that system generates many, many more organic compounds. Some of the compounds involving elements other than just H and C are various oxides (O_3 and peroxides), oxides of nitrogen (such as peroxyacetyl-nitrate), and, if present, oxides of sulfur. Some of these multiple reactions have been documented by Atkinson’s group at the Statewide Air Pollution Center, UCR (see Atkinson 1990, Atkinson and Aschmann 1993, Atkinson 2000, Aschmann et al. 2002).

Table 1-1. Effect of fumigation with hydrocarbon, ozone, NO_2, SO_2, alone and in combination, without and with sunlight on plants (adapted from Haagen-Smit et al., 1952; Table I, 27 and Table IV, 31).

Mode of fumigation	Injury of leaves				
	Spinach	Endive	Beets	Oats	Alfalfa
Hydrocarbon 8.5 ppm x 5h	0	0	0	0	0
Ozone 0.2 ppm x 5h	A	A	0	0	A
Hydrocarbon 3.4 ppm + Peroxide 0.28 ppm + Ozone 0.2 ppm x 5h	T	T	T	T	T
Hydrocarbon 4 ppm + NO_2 4 ppm x 5h	0	0	0	0	0
NO_2 0.4 ppm + Sunlight	0	0	0	0	0
Hydrocarbon 4 ppm + NO_2 0.4 ppm + Sunlight x 2h	t	T	T	T	T
Hydrocarbon 4 ppm + SO_2 0.1 ppm + Sunlight x 5h	0	0	0	0	0

0: no injury; A: atypical damage; T: typical smog damage; t: less severe typical smog damage

1.5 Research in other institutions

In the 1950s the main location of auto exhaust emission was near downtown Los Angeles, so the concentration of O_3 was greatest there in the morning (with the highest amounts observed in the late morning). As the on-shore breezes increased during the afternoon, the polluted atmosphere blew into Riverside such that high peaks were observed at 3.00–4.00 PM (some as high as 0.4ppm in 1970).

Mark Dugger, C. Ray Thompson, O. Cliff Taylor, Irwin Ting, William Thomson, Brian Mudd, and Lawrence Ordin at UCR (the University of California at Riverside) began a series of research endeavors which tried to determine what was happening within the plant to cause such damage. Dugger and Ting (1970) wrote an Annual Review of Plant Physiology article, which shifted the discussion from how individual plants showed the visible effects of the oxidants to what physiological processes were involved. One of the early discoveries was that the stomata controlled much of the injury, see Fig.1.3. As the conductance increased in the light (from 0.067 initially to 0.20 cm/sec), the visible injury increased from near zero to 25%. Of course, this was argued by others as not very absolute in terms of cause and effect, but gradually the role of the stomata was proven to be significant (Musselman et al. 2006; Grulke et al. 2007).

Initially, the plants used were of obvious agricultural importance: the visible injury that was produced lowered their economic value and production. After many observations, it was equally clear that plants in an ecological setting were likewise being altered (observed in the mountains surrounding the Los Angeles basin) (see Bytnerowicz et al. 2008; Sandermann et al. 1997). This alteration was often to plants that had a low economic value and were thus unimportant to political consideration. Later, the alterations were shown to weaken the plant, such that other diseases or insect pests could kill it and thus lower its relation to different ecological parameters leading to a collapse of the full system. All countries realized that urban air, by moving into important ecological areas for tourists and harvesting trees, needs detailed studies.

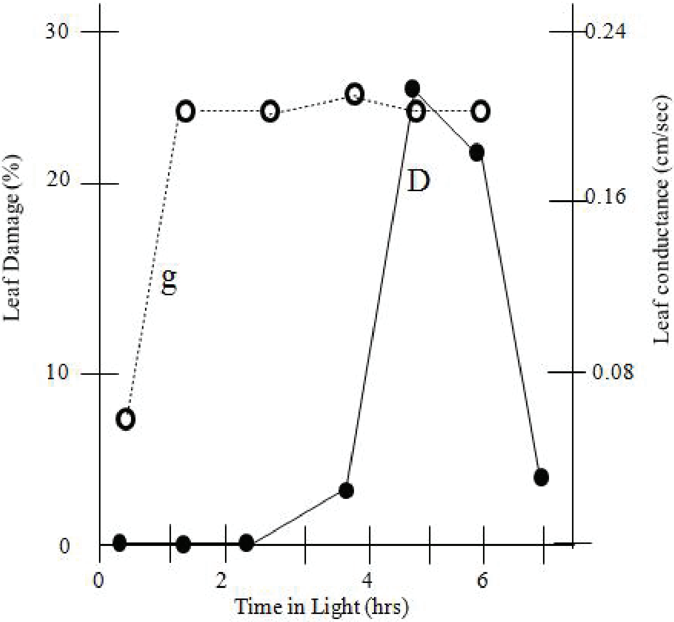

Fig. 3. Leaf damage from O_3 as a function of time in light (6×10^4 ergs/cm^2- sec). Three-week-old cotton plants were treated with 0.75 ppm O_3 for 1 hr at the time indicated. Plants were in the dark for 12 hrs before the light period. The authors used leaf resistance, determined with a resistance hygrometer as a measure of stomata opening, before O_3 treatment, but here that has been converted to conductance, the inverse of resistance, and today's standard. Redrawn from Dugger and Ting, 1970, Fig. 3

Other research groups in the 1960s were those found at North Carolina State University, which included Walter Heck, Arthur Heagle, and Howard E. Heggestad at the US Department of Agriculture at Raleigh along with Ellen Brennan and Eva Pell at Raleigh University (Pell later went to Penn State to join John M. Skelly). A strong association was formed at the University of Minnesota, which was dominant in the editorship of the journal *Environmental Pollution*, by William J. Manning and Sager V. Krupa. Later, the formation of an Environmental Protection Agency Center at Corvallis, Oregon, generated a large group of scientists, which included David T. Tingey, William E. Hogsett, and David M. Olszyk (who was previously at UCR's Statewide Air Pollution Center), who cooperated with many others across the US.

At this time, ecologists were beginning to see injury patterns suggesting O_3 injury within the forest near the LA basin and far removed from cities, such as the Sierra Nevada Mountain Range of California. The visual patterns were needle bleaching or mottling, leading to needle loss, summarized in Sandermann et al. (1997) and Bytnerowicz et al. (2008). A group formed at the Southwest Regional Headquarters of the Forestry Service in Riverside, called the Fire Lab since they were working on forest fire prevention in the local mountains, was led by Paul Miller, Andrej Bytnerowicz, and Nancy Grulke.

Also in the USA, the idea of an Air Pollution Workshop was conceived in mid-1968 at the meetings of the American Phytopathological Society at Columbus, Ohio, by Norman Lacasse, Richard Reinert, William Feder, and Gabriel Seldman. This workshop would consist of an informal meeting ground for those researching air pollution and plants[5]. Of especial note was the primary purpose of communicating research among the young scientists new to air pollution research and the older scientists who had been studying air pollution and vegetation effects since the mid-1950s. An air pollution workshop's plan was a need for "*greater communication among scientists involved in air pollution research in agriculture in the United States and Canada*." The workshop's purpose was to "*bring together all interested persons involved in air pollution research related to agriculture for an informal exchange of ideas and information*." A tentative agenda was suggested with two time periods devoted to the intensive discussion of 5 topics. The first air pollution workshop was held on March 17-19, 1969, at the Nittany Lion Inn, Pennsylvania State University, College Park, PA, and run by Norman Lacasse. Drs. Michael

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[5] From a note written by Richard Reinert and passed out at the 1997 Air Pollution Workshop.

Treshow and Clyde Hill ran the second workshop at the University of Utah in Salt Lake City in March 1970. A newly formed Steering Committee met on May 14, 1970, following the 2nd annual workshop and decided that the 3rd and 4th Annual Air Pollution Workshops would be held at Riverside, California (west) and Raleigh, North Carolina (east); the concept of rotating meeting sites from west to east was then formed. This workshop proved to be very effective in research communication but was lost in about 2016 due to funding problems and a lack of active researchers.

1.6 Europe: Reaction to the Problem

In southern Germany in the early 1980s, especially in the Black Forest, symptoms of forest decline (Waldsterben) were observed on silver fir (Abies alba Mill.) and Norway spruce (*Picea abies* Karst.) (Schütt and Cowling 1985, Krause et al. 1986). Scots pine (*Pinus sylvestris*) and deciduous trees such as beech (*Fagus sylvatica*) and oak (*Quercus robur*, *Q. petraea*) were also affected (Schütt and Cowling 1985, Krause et al. 1986). Symptoms of tree decline were also observed in different countries of western and central Europe. In France, as early as 1983, severely defoliated coniferous stands were identified in the Vosges Mountains (Landmann and Bonneau 1995). However, extensive dieback of forests throughout western and central Europe did not occur, which led the German scientists to introduce the concept of "novel forest decline" (*neuartige waldschäden*, Krause et al. 1986, Matyssek et al. 1997). Forest status was thus based on crown transparency, linked to leaf loss and foliage yellowing. Experiments were carried out on slightly damaged mature Norway spruce trees with yellowing needles in France and Germany in a collaboration between French and German scientists (Arndt et al. 1993). In parallel, an attempt was made to differentiate the factors linked to this novel forest decline from the known factors, usually causing tree declines such as climatic and biotic constraints and acidic smoke injury observed since the beginning of the industrial revolution (Krause et al. 1986).

It appeared there was not one unique cause of this novel forest decline in Europe (Schütt and Cowling 1985; Landmann 1995). In the mountainous regions, forest trees generally grew on superficial, rocky soils with a poor water reserve and possible nutritional deficiencies. Furthermore, the plantings were often very dense. These predisposing factors would allow climate events, such as drought episodes and air pollution, to contribute to the observed forest damages (Landmann and Bonneau 1995). Also, high amounts of SO_2 in the atmosphere and acid rains linked to SO_2 and NO_2 were related to tree damages (Ulrich 1984, Krause 1988, Darrall 1989). A similar effect was observed in agriculture and forestry (Roberts 1984).

The SO_2 atmospheric levels were high in the period 1960–1980, especially in central and eastern Europe, with a clear relationship between the industrial source's proximity and the observed damages on plants. Since the mid-1980s, a decrease in SO_2 pollution has occurred due to the reduction in emissions. In the Vosges Mountains, SO_2 reached up to 100 ppb in the winters of 1986 and 1987, but since then, the atmospheric concentration of SO_2 has drastically decreased (Fig. 1.4).

By contrast, the O_3 concentration was high every year during the spring-summer period (Fig. 1.4), being at least in part responsible for the symptoms of damage observed on trees mainly at relatively high altitudes. As early as 1975, it was suggested that photochemical O_3 might be transported in continental Europe, away from its region of production, into isolated areas where plants may suffer the effects of this pollution (Cox et al. 1975). A consensus thus emerged in the scientific community between 1985 and 1995: damage could result from a range of predisposing stress-inducing factors followed by secondary abiotic and biotic factors. Several severe climatic episodes (as drought stress in 1976, severe cold periods) would have contributed to weakening trees planted too densely on poor soils. Air pollution was then responsible for foliar damage to these trees. Acidic deposition, mainly observed in the eastern countries near industrial settlements, was thus incriminated with SO_2 as the main responsible pollutant. O_3, transported by winds and present at high altitude, was later named as a secondary causal factor that aggravated the situation and allowed insects’ and pathogens' attacks on weakened trees (Landmann and Bonneau 1995).

The abundant alarmist comments in the press about the visibly diseased forests in Germany and France contributed to the formation of national research programs on this problem, such as in Germany (PEF, Projekt Europaisches Forschungzentrum für Massnahmen zur Luftreinhaltung 1984–1998) and in France (DEFORPA, Dépérissement des Forêts et Pollution Atmosphérique 1984–1991). These programs were devoted to the study of natural and anthropogenic factors which were capable of causing forest decline. Fruitful cooperation first developed between the two countries, followed by European programs (EUROSILVA, 1987–1994). As mentioned above, serious damages to coniferous trees were also observed in the western USA at the end of the 1950s, which led, as early as 1983, to a German-USA scientific exchange on forest decline, sponsored by the Bundesministerium für Forschung und Technologie in West Germany and the Environmental Protection Agency in the USA. This program allowed a comparison between the symptoms analyzed in the two continents. The European Union has always supported the study of air pollution and plants over the last 30 years by directly funding research programs and allowing exchanges between researchers through European Cooperation in Science and Technology (COST) programs. These COST programs, among them "ICAT, impacts of elevated CO_2 levels, climate change and air pollutants on tree physiology (1991–1997)" and "Climate change and forest mitigation and adaptation in a polluted environment (2009–2013)", largely contributed to the improvement of knowledge through facilitated scientific cooperation.

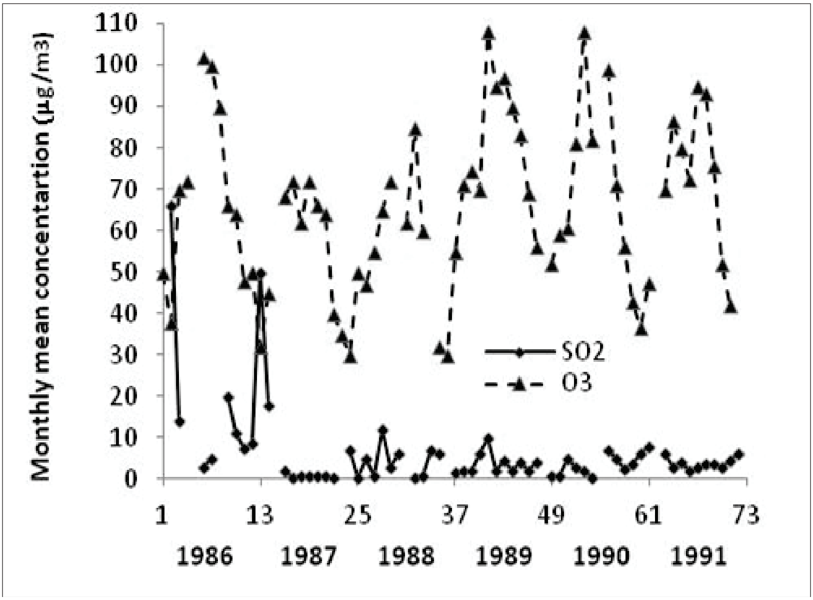

Fig. 1.4. Monthly mean concentrations of SO_2 and O_3 in the Vosges mountains at the Donon pass (700 m asl).
(After data from the Association pour la surveillance et l'étude de la pollution atmosphérique, Alsace, France)

1.7 Ozone concentration and symptoms of injury

The symptoms of damage caused by smog in the Los Angeles basin, thereafter attributed to photooxidants and O_3, were first observed by Middleton et al. (1950). They were characterized by silvering, bronzing, and necrosis, principally on the lower leaf surfaces of crops and weeds.

The conifers showed the yellow chlorotic mottling of needles (Parmeter et al. 1962). These visible damage symptoms are typically linked to high concentrations of O_3, causing acute injury and leading to cell and tissue death. Lower concentrations of O_3 delivered during weeks cause chronic injury, characterized by the reduction of growth, often in the absence of visible symptoms. This hidden injury may occur by changes in carbon metabolism (assimilation and catabolism) at the enzyme level, allowing the cell to accommodate the oxidative stress (see paragraph 1.15.4). If the O_3 exposure persists, the cell will not cope with the negative impact of the oxidative stress, which will ultimately lead to cell and plant death, as recently reviewed (Vollenweider et al. 2019).

Acute O_3 exposure can be considered as a short duration exposure of 200 ppb to 2 ppm O_3 from 1 hour to 3 days. In contrast, chronic exposure applies to more realistic long-term exposures (weeks or months) to lower O_3 concentrations of 50 to 150 ppb (Renaut et al. 2009). The effects are not linear in that 0.4 ppm of O_3 for 1 hour does not resemble 0.1 ppm of O_3 for 4 hours. Generally, the lower the dose, the much fewer are the symptoms, while higher levels will damage the leaf (Fig. 1.5C). Also, much of the visible injury pattern is a pattern across the leaf where some regions (both small and large) look normal, while others are chlorotic or necrotic. A larger selection of photos of vegetation is in Jacobson and Hill (1970).

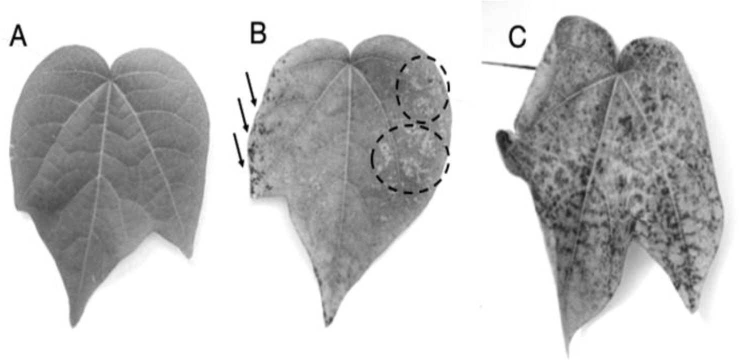

Fig. 1.5. Visible Injury Patterns on the Leaves of Cotton Plants. Cotton seeds were germinated and allowed to grow in a greenhouse for 3 weeks until the first true leaf was well developed. The plants were then exposed in a closed chamber (similar to Fig. 1.9) in a greenhouse to 3.0 ppm O_3 for 20 minutes near noon, removed, and returned to the greenhouse for an additional day. The left photo (A) is the control without O_3 exposure, and the others (B and C) are randomly chosen leaves. An electronic camera took the pictures with the leaf held over a white sheet of paper in the greenhouse. Solid ellipses represent chlorotic regions, while arrows represent necrotic regions – unpublished data from David Grantz and Robert Heath

The variation in visible injury is easily seen in the first true leaf of a cotton plant exposed to high O_3 levels for a short period in the middle of the day (see Fig. 1.5B). A typical plant's uniformity is not seen, but relatively small necrosis areas generally appear near the margin. As the level or duration of O_3 exposure increases, the size of the necrosis increases until it covers nearly all the leaf; yet the veins seem to be more resistant to atmospheric O_3, presumably because of a lowered density of stomata. Many leaves exhibit chlorosis, again between the veins but with very little uniformity. With such variation, visible injury is usually given to estimate the area in which either chlorosis or necrosis occurs. Again, the density and aperture of the stomata seemed to play a part, and it has been noted that not all stomata behave in the same manner across a leaf (Cheeseman1991; Mott and Buckley 2000).

Early on, three varied symptoms were described by researchers: 1) visible injury, 2) loss of productivity, and 3) invisible injury. As described above, the visible injury was necrosis, chlorosis, or waterlogging that could be seen but was generally erratic across the leaf's surface. Loss of productivity was a long-term result that required producing a "product" of the crop, e.g., seed production. The invisible injury was defined as no apparent visible injury but an alteration of the plant's "normal" growth pattern. One prominent "alteration" was an increased pathogen attack (Conklin and Barth 2004).

Plants' visible injury was not simple: there was a wide range of apparent patterns, and the leaf surface was not uniformly damaged. How could that be? Bobrov (1952, 1955), using cytological microscopy, did extensive studies, summarized by Middleton (1961).

> *Her [Bobrov] elegant studies of living tissue have shown that the first response of cells is their engorgement, especially of the guard cells and surrounding epidermal cells. As the epidermal cells become stretched, unusually through the guard cell's distention, they frequently rupture and collapse. This observation accounts for the change from the epidermis's blister-like appearance to that of the water-soaked appearance in which the plasmolysis of cells in the spongy parenchyma occurs. The silvering and glazing of the leaf surface are attributed to the dehydration and shrinkage of many of the mesophyll cells through the formation of enlarged, air-filled, intercellular spaces.*

It was recognized that these injury patterns were linked to the stomata and to how the oxidants entered the leaf through the stomata or the cuticle:

> *...exposed to low concentrations of oxidant for short periods, they frequently appear chlorotic rather than expressing the typical glazed lower*

> *surface. Bobrov demonstrated that this yellowing is due to the plasmolysis of a limited number of cells, such that several chloroplasts are destroyed while adjacent cells may remain intact.*

The injury patterns were not just the apparent visual change but also a mechanism deeper within and linked to the plant. In the 1950s, plant physiology and biochemistry were beginning to expand; yet both fields had a long way to go.

> *Tomato plants that were given limited water supply resisted injury compared to those receiving abundant water. They also demonstrated that transpiration and water uptake rates of tomato plants were lowered by exposure to oxidant. (Hull et al. 1954)*

The full plant was involved as it was not just the leaf appearance that was affected but also the fruit both by productivity and early senescence.

> *As the exposure of lemons to both reaction products and ambient air containing oxidants was extended, there was premature senescence and drop off the older lemon leaves. (Taylor 1958)*

We can now see how those symptoms may be related, but not in a linear relation. Visible injury lowers the photosynthetic productivity of the leaf by a loss of productive area. The area of damage is walled off and so does not spread into the leaf's remainder, but less area means less productivity. Loss of productivity is a full-plant response that suggests a loss of photosynthetic productivity and a loss of translocation to the plant's productive part, e.g., seed production within the reproductive organs. It may also suggest a poor functional ability to move nutrients from the soil to the organ that requires them and a transfer of energy and carbon from one use to another, e.g., a wounding or pathogen response.

There is another more general response that is more global: forest decline. Operationally it is a visual response inventory – chlorosis of needles with a loss of needles (or rather the lack of foliar retention) and a change of morphology of the structure of the collection of needles (the whorls) (see Chapter 11 in Sandermann, Wellburn and Heath 1997, Grulke and Heath 2020).

Too often, we do not see that these different responses are interlinked. The study of one response cannot easily lead to or predict the function of another. For detailed studies, one must dose the leaf with a known amount of O_3 flowing about the leaf, find what regions are affected, perhaps by chlorophyll fluorescence, and then study physiological changes across the leaf within small sections of that leaf.

1.8 Oxidation as part of life

Chemical reactions often occur due to the movement of electrons from one atom (the reductant) in a compound to another atom (the oxidant) in another compound, called oxidation. Generally, this involves a flow of energy. All of life requires energy movement to convert one type of compound into another, generally a varied carbon state. Early life (4-3 BYA) in the Earth's seas used existing reduced compounds such as H_2S or ferrous compounds for electrons to reduce carbon. Early life seemed to do quite well without oxygen as it is a powerful oxidant – it "steals" electrons from many compounds. When photosynthesis using sunlight arose, it formed oxygen as a waste product and forced all life forms to develop techniques to contain/control their own oxidative status. Evolution found that oxygen was good and evil. It was a marvelous sink for electrons coming from other nutrients, allowing a secondary energy capture but ruthlessly attacked other unrelated molecules. Not surprisingly, these mechanisms used to contain unwanted oxidations are often crucial in controlling foreign oxidants, such as O_3.

> *Plants ... have a love/hate relationship with light. As oxygenic photoautotrophic organisms, they require light for life; however, too much light can lead to increased production of damaging reactive oxygen species as byproducts of photosynthesis. In extreme cases, photooxidative damage can cause pigment bleaching and death… (Müller et al 2001)*

1.8.1 Photosynthesis

Once early life learned how to break down water into oxygen and "reduced hydrogen" using sun power, an infinite source of reducing power became available. Photon capture using chlorophyll set up as an energy source that can remove electrons from water, thus generating oxygen and allowing CO_2 to be reduced to carbohydrates (and many other compounds). Two systems of photosynthesis are linked: the light reactions (in which light is captured and used to generate ATP and NADPH and releases oxygen) and the dark reactions (or Calvin Cycle Reactions in which ATP and NADPH are used to convert CO_2 to a simple carbohydrate) (Fig. 1.6). The light reactions are the most dangerous because of the many reduction/oxidation units within them. Furthermore, this is within a membrane system that can be damaged by oxidants. Photosystem II within the light reactions is the most oxidizing unit because it must split water into O_2 and active H. For this, many oxidation controls exist, such as glutathione, ascorbate, tocopherol, and superoxide dismutase.

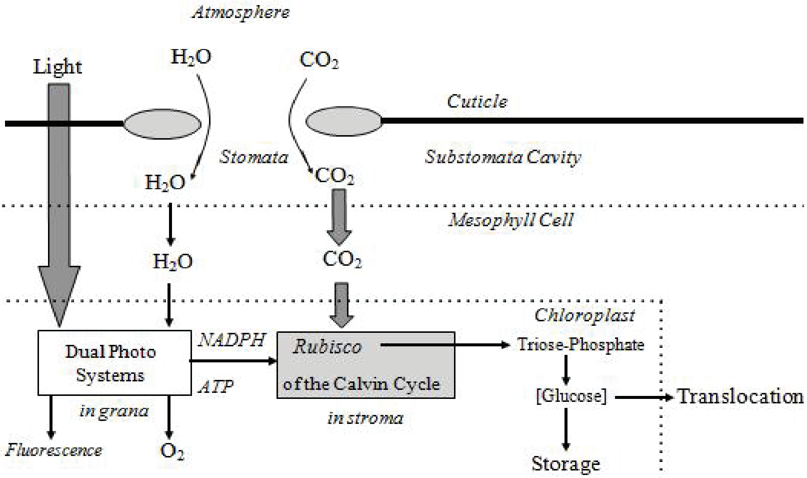

Fig.1.6. Basic processes of photosynthesis in a leaf diagram. The photosynthetic apparatus is contained within the chloroplast from which sugar is transported out to be used in other portions of the cell. The leaf structure (shown upside down with the stomata on the top) allows gases to flow into the leaf. The varied processes are discussed in detail below

The light and gases from outside the leaf must penetrate the leaf's cuticle and epidermis to reach the region of the mesophyll cells. The photosynthetic processes occur within the chloroplast (denoted as the gas exchange). The size of stomata will generally limit all gas exchange as the cuticle represents a near-zero gas exchange capacity. Similarly, the amount of light entering the leaf will be governed by the cuticle via some reflection (ca. 3–6%). Typically, the concentration of gases within the leaf is different from those outside – water vapor is higher due to the movement of water from the cell to the substomatal cavity and then out to a drier atmosphere, while CO_2 will be lower within the substomatal cavity due to CO_2 capture by photosynthesis. O_3 will also move along the gas exchange pathway.

All three environmental units (light and gases) must then pass through the plasma wall and membrane of the mesophyll cell and the chloroplast membrane before photosynthetic reactions occur. Once the light reactions and dark reactions produce a triose-phosphate molecule, that molecule can be converted into many other carbohydrates, which can be used for starch storage and sucrose transport out of the chloroplast and into a vascular system (translocation). The sugar product, indicated as [glucose] in the figure, can be [1] transformed into other carbohydrates, [2] converted into amino acids with nitrogen units, [3] stored within the chloroplast as starch, and [4] moved out of the leaf as sucrose by translocation to other portions of the plant. Again, these varied pathways are highly regulated, and any disruption, such as by oxidants, alters the normal metabolic changes of carbohydrates. O_3 can react with any of these biological processes.

The dark reactions center on the enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), which "fixes CO_2" (Fig. 1.6). Rubisco is highly regulated by the energy level of the chloroplast and by the flow of various sugars. It was found that Rubisco was a critical part of how O_3 altered plant productivity (see later). Rubisco is a key to produce the triose-phosphate, which is the beginning of carbohydrate metabolism.

Two major processes of the gas flow in green plant photosynthesis occur with the movement of CO_2 into the leaf through the stomata in the cuticle's outer layer (see the top of Fig. 1.6) and the actual reduction of CO_2 to carbohydrate as in the Calvin Cycle, above. Oxidants can interfere with the two processes in entirely different ways, and therefore observed effects upon photosynthesis depend upon each flow system separately.

1.8.2 Production and reactions of O_3

Experimentally, O_3 can be produced by either a reaction of oxygen with ultraviolet radiation or a high voltage spark discharge. Ozone thus produced is in high concentration within the gas stream and must be diluted for use. However, O_3 is highly reactive and must be transported within stainless steel or Teflon containers and tubes. Commercially, high concentrations of O_3 are used to sterilize solutions of water in water purification treatments (Hoigre and Bader 1978), and thus many reactions of compounds with water have been well studied (Bailey 1958, 1982). On the other hand, the production of O_3 within atmospheric regions depends on what is injected into the atmosphere by partially combusted gasoline and the atmosphere's conditions, including humidity (Stone et al. 2012).

The most common unit used in the current literature is parts per million (ppm, vol/vol) or parts per billion (ppb). Ppm and ppb are equivalent, respectively, to μLL^{-1} and nLL^{-1}. Calculations to convert to other units such as micrograms per cubic meter ($\mu g.m^{-3}$) of air rely upon the pollutants' molecular weight and the perfect gas constant (at 0°C, 22.4 liters/mole). At a temperature of 20°C and pressure of 1013 kPa, an O_3 concentration of 1 nLL^{-1} or 1 ppb is equivalent to 2 $\mu g.m^{-3}$.

The chemistry that O_3 causes as it interacts with plants has been challenging to measure for two significant reasons: [1] O_3 is highly reactive with many compounds, especially in a water milieu and the compounds thus produced (typically H_2O_2 and $HO^{\cdot}$) are likewise short-lived and highly reactive (see below); and [2] Ozone is three atoms of oxygen, and oxygen does not have an easily obtained radioactive isotope, which can be followed.[6]

Using chemical data from air reactions and water-solvent reactions of O_3 with varied purified compounds (Alder and Hill 1950, Atkinson 1990, Atkinson and Aschmann 1993, Atkinson et al. 1992, 1994, Stone et al. 2012), studies have made predictions of how O_3 would react with biochemicals on the surface of and within plants (see Heath 1979, 1987b, 1996b, 1996c, 1999). In general, an oxygen atom or hydroxyl group is transferred to the biochemical with several free radicals produced transiently. Stone et al. (2012) indicate many of the simpler types of reactions revolving around hydroxyl radical and excited O_2 states; however, many of these compounds are very transient but reactive, so multiple pathways often exist.

To date, there have been few biochemical studies to find which of the various postulated compounds are present in plants after O_3 fumigation. Runeckles and Vaartnou (1997) attempted to measure free radicals by electron spin resonance. They were frustrated by the high concentrations of free radicals normally produced by photosynthetic processes. No real conclusion was reached about the subtractive spectra.[7]

1.9 Exposure Indices

While visible injury shows damage to the plant, the question rapidly became, how much O_3 in the air does not lead to injury, visible or otherwise? Some argued that any amount is toxic, yet background O_3 in the Antarctic is about 0.02 ppm, indicating that level is the background level for which plants have compensated. On the other hand, one should assume that the amount entering the plant through the stomata is the dangerous concentration – the level due to flow into the cellular system that could not be detoxified (see Dugger and Ting 1970, Heath 1980). Typically, the relationship would be given as:

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[6] $O^{16}$is a stable isotope, but other stable isotopes (O^{17} and O^{18}) are present in small concentrations; all can be followed by mass spectrometry.

[7] Subtractive spectra produced by subtraction of the spectra observed with O_3 exposure from the control spectra (that without O_3).

$\text{Injury} = g\,[O_3]_{\text{external}}$ [1-2]

where g is the stomata conductance (in units of m sec^{-1}). Here the amount of injury would be given as mole m^{-2} sec^{-1} since the concentration would be in mole m^{-3}. The level of injury (e.g.,% of visible injury) would have to be related to the amount of O_3 (moles) that caused that injury. The one-to-one relationship of this injury pattern has been difficult to determine.

However, the above equation means that injury would increase linearly with a constant conductance. Many studies have shown that any measure's injury does not increase linearly (see Fig. 1.3 and Heagle et al. 1988). Yet this was not the only point of controversy; some believed there might be a threshold of O_3 to induce injury ($[O_3]^T$). This threshold may be the plant's ability to detoxify any entering O_3 up to a certain level.

$([O_3]^T < \text{no Injury})$ [1-3]

This could then mean that it is a linear development of injury to the amount of O_3 above the threshold ($[O_3]^T$). Here the injury is linearly related to the concentration above the threshold.

$\text{Injury} = A\ \{\ [O_3] - [O_3]^T\ \}$ [1-4]

On the other hand, there could be a more complex power law (e.g., n) response to injury, such as:

$\text{Injury} = A\ \{\ [O_3] - [O_3]^T\ \}^n$ [1-5]

Once this type of query was started, the number of possible mathematic responses seemed to spread, so almost any response relation was explored (see later).

This is not just an academic discussion. Politically, if a group wishes to control the level of O_3, one must explain why a certain level was chosen for a control point. Is there a real threshold below which one does not have to be concerned about any injury or damage? If it goes above that threshold, does the injury become greater, faster, or more extreme, or does the actual injury slow its further production rate? Two other concepts give problems in such discussions. One is the idea of an "invisible injury" in which no visible injury is observed, but the plant does not thrive, and its net production can be reduced. Production takes a long time to measure, as one must go through a growing season while a typically visible injury develops in a few days. The second one concerns an accumulated dose being the problem; damage or a loss of productivity will gradually develop with the total dose (amount per unit time entering the plant *X,* the plant's total time in that particular atmosphere). This accumulated dose must be subjected to the same type of arguments as above. How do you calculate the dose which is being accumulated, and can the plant reverse the injury if the accumulated dose is small? There have been many arguments along this line, and various countries have argued for and against most mathematical models.

1.10 Atmospheric concentrations: now and models

A rapid increase in O_3 concentration was observed across western Europe using measurements from several locations, beginning with the Pic du Midi in the 1870s followed by several high altitude sites in Switzerland, Germany, and France from the 1930s through to the early 1990s (Marenco et al. 1994). This increase was by a factor of 5 between the late 1800s and the early 1990s and by a factor of 2 between the 1950s and the early 1990s. Similar conclusions were drawn from additional data sets from the 1930–1950 period in central Europe (Staehelin et al. 1994). Routine monitoring of the composition of atmospheres developed in the 1980s at rural and remote locations for detecting long-term changes throughout the USA (Lefohn et al. 2010; Cooper et al. 2012). For a complete picture of how O_3 levels have changed within the Los Angeles basin and in the mountains surrounding it, see Lee et al. (2003). More recently, a study of a time series from 20–40 last years showed a leveling off or a decline from earlier O_3 increases in the mid-latitudes of the northern hemisphere (Oltmans et al. 2013). A complete overview of the global distribution and trends of tropospheric O_3 confirmed this plateau in the northern hemisphere (Cooper et al. 2014; Fig. 1.7).

In the late 1950s to early 1960s, the yearly average O_3 concentrations in Europe were in the 15-20 ppb range, which almost doubled at the beginning of the 20th century (Vingarzan 2004), but no positive trend has been observed since then (Fig. 1.7). This stabilization in annual mean surface O_3 concentrations in Europe and North America is predicted up to 2050 while a continued increase would be observed in South and East Asia (Cho et al. 2011, Lei et al. 2012, Li et al. 2019, Ainsworth et al. 2020).

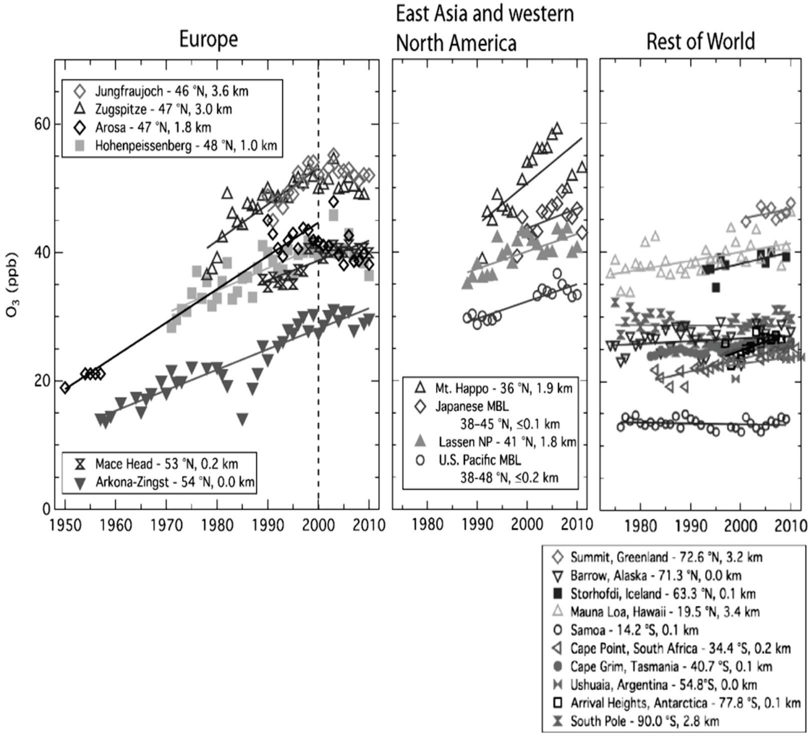

Fig. 1.7. Surface O_3 time series at several rural sites around the world. Trend lines fit through the yearly average O_3 values using the linear least-square regression method (modified by Cooper et al. 2014, Figs.1, 5, from the original that appeared in **IPCC 2013**

1.11 Exposure Methodology

Early studies were done in separate fields with different atmospheric levels of O_3, which were agronomically close (Musselman and Hale 1997). Clearly, these initial studies were useful but difficult to compare. Plants subjected to different water potentials and nutrients in the soil could not be easily compared to productivity. Naturally, this method has fallen out of use, except for ecological studies. Large trees in the wilderness cannot be moved or covered with atmospheric chambers, so the field and atmospheric conditions must be similar but taken into account.

Currently, we know more about how conditions affect plant growth and, therefore, can compensate for field variation (this is now what is referred to as a "big data" investigation). Furthermore, as we understand how O_3 interacts with plants and how to model exposure indices, varied fields of plants can be more easily compared.

1.11.1 Open top chambers

Studies of varied fields did not lend themselves to a complete understanding of the injury patterns, and therefore, predictions of which injury would occur could not be easily accomplished. Plants have to be exposed to O_3 in the same field conditions as a control. A leap in experimental evidence of O_3-derived injury patterns was made with a better fumigation system, an exposure system that enveloped the crops – one chamber had added O_3, and another had an unchanged atmosphere. In the 1970s, open-top chambers (OTC) were standardized to study the dose-response effects on productivity and physiological parameters (Heagle et al. 1973; Musselman and Hale 1997). Plants in OTCs were fumigated with various concentrations of O_3, and charcoal-filtered chambers served as a control. The "Open Top Chambers" were used routinely thereafter with many small modifications (e.g., a rain shield over the gases' exit at the top). Figure 1.8 shows a diagram of such a chamber. The steel structure of the chamber was lined inside with transparent Teflon sheets.

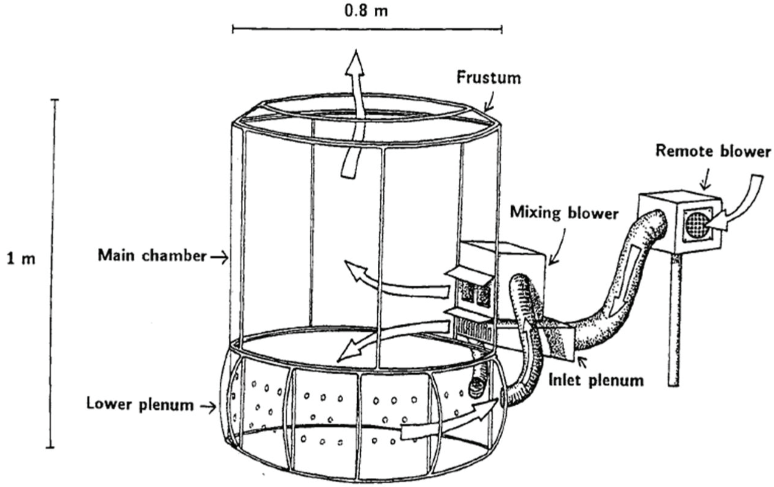

Fig. 1.8. Diagram of an Open Top Chamber detailing the flow of air. Air is drawn into the remote blower and blown through the inlet plenum into the chamber. The mixing blower draws air from inside the chamber through the lower plenum's perforated inner wall and blows it back into the chamber. Air exits the chamber through the frustum (Drake et al. 1989).

Ozone does not react with stainless steel metal, glass, or Teflon: but it does react with most other plastics and metals. Yet every surface produces some breakdown of O_3, and so any chamber must be monitored extensively as to the actual O_3 concentration within. Ozone is introduced at a high concentration into a wind generated by a large fan leading into the chamber with the airflow exiting through the top opening. A door is provided so the researcher can enter to take samples. In these studies, an unenclosed area of plants is used to show if the enclosures alter plants' growth. The unenclosed plants are generally found to be different from the control enclosed plants. The enclosure does alter the light quantity and quality and changes the atmosphere itself due to wind and evaporation from the soil. Such a field of OTC is shown in Figure 1.9.

Fig.1.9. Loblolly pine saplings growing in open-top chambers (with Rain Shields at the top) treated with charcoal-filtered air, non-filtered air, and 2.0 x ambient O_3 concentration (Photo taken by Pierre Dizengremel, 1990 at Duke Forest site, Durham NC)

> *The typical experiment was performed in Duke Forest, Durham, North Carolina, USA. Loblolly pine seedlings were transplanted to the field plots in June 1987. Open-top polyvinyl field chambers, 5 m in diameter and 5 m tall with rain exclusion caps were erected in March 1988. The trees were thinned to 8 per chamber in 1989 and to 4 per chamber in 1990. The overall experiment consisted of 54 chambers arranged in 3 blocks with charcoal-filtered air (CF), non-filtered air (NF), or 2.0 x ambient* O_3

> *concentration. Charcoal-filtering removed approximately 80% of ambient O_3, while NF chambers tracked 5 to 8% below ambient O_3 concentrations. The 12-h daily mean O_3 concentrations were 8 ppb in CF chambers, 45 ppb in NF chambers, and 86 ppb in 2.0 x ambient O_3 chambers. The trees were fumigated from 08:00 to 20:00h (EST), from March/April to mid-November each year. As saplings, loblolly pines produce three to five flushes of growth per year. The cumulative O_3 exposures received by each tree's flushes were calculated. The cumulative O_3 dose is the summation of l-hr mean concentrations for each of the 12 exposure hours each day during the exposure season. (Dizengremel* et al. *1994, 301-302)*

The open-top chambers have often been built within a greenhouse (often with added lights) to provide better light and temperature controls during repeated days of exposures. Here too, the chamber modifies the exposure conditions compared to the greenhouse alone. To better measure mature trees' response to O_3, large branch chambers were developed (Ennis et al. 1990, Houpis et al. 1991, Teskey et al. 1991, Havranek and Wieser 1994). This system, which is easy to use, can also be criticized since the isolated part of the plant treated with O_3 is under different environmental conditions than the rest of the tree.

1.11.2 FACE units

The problem with most exposure systems is that they must be artificial compared with the normal environmental conditions.To mix the air with any short-lived pollutant requires rapid mixing followed by a fast delivery into an area and requires a wind velocity well beyond normal atmospheric exposures. In addition to the modification of some microclimate parameters, the OTCs can only contain tree seedlings even for the larger chambers. Thus, a new system, called FACE for “Free Air Controlled Exposure” or “Free Air Carbon dioxide Enrichment," was developed at the beginning of the 1990s (McLeod et al. 1992, Wulff et al. 1992; Hendrey and Kimball 1994, Musselman and Hale 1997).

Fig. 1.10. Aspen FACE at the Northern Forest Ecosystem Experiment (NFEE), funded by the US Forest Service. The fumigation sites are observed by the series of circles with vertical tubes. Original trees were harvested during 2009, and a new forest vigorously sprouted under the FACE treatments in 2010 before treatments were discontinued (http://aspenface.mtu.edu/index.html)

This system requires a relatively large and flat area, which will be the planting area. Arranged around this area in a circle is a series of pipes that have holes at a predefined spacing from which the mixture of gases that need to be part of the atmosphere is emitted. The wind speed and direction across the area are measured, and the injection rate of each pipe is controlled by a computer so that a given concentration is formed from that flowing across the area. As the wind changes in speed or direction, so does the amount of the gases released coupled with the varied pipes used. Thus, the ambient atmosphere and wind conditions are used for the plants within the circle, and the mixed gases within the atmosphere of the plants is very similar to the environment, save for the added gases. Moreover, several gases can be used simultaneously, such as O_3 and more CO_2 (Fig. 1.10). Thus, the FACE system, simulating field plant growth conditions better than OTCs, was used to research the effects on plants of O_3 and high CO_2 (Matyssek et al. 2007).

In the upper part of Michigan in the USA, one free-air fumigation study was conducted within the canopy of tall sugar maple trees, although the O_3 fumigation was restricted to clusters of sun and shade foliage (Tjoelker et al. 1995). Another experiment, Aspen FACE, with elevated CO_2 and O_3, was carried out in Wisconsin from 1998 to 2010 with trembling aspen alone or mixed with either paper birch or sugar maple (Karnosky et al. 2003). After seven years, the presence of elevated O_3 hastened the conversion of stands to paper birch, whereas the presence of elevated CO_2 delayed it. Elevated O_3 modified the relative importance of aspen and maple by -2% and +5%, respectively, and with elevated CO_2, the changes were +9% and -20%, respectively. Thus, elevated O_3 slightly increased the conversion rate of aspen stands to sugar maple, but the opposite effect is observed under elevated CO_2 (Kubiske et al. 2007).

In Europe, a FACE system studied young birch trees' growth in Finland (Oksanen 2001). More recently, an example of a FACE project was carried out in Kranzberg Forest (Bayern, Germany). This research project, CASIROZ "Carbon Sink strength of beech in a changing environment: experimental Risk assessment of mitigation by chronic O_3 impact," was supported from 2002 to 2006 by the European Commission under the Fifth RTD Framework Programme (Matyssek et al. 2007). The project studied the vulnerability to chronic O_3 of adult beech trees growing in under-stand conditions. A "Free-Air Canopy O_3 Exposure” system, creating an experimentally enhanced O_3 regime within the canopy (relative to “control” trees in unchanged air), was used for analyzing O_3-induced responses. For relating tree performance to effective O_3 doses rather than O_3 exposure, the O_3 flux concept into leaves has been examined and validated against the AOT40 standard. Besides, branch cuvette fumigations and exposure of young beech plants inside the stand canopy would validate the ecological significance of former O_3 studies in phytotrons, open-top chambers, or on single branches in tree crowns. Mechanistic modeling for scaling to the stand level and quantifying the O_3 impact for "Global Change" scenarios was the goal. The main results were that the doubling of O_3 concentrations over five years did not substantially weaken beech trees, thus not the most O_3 sensitive tree species. Nevertheless, at cell and leaf level, many O_3 responses were detected, and O_3 intake rather than external O_3 concentration was relevant when assessing the risk potential. O_3 responses varied between years, sun and shade leaves displaying similar O_3 sensitivity, and O_3 effects on roots and mycorrhiza were detected.

A FACE system was recently used in Japan to study the effect of O_3 on larch (Agathokleous et al. 2017).

1.12 Ecophysiological Systems Affected by O_3

In many of the chapters within this book, more details are given about how O_3 alters plants' normal physiology, as these are constantly being improved in their detail. As is now understood, many of the processes are interlinked through signal transduction pathways and small biochemicals altering speeds of protein synthesis and/or stability of produced proteins.

It was understood that genetic variations would alter plant responses (Engle and Gabelman 1966, Berge 1973, Cameron and Taylor 1973, Cameron 1975), but our understanding of genetics was not good enough to connect the varied ideas. The notion that some cultivars had different responses was used to test those variations (Elkiey and Ormrod 1979, Dijak and Ormrod 1982) and develop bioassays for the field (Berge 1973). We have arrived at our understanding today because of the great strides forward in such fields of genetics and system biochemistry. Moreover, such systems understanding depends upon new sensitive technology.

By the 1990s, several plant systems were becoming sufficiently understood to describe how O_3 could alter the plant's net pollutant responses in terms of gross metabolism and visible wounding of the leaves. These responses were listed by Heath (1999) and, with some modification, still go far in explaining what is happening at the physiological level (Table 1.2).

Table 1.2. Current theories of oxidant-induced alterations of plants. Adapted from Table 1, Heath (1999) they are listed by the physiological process but arranged into special processes or biochemicals

1. Loss of Photosynthetic Capacity
    - Stomatal Response
    - Photosystems
    - Carboxylation
    - Translocation
2. Membrane Dysfunction
    - Loss of Ion Channels (K^+, Ca^{2+})
    - Loss of Permeability
    - Alteration of Normal Ionic Channels
    - Membrane Structure
3. Loss of Signal Transduction Receptors
4. Antioxidant Protection
    - Superoxide Dismutase (anti-superoxide)
    - Peroxidases and Catalase (anti-hydrogen peroxide)

- Ascorbate and Glutathione (anti-superoxide, hydrogen peroxide, hydroxyl radical)
- Tocopherol (anti-peroxides)

5. Wounding Response
    - Wounding Proteins (e.g., chitinase, β-glucan synthase)
    - Activated Oxygen (ROS)
    - Ethylene Production
6. Premature Senescence
    - Early Loss of Productivity
    - Inability to Remobilize Nutrients

1.12.1 Effect on yield, growth, and productivity

One of the USA's largest projects was the National Crop Loss Assessment Network (NCLAN) in the mid-1980s. Headed by Walter Heck, it included many of the researchers who were active at that time in a set of studies using virtually identical systems to expose varied crops to O_3 (generally using open-top chambers) but in different USA regions. These studies were funded by the US Environmental Protection Agency and were summarized at the end by a symposium (See Heck et al. 1988). While the emphasis was on plants' growth and the production of seeds (yield), side studies were done on what changes were forced by the O_3 exposure. The effect of O_3 was thus also studied not only on the quantity but also the quality of the harvested product (Pell and Pearson, 1984). This was one of the first major attempts to generate a workable metric that the EPA could use to regulate O_3 levels in varied sections of the USA. For a relatively complete but short summary of findings, see Heagle et al. (1988).

1.12.2 Sensitivity and tolerance

It was discovered early on that varied species of plants demonstrated effects and tolerated O_3 differently, e.g., Gillespie and Winner 1989. Table 1.3 lists some varieties classified by their relative sensitivity to O_3. Of course, how sensitivity is ranked (e.g., visible injury, growth rate, or productivity of seed production) will change some of these rankings. Sensitivity has not yet had a coherent and easily observed measure.

Crops are more sensitive than trees, and annual grasses are more sensitive than perennial ones. It is noteworthy that C4 plants are more tolerant than C3 plants (see also paragraph 1.14). Concerning trees, recent comparisons generally show that angiosperm trees (deciduous) are more sensitive to elevated O_3 than gymnosperms (evergreen). The total biomass of angiosperms was reduced by 23% at an average O_3 concentration of 74 ppb, while the total biomass of gymnosperms was reduced by 7% at a higher mean O_3 concentration of 92 ppb (Wittig et al. 2009).

Table 1.3. Classification of plants in the function of their sensitivity to O_3 (adapted from Mills et al. 2007, Table 1; Wittig et al. 2009, Fig. 4 and Fig. 8; Castell and Le Thiec 2016, Table 1)

	Ozone-sensitive species	Moderately ozone sensitive species	Ozone tolerant species
Increasing tolerance ↓	wheat, turnip, soybean bean, pea, cotton tomato	beet, colza, tobacco, rice, potato maize, sorghum	barley, rye
	poplar, black cherry	beech, birch, sugar maple	oak
	larch		
	ponderosa pine loblolly pine maritime pine	scots pine, Austrian pine white pine, fir	Douglas fir Norway spruce

However, a specific species can have multiple cultivars that also have different sensitivities to O_3 (see above). In the past, the definition of sensitivity was generally demonstrated by how much visible injury was observed after a specific amount of exposure to O_3. That amount could be varied greatly by the researching team since the exposure characteristics and times of post fumigation vary. Clearly, all these variables can influence the definition of a sensitive cultivar. Some cultivars have been very useful in understanding why variations existed, such as the O_3-sensitive tobacco Bel-W3 and the tolerant Bel-B (Heggestad 1991). Heagle et al. (1991) have used various clover cultivars to examine physiological differences and develop plant-based sensitivity tests.[8]

On the other hand, as economists often state, the only thing of value in a forest is the trees which are to be logged (see chapters 19-23 in Heck et al. 1988). In this, much of the forest has no monetary assigned value since much of a forest is just beauty and peacefulness. Yet early on, it was observed that woods near a polluted area had major problems (Evans and

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[8] The sensitivity tests are bio-assays in that a specific sensitive cultivar is grown under uniform conditions in a pot. Then those pots are set out in an environment that is believed to have O_3 in the atmosphere. After only a few days, the visible injury appears, and that injury is correlated with the amount of O_3.

Miller 1972). In a later attempt at summarizing what was happening in the varied US and European forests, Sandermann et al. (1997) edited a book on Forest Decline, which summarized much of what was then thought about how forests were being altered, if not destroyed, by atmospheres with oxidants. Much of the O_3 was being produced elsewhere but transported by normal atmospheric flow to the mountains and rural valleys. Once again, California was at the forefront of research led by Paul Miller, Patrick Temple, and André Bytnerowicz (see chapter 2, Miller et al. 1997). An excellent map shows the urban air basins of San Diego, Los Angeles, and San Francisco, which, producing the polluted atmospheres, are upwind of the regions of forests in the mountains of the Sierra Nevada and the Coast Range (see Figure 2.1, 41 of Sandermann et al. 1997). In these studies, the numbers of whorls and needles in each of the pine trees declined with O_3 exposure along with a mottling[9] of many of the needles of a whorl, which is a combination of developmental/production alteration combined with visible injury (see Miller et al. 1997).

1.12.3 Alteration of gaseous exchanges

There are two main sites on the leaf where O_3 could impact the cuticle and enter the mesophyll layer through the stomata (see above regarding the dose). The cuticle data are somewhat contradictory, but in general, there was no real evidence that the cuticle was being altered by the O_3 exposure at reasonable levels (Kerstiens and Lendzian 1989). Indeed, O_3 at the cuticle surface could breakdown with no alteration of the productivity of the leaf, but its breakdown could lower the concentration near the surface of the leaf, and thus, the amount of O_3 entering the mesophyll region would decline (see Aphalo and Jarvis 1993, Grantz and Vaughn 1999, Morison et al. 2007 for diagrams of gaseous flow near the stomata).

The stomata were tagged as a possible control of injury to the leaf very early on (Dugger and Ting 1970; Brennan 1975; Heath 1980; Winner et al. 1991). The exact role has always been challenging to understand due to the ability to measure actual flow due to then-current technology. The leaf, as an interface between the atmosphere and plant, is the main target for pollutant attack. In its gaseous exchanges, all flux into and out of the mesophyll cells is regulated by stomata (see Fig. 1.6). Air pollutants, such as O_3, by entering the leaf through the stomata, can disturb sugar production and cellular metabolism directly by altering the stomata's

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[9] Mottling is defined as loss of chlorophyll non-uniformly along a needle; only chlorosis in regions.

ability to open and so lowering the internal CO_2. Yet that would also lower the dose of O_3 into the leaf. Succinctly put, if the stomata are closed, there is no problem with O_3 entry, and so the plant is protected. However, then the ability to fix carbon through photosynthesis would be deeply impacted (Reich and Lassoie 1984). Yet the boundary layer cannot be ignored as air flows faster and more turbulently around the margin, leading to a greater exposure of pollutants there (Woodrow, Ball and Berry 1990). In fact, boundary layers about the leaf can explain why the margin of the leaf (with its higher, more turbulent airflow) is more likely to show visible injury (Fig. 1.5). The alteration of airflow near the veins and the stomatal density near the veins can partially explain why the vein regions are the last to show visible injury; a large boundary layer will slow the O_3 flow into the interior of the leaf.

A series of papers (Olszyk and Tibbitts 1981, Tingey and Hogsett 1985) began to address the role of water stress upon injury by O_3 to leaves. This, of course, pointed to stomata's role in controlling the flow of the pollutants into the leaf since water stress slows gas flow by closing stomata. Water stress normally develops later in the day (midday depression, Heath et al. 1985). Thus, stomata opening and O_3 entry are not constant throughout the day. This O_3 impact with water status was nicely summarized by Pearson and Mansfield (1993). During the last few decades, one question has been repeated: how much O_3 actually penetrates into the full mesophyll where any gas injury would be expected and could be measured? In several experiments, it was observed that photosynthesis could be lowered without a concurrent decrease in stomatal conductance (Saxe 1991, Heath and Taylor 1997, Grulke et al. 2007). A recent meta-analysis on trees clearly showed that even though both the photosynthetic rate and the stomatal conductance were decreased progressively in the chronic O_3 dose's function, the stomatal conductance was systematically less altered than the photosynthetic rate (Wittig et al. 2007).

Short-term exposure to acute high concentrations of O_3 (above 200 ppb) generally causes some degree of closure of stomata (Hill and Littlefield 1969, Rosen et al. 1978, Olszyck and Tibbitts 1981). At concentrations below 200 ppb O_3, a diversity of stomatal responses has been reported with opening, closure, or no change in the function of the studied species (Bennett et al. 1974, Darrall 1989). As the understanding of how regulations to control the stomata aperture progressed, it became clear that stomatal regulation was highly complex, and there were several sites in which O_3 could alter the net behavior of stomata themselves (Fig. 1.11).

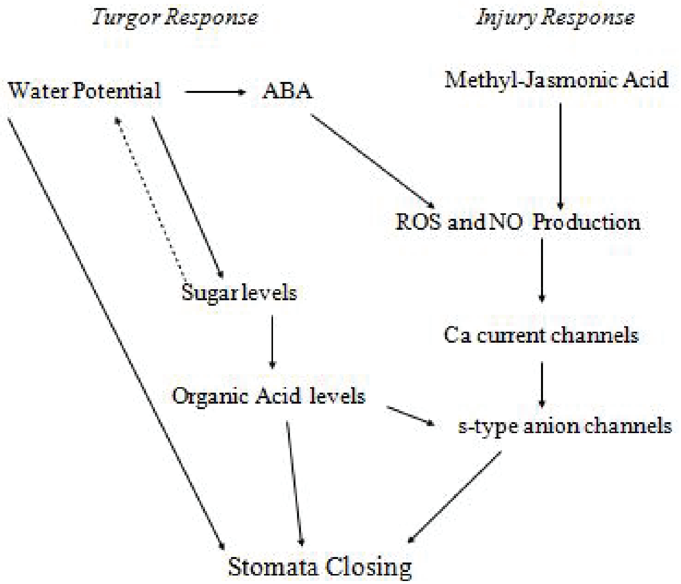

Fig. 1.11. Processes' interaction to alter the aperture of the stomata. Adapted from Munemasa et al. 2007. The responses can be separated into turgor-induced and injury-induced. Both are connected by abscisic acid, the stomata phytohormone, and organic acids, closely linked metabolically to sugars via the Krebs Cycle. Note the involvement of varied channels (of Ca^{2+} and anions) within the guard cells' membrane. ROS: Reactive Oxygen Species

Of course, the leaf's water potential played a dominant role in the aperture of the stomata; highly negative water potential meant that water would be withdrawn from the stomata guard cells, and they would become flaccid and close. Well-watered plants maintained an open aperture, and gas flow would be high for CO_2 and O_3 (Heath et al. 1985). Later it was shown that ion fluxes (especially Ca^{2+} and anions) and the pH within the cell and cell walls of stomata could alter the water balance and the aperture size (Outlaw and Lowry 1977, Outlaw and Kennedy 1978). Recently, interactions of varied phytohormones, such as ABA, auxin, and varied jasmonic acid species, could alter the ion fluxes' response and alter how stomata responded.

Early in the 21st century, it was clear that while turgor pressure, including its alterations by ions and sugars and hormones (Beardsell and Cohen 1975, Assmann 2003), was critical, there were many other players in the functioning of the stomata. Of surprising note was the role of H_2O_2

and general ROS biochemicals (Munemasa et al. 2007, Pei et al. 2000). It was found that abscisic acid (ABA) and methyl-jasmonate could stimulate the production of H_2O_2, thereby altering normal calcium channels, and therefore change the flow of anions, especially K^+. The normal reactions of O_3 with water can produce these various ROS chemicals. They would be able to interfere with normal stomata control, so depending upon many variables, the stomata could open, close, or change the speed of responses by O_3 exposure. This may explain the confusion of earlier observations in the field.

1.12.4 Effect on Photosynthesis

The first studies conducted on the effects of O_3 on photosynthesis used high concentrations of O_3 (between 400 ppb and 1 ppm) delivered over a few hours, leading to a marked decrease of photosynthesis (Erickson and Wedding 1956, Todd 1958, Todd and Propst 1963, Hill and Littlefield 1969, Botkin et al. 1972). In some cases, the fumigation was repeatedly made for several days (Taylor et al. 1961). Visible plant damage symptoms are often a consequence of this acute concentration (Todd and Propst 1961, Botkin et al. 1972, Taylor et al. 1982). Sometimes, visible symptoms appear a few hours after the end of very short-term fumigations with high concentrations (440 ppb) of O_3 (Todd 1958). With slightly lower concentrations (300 ppb) delivered to beans for 3 hours, photosynthesis is immediately decreased, but visible leaf injury symptoms develop only after one day (Pell and Brennan 1973). All these experiments were done on young crops and tree seedlings, thus beginning the "cause and effect" argument about which process affects which other processes.

Several experiments used fumigations with more realistic O_3 concentrations, up to two times the ambient concentration. These long-term chronic O_3 exposures, conducted in the same period on tree seedlings, caused a decrease in photosynthesis without any visible symptoms of needle injury, at least in the first stages of the time course (Miller et al. 1969, Barnes 1972). Table 1.4 summarizes the two latter studies' results using 150-300 ppb O_3 delivered up to one month. A chlorotic mottle of needles was observed only for the higher O_3 treatment applied during the longest time (Miller et al. 1969).

Plant species (age)	Treatment	Reduction in photosynthesis	Symptoms	Reference
Pinus Ponderosa (3-year-old)	150 ppb 10 days	4%	No	Miller et al. (1969)
	150 ppb 30 days	10%	No	Miller et al. (1969)
	300 ppb 10 days	15%	No	Miller et al. (1969)
	300 ppb 30 days	67%	chlorotic mottle	Miller et al. (1969)
Pinus elliottii	150 ppb 36 days	23%	No	Barnes (1972)
Pinus serotina	150 ppb 36 days	6%	No	Barnes (1972)
Pinus taeda (8-month-old)	150 ppb 36 days	14%	No	Barnes (1972)
Pinus strobus (2-year-old)	150 ppb 36 days	22%	No	Barnes (1972)

A decline in photosynthesis under moderate concentrations of O_3 was observed in many other experiments on young crops (Ormrod et al.1981; Reich et al. 1986) and trees (Kuno 1980, Reich 1983, Yang et al. 1983, Arndt and Kaufman 1985, Keller and Häsler 1987, Küppers and Klumpp 1988, Tseng et al. 1988, Sasek and Richardson 1989, Wallin et al. 1990). In the field, sapling trees (*Pinus ponderosa*) experiencing air oxidants in the San Bernardino mountains (Coyne and Bingham 1982) and 25-year-old white pine trees under the ambient pollutant level in Tennessee (McLaughlin et al. 1982) also showed a reduction in photosynthesis. A clear separation can thus be made in plant photosynthesis responses to acute and chronic O_3 exposures (Darrall 1989). In the latter case, a parallelism between the reductions of photosynthesis and growth is generally observed (Reich and Amundson 1985).

As for growth and yield, the degree of photosynthesis sensitivity to O_3 depends on the studied species. In a study on seven species (crops and trees), long-term exposure to O_3 caused a linear decrease of the photosynthetic rate without visible injury (Reich and Amundson 1985). In non-fumigated conditions, the crop species (clover, wheat, soybean) and hybrid poplar present higher photosynthesis rates than sugar maple, red oak, and white pine. They experienced a stronger decline in photosynthesis per unit O_3 dose (Fig.1.12). A 10% reduction of photosynthesis was observed for 5 ppm.h for wheat and clover, whereas 25 ppm.h were necessary to decrease red oak and 30 ppm.h for white pine.

A question arises on the concomitance between the reductions in the photosynthetic CO_2 uptake and the stomatal conductance. The decreased photosynthetic rate observed in O_3-fumigated needles of loblolly pine trees growing in OTC for 3 years in two times ambient O_3 (92 ppb, 12 h seasonal mean) appears to be not due to higher stomatal resistance (Sasek and Richardson 1989). The decreasing effect of O_3 on the photosynthetic rate would better correspond to a decrease in the Rubisco-mediated carboxylation efficiency rather than changes in stomatal conductance (Dann and Pell 1989, Heath and Taylor 1997, Matyssek et al. 1991, Pell et al. 1992).

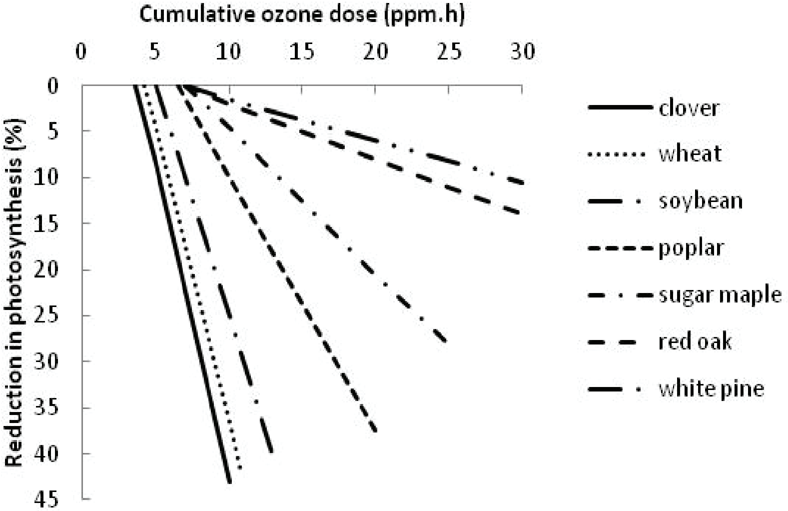

Fig. 1.12. Reduction in photosynthesis of several crops and trees in relation to the cumulative dose of O_3 (adapted from Reich and Amundson 1985, Fig. 1 right, 568)

1.12.5 Effect on Respiration

By contrast with photosynthesis, O_3 generally increased respiration (Dizengremel and Citerne 1988, Dizengremel 2001). Under exposure to high O_3 concentrations during a few hours, this increase was generally accompanied by visible injury (Todd 1958, Macdowall 1965, Pell and Brennan 1973, Furukawa and Kadota 1975). With lower concentrations of O_3, more typical of ambient polluted air, and delivered during weeks, an increase of respiration was observed without symptoms of visible injury (Barnes 1972, Küppers and Klumpp 1988, Wallin et al. 1990). A realistic concentration of O_3 (100 ppb) delivered for 2 weeks resulted in a strong decrease of photosynthesis and photorespiration and a slight respiration stimulation in poplar leaves (Kuno 1980). The opposite effect of O_3 on photosynthesis and respiration was clearly demonstrated in a study on leaves of young poplar cuttings treated with 125 ppb during three months (Reich 1983; Fig. 1.13).

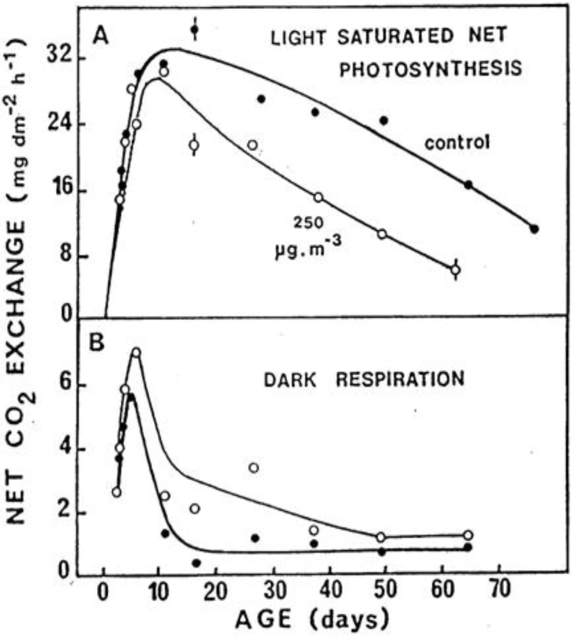

Fig. 1.13. Effect of O_3 on photosynthesis and dark respiration of hybrid poplar leaves (redrawn after Reich, 1983, Fig.1, 293)

By contrast, photorespiration is generally shown as being decreased under O_3 exposure, probably in relation to the damage caused at the Rubisco level (Aarnes et al. 1993, Booker et al. 1997, Dizengremel 2001, Bagard et al. 2008).

1.12.6 Assimilate Distribution

Recent reviews and meta-analyses were compiled on the effect of O_3 on photosynthesis, growth, and yield of crops (Morgan et al. 2003, Feng et al. 2008) and trees (Wittig et al. 2007, 2009). Of course, growth and yield are coupled to photosynthesis by translocation. Translocation is dependent upon photosynthesis products and the water potential of the leaves and other organs to which the sugars are transported. The full system is complex. There are few studies on the O_3 impact upon translocation *per se*, but a useful review of translocation by Cooley and Manning (1987) should be consulted.

A depression of photosynthesis resulting in a reduction of photosynthates and stimulated respiration providing carbon skeletons for repair and detoxification would restrict carbohydrates available for growth (Barnes 1972, Heath 1996a, Dizengremel 2001). Furthermore, greater retention of photosynthates in the leaves was observed concomitantly with a decrease in phloem loading and allocation of carbohydrates to roots leading to a decrease in root-shoot ratios (Adams et al. 1990, McLaughlin et al. 1982, McLaughlin and McConathy 1983, Darrall 1989, Spence et al. 1990, Einig et al. 1997). Clearly, one must take into account the full carbon balance of the entire plant.

1.13 Effect on Enzymes of Carbon Metabolism: Biochemical Mechanisms

1.13.1 RuBP carboxylase (Rubisco)

Even though O_3 can reduce CO_2 assimilation by indirectly inducing stomatal closure, many experiments show that O_3 can directly alter photosynthesis functioning (Pell and Pearson 1983, Dizengremel 2001). It is generally admitted that the carbon reduction part of photosynthesis (Calvin cycle) is impacted before any alteration in the photochemical dependent light-use efficiency (Dizengremel 2001, Heath 2008). A decrease of Rubisco activity was first demonstrated in rice leaves treated with O_3 (Nakamura and Saka 1978) and was further observed in a large number of crops and trees (Pell et al. 1992, Pell and Pearson 1983, Dizengremel et al. 1994, Fontaine et al. 1999, Pelloux et al. 2001, Degl’Innocenti et al. 2002).

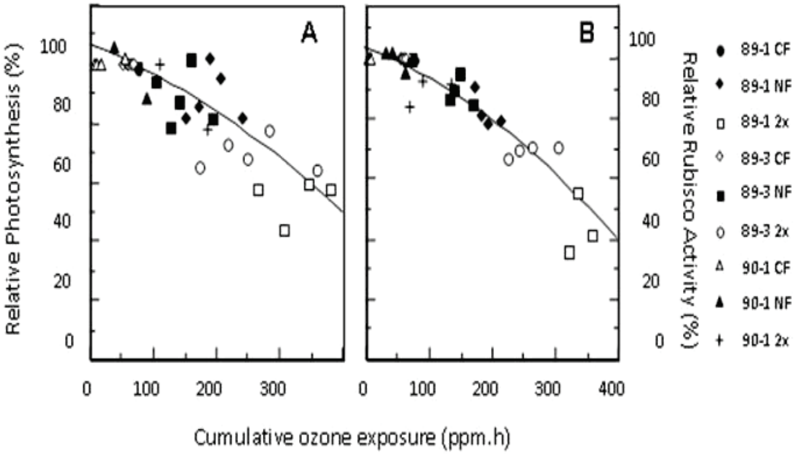

Fig. 1.14. Photosynthesis (A) and Rubisco activity (B) expressed relative to the charcoal-filtered treatment in relation to the cumulative O_3 exposure for loblolly pine needles exposed to charcoal-filtered air (CF), non-filtered air (NF), and 2 × ambient O_3 in the 1989 first flush (89-1), third flush (89-3) and 1990 first flush (90-1). Values are from each month from May to August 1990. The photosynthetic rates and the Rubisco activities were expressed on the same basis (needle surface area). Adapted from Dizengremel et al. 1994, Fig.2, 303)

Rubisco's implication in the O_3-induced decrease of photosynthesis was demonstrated in loblolly pine needles with a parallel reduction in photosynthetic rate and Rubisco activity with increasing cumulative O_3 exposure (Fig. 1.14, Dizengremel et al. 1994). The 3-year-old trees were placed in OTCs with charcoal-filtered air (CF, 8 ppb O_3, 12 h daily mean), non-filtered (NF) ambient air (45 ppb O_3), and 2 × ambient O_3 (86 ppb O_3). Data obtained from three different flushes at various sampling dates were expressed in terms of cumulative O_3 exposure in ppm.h. By mid-August 1990, the 1989 first flush had accumulated an O_3 dose of 380 ppm.h in the 2 × O_3 treatment compared with 125 ppm.h in the CF one. In contrast, the younger 1990 first flush had only accumulated 120 ppm.h in the 2 × O_3 treatment and less than 20 ppm.h in the CF treatment.

A similar pattern of changes in photosynthetic rate and Rubisco activity under O_3 is also well illustrated with the diurnal changes in these two parameters measured during a sunny day on needles of trees submitted to different doses of O_3 (Fig. 1.15).

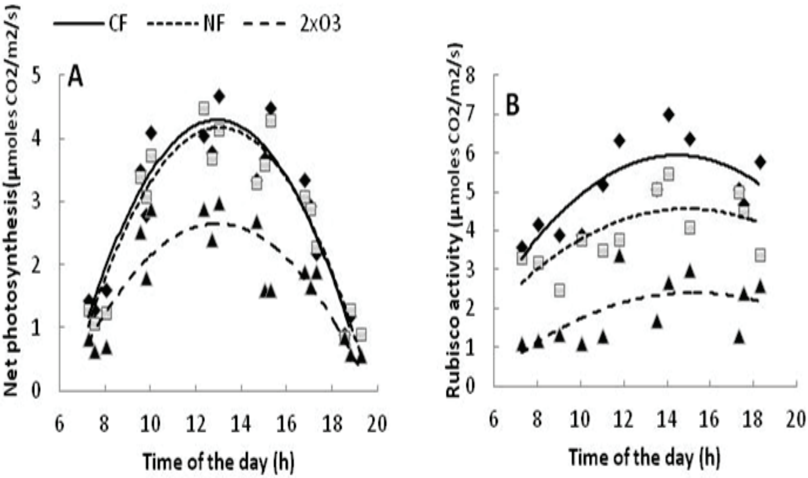

Fig. 1.15. Effect of O_3 on the diurnal photosynthesis and rubisco activity. Measurements were made on a sunny day in August 1991 on the 1st flush 1990 of 4-year-old loblolly pine seedlings submitted to O_3 in OTCs. The cumulative O_3 doses were 120 ppm.h, 200 ppm.h, and 380 ppm.h in charcoal-filtered air (CF), non-filtered air (NF) and two times O_3 ($2xO_3$) (Dizengremel, Brown, Sasek and Richardson, unpublished data)

In a later paper, Pell et al. (1994a) discusses the idea that one of the toxic products of O_3 fumigation has altered Rubisco. The observed decrease in Rubisco activity by O_3 is due to a reduction in quantity (Pell and Pearson 1983, Landry and Pell 1993, Fontaine et al. 1999, Pelloux et al. 2001, Gaucher et al. 2003, Bohler et al. 2007) and that of Rubisco activase (Pelloux et al. 2001, Degl'Innocenti et al. 2002). Decreased levels of the mRNA transcripts for Rubisco's two subunits were observed in tree leaves (Bahl and Kahl 1995, Brendley and Pell 1998, Pelloux et al. 2001). However, the O_3-induced reduction in the Rubisco activity and quantity may involve several mechanisms, such as an inhibition of the protein synthesis, as described above, an increased rate of protein degradation or alteration of the protein through an oxidative process (Landry and Pell 1993, Junqua et al. 2000). These processes could occur together or separately, depending on O_3 concentration, exposure time, and leaf age (Junqua et al. 2000).

Yet Williams et al. (1994) have developed a correlation between the levels of ABA after water stress in *Arabidopsis thaliana*/leaves and the loss of rbcS. This work suggests that water stress may alter the relationships much more than merely closing the stomata. If ABA is lowering rbcS by whatever mechanism, rbcS may not be worth much as a marker of O_3 fumigation except under very controlled conditions. Harris and Heath (1981) showed that conductance was not the only point where water potential played a role: root and water delivery systems could play a major contributory role in determining foliar O_3 sensitivity. Heath (1994a) summarized what was known about the mechanisms of O_3 injury on Rubisco, but he pointed out that, like many processes of plants, that enzyme is highly regulated by a relatively complex transducer. For example, the two subunits of Rubisco (rbcS and rbcL) are genetically related, and the enzyme activity is regulated by other polypeptides, such as Rubisco activation. So, the loss of subunits' production by O_3 exposure may be controlled by different processes and not a simple cessation of DNA transcription (Galmès et al. 2013). Like stomata aperture size, the activity and/or levels of Rubisco may be subjected to events far removed from O_3 halting DNA systems. An excellent and exhaustive review of photosynthesis and O_3 exposure has been provided by Saxe (1991).

The lowered plant productivity (Heck et al. 1988) measured over a growing season is believed, but not fully proven, to result from photosynthesis inhibition with the actual mechanism still being actively debated.

There are two probable causes: a forced closure of the stomata and/or an actual inhibition of one or more carbon fixation steps. Ozone may cause both effects either simultaneously or subsequently.

As discussed in both Chameides (1989) and Heath (1994b), the detoxification system used to remove O_3 or its products from the tissue is critical. Any system can be overloaded if O_3 enters the cell rapidly. Detoxification systems are generally enzyme-based and with a limited amount of co-substrate (e.g., ascorbate) and enzyme (e.g., superoxide dismutase) present. Again, the timing and amount of the oxidant exposure may be closely linked to the effects observed due to multiple pathway involvements.

There are at least three possible O_3 pathways within the tissue to three different cell types (Heath 1994a). The cells closest to the stomatal aperture are the guard cells, and the short path to those cells would serve to minimize the breakdown of O_3 and maximize the exposure of those cells. Thus, the guard cell should be exposed to the highest level of O_3.

The cells next closest are generally the subsidiary cells (Esau 1965). These would be subjected to the next highest level of O_3. The mesophyll cells containing the chloroplasts are often the furthest from the entry point and should receive the lowest exposure due to the breakdown of O_3 during its travel. It seems likely that these questions will not be answered on a macroscopic investigation; the types of oxidants and where they occur microscopically must be investigated.

1.13.2 Phosphoenolpyruvate carboxylase and enzymes of carbohydrate breakdown

Phosphoenolpyruvate carboxylase (PEPc) is a cytosolic enzyme with low activity in C3 plants in which it contributes to the refixation of respiratory CO_2 and the replenishment of the tricarboxylic acid cycle (TCA) in an anaplerotic pathway (Latzko and Kelly 1983, Chollet et al. 1996). Increased activity of PEPcase in conifers exposed to O_3 was observed for the first time at the beginning of the 1990s (Lüethy-Krause et al. 1990).

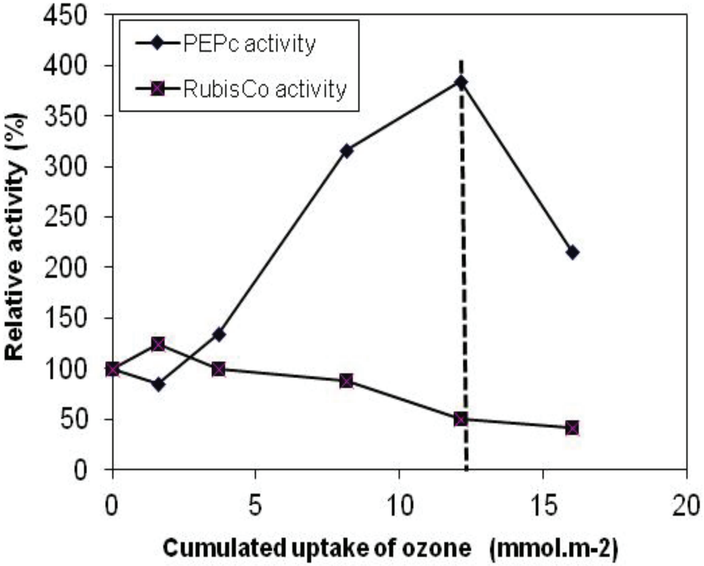

Fig. 1.16. Changes in RuBisCO and PEPcase activities of mature poplar leaves fumigated for 1 month with 100 ppb O_3. The dotted line shows the maximal PEPcase activity (adapted from Renaut et al. 2009, Fig.5, 503)

A high stimulation of the PEPcase activity was afterward found in deciduous and coniferous trees (Saurer et al. 1995, Sehmer et al. 1998). During chronic O_3 exposure of C3 plants, a relatively substantial increase in PEPcase activity accompanied a progressive decrease in Rubisco (Landolt et al. 1994, 1997, Fontaine et al. 1999, Lütz et al. 2000, Dizengremel 2001, Gaucher et al. 2003, Inclan et al. 2005, Leitao et al. 2008, Gandin et al. 2009, Renaut et al. 2009, Dghim et al. 2013, Fig. 1.16). Increases in the PEPc protein amount and the corresponding mRNAs were also observed (Fontaine et al. 2003). The ratio between Rubisco and PEPcase activities is decreased 3- to 20-fold in various species (Dizengremel et al. 2009). The contrasting behavior of the two carboxylases was also observed in the field where needles of declining trees in O_3-polluted areas showed lower Rubisco/PEPc ratios than needles of healthy trees (Wild and Schmitt, 1995, Dalstein et al. 2002).

Under chronic O_3 exposure, PEPc could supply organic acids to the TCA cycle, thus providing precursors for amino acid synthesis through the anaplerotic pathway (Dizengremel et al. 2009, 2012). The stimulation of PEPc activity is related to the increased activities of several enzymes of glycolysis and the pentose phosphate pathway as well as of cytosolic enzymes delivering NAD(P)H (Dizengremel et al. 1994; Sehmer et al. 1998, Dizengremel et al. 2008, 2009, Dghim et al. 2013). The resulting higher amount of reducing power would serve for repair and detoxification mechanisms. In fact, phosphoenolpyruvate is at the crossroads of four metabolic routes, which all increase under O_3 exposure: mitochondrial respiration, the anaplerotic pathway, the pathway for isoprenoid synthesis, and the shikimate-phenylpropanoid pathway (Dizengremel et al. 2012). However, it is interesting to note that the increase in PEPcase activity would be transient (Fig. 1.16).

In the mitochondria, some TCA cycle enzymes were increased in activity (Dizengremel et al. 1994, Sehmer et al. 1998). Using a concentration of O_3 at the limit of acute on tobacco, Ederli et al. (2006) showed a decrease in the capacity of cytochrome oxidase and an increase in the capacity of alternative oxidase (AOX), the non-phosphorylating terminal oxidase of the respiratory chain. An increase in the alternative pathway's capacity in leaves of *Erythronium Americanum* exposed to 80 ppb O_3 for 3 weeks was also observed (Gandin et al. 2009). A net increase in the level of mRNA transcripts for AOX was found in leaves of poplar trees treated with 100 ppb O_3 for 4 weeks (Hasenfratz-Sauder et al. 2000) as well as in tobacco leaves treated for a few hours with 150 ppb O_3 (Ederli et al. 2006). Increased participation of AOX could limit the production of reactive oxygen species (ROS) (Purvis and Shewfelt 1993, Maxwell et al. 1999), which follows the uptake of O_3 by leaves.

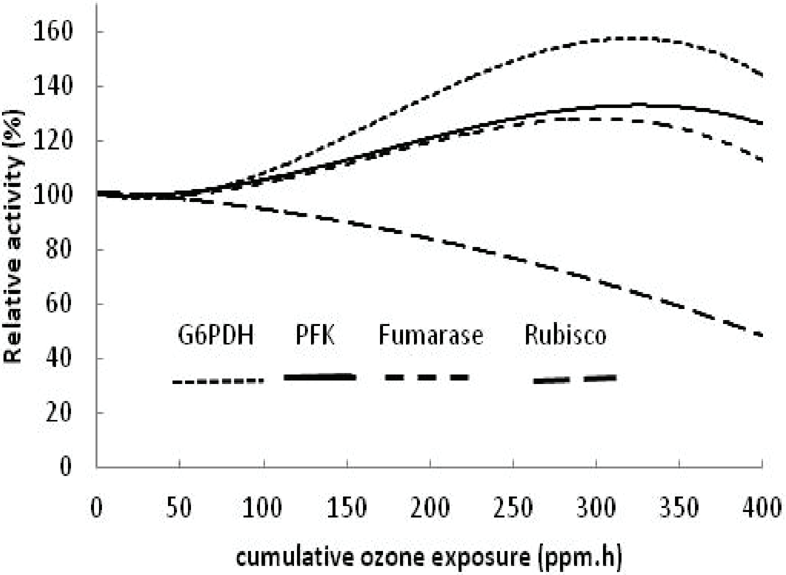

Fig. 1.17. Relative activities of three enzymes of carbohydrate breakdown and Rubisco in the function of the cumulative O_3 exposure for loblolly pine needles exposed to CF, NF, and $2xO_3$ ambient air (see Fig. 1.14 for details on studied pine needles). G6PDH: glucose-6-phosphate dehydrogenase (HMP pathway); PFK: phosphofructokinase (glycolysis); Fumarase (mitochondrial Krebs cycle). Adapted from Dizengremel et al., 1994, Fig. 4, 304

A careful examination of the results obtained in a range of experiments shows that the substantial increase in PEP case activity reaches a maximum, which is transient (see an example in Fig. 1.16 with the dotted line). More generally, the activities of several enzymes implied in carbohydrate breakdown show an increase more or less pronounced before a plateau and a decrease in function of the course of fumigation and, thus, in the function of the cumulative uptake of O_3 (Fig. 1.17; Dizengremel et al. 1994). This decrease, therefore, accompanying the reduction in Rubisco activity, would correspond to visible symptoms of injury and cell death.

1.14 Case of C4 plants

Even though a general decrease in crop yield is described, it is rarely mentioned that C4 plants are less sensitive than C3plants (Fig. 1.18). This better tolerance could be due to the well-known lower stomatal conductance of C4 plants compared to C3.

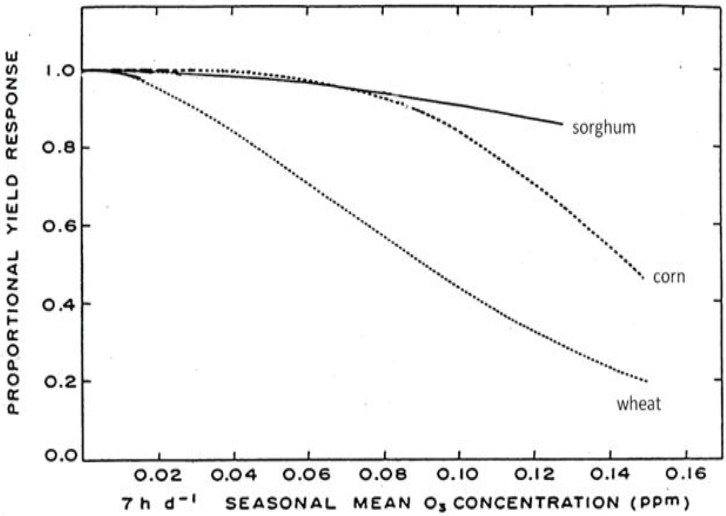

Fig. 1.18. Effect of O_3 on the yield of C3 (wheat) and C4 crops (corn, sorghum). Adapted from Heagle et al. (1988), Fig.7.6, 159

The photosynthesis rate is generally more reduced in C3 than C4 plants exposed to O_3 (Miller 1988). However, in the two types, a good relationship between the reductions in photosynthesis and yield was observed (Miller 1988), as already mentioned for a series of C3 plants (Reich and Amundson 1985). More recently, a similar decrease of the CO_2 assimilation rate and Rubisco activity of C3 and C4 crops was observed when expressed in the function of AOT40 (Accumulated amount of O_3 above a Threshold value of 40 ppb), maize presenting the lower reduction compared to wheat (Bagard et al. 2015).

However, when expressed on a POD (Phytotoxic O_3 Dose) basis, maize's response curves become more pronounced than those of wheat (Bagard et al. 2015). Fig. 1.19 compares the changes in the photosynthetic rate of the two crops expressed on an AOT40 and POD0 basis. The difference is that AOT40 only considers the external O_3 concentration while POD integrates the uptake of O_3, as discussed in paragraph 1.17.

As shown in Fig. 1.20, Rubisco's activity decreased along with the POD0 values for wheat, poplar (C3), and maize (C4).

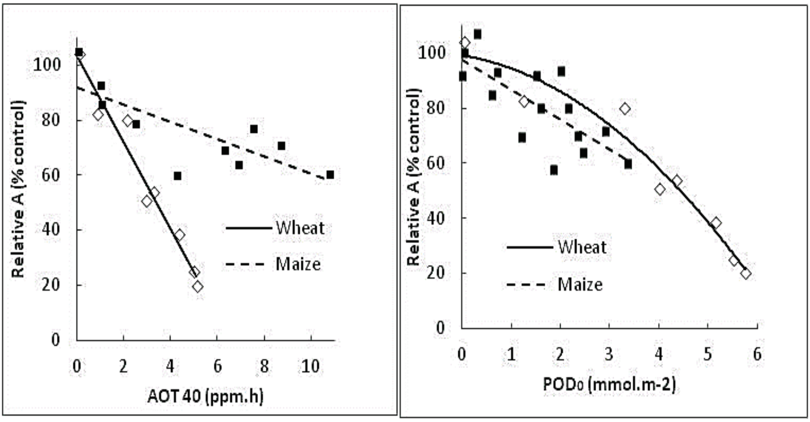

Fig. 1.19. Comparison of the relative photosynthetic rates of wheat and maize expressed on AOT40 and POD0 (adapted from Bagard et al. 2015, Fig. 2, 415)

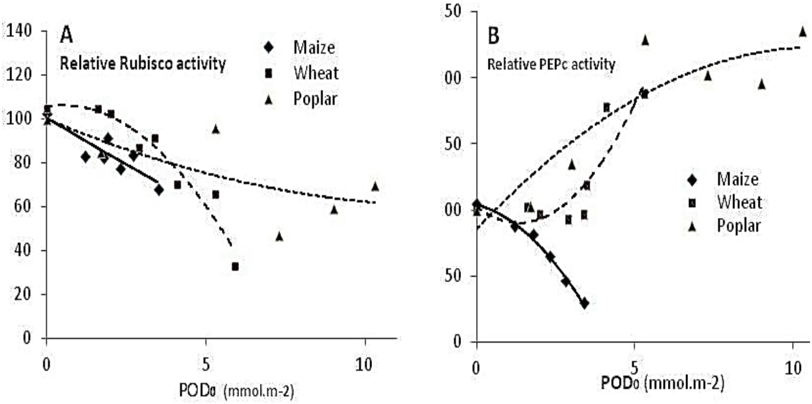

Fig. 1.20. Dose-response relationships for Rubisco activity (A) and PEPc activity (B) of maize, wheat, and poplar exposed to O_3. Wheat seedlings were exposed to charcoal-filtered (CF) air, CF air + 40 ppb O_3, and CF air + 120 ppb O_3 for 18 days during the 14-h light period. Maize seedlings were exposed to CF air, CF air + 40 ppb O_3, and CF air + 80 ppb O_3 for 32 days, 12 h daily during the light period. Hybrid poplar trees (Populus tremula x Populus alba, INRA 717-1B4 clone) were exposed to CF air, CF air + 40 ppb O_3, and CF air + 120 ppb O_3 for 35 days, 13 h daily during the light period (adapted from Bagard et al. 2015, Fig.3, 416)

The activity of PEPc was increased in treated leaves of wheat and poplar, which is a typical response of C3 plants to O_3; this stimulation is related to a stronger demand in reducing power for detoxification and repair processes. By contrast, in maize leaves, the activity of PEPc, which is in charge of the primary step of CO_2 fixation, is strongly reduced in response to O_3, as previously shown by Leitao et al. (2007). This decrease is even stronger than that observed for Rubisco, PEPc being more exposed in mesophyll cells while Rubisco would be more protected in bundle-sheath cells. In the field, the least sensitivity of maize, compared to wheat, for similar atmospheric O_3 concentrations, is probably largely due to its smaller stomatal conductance, which considerably reduces the uptake of O_3.

1.15 Biochemical mechanisms of O_3 impact

Investigations of which biochemical systems were altered by O_3 exposure began as soon as it was clear that exposure lowered productivity (see above). Of course, in the 1950s the ability to measure the varied biochemicals was severely limited by the available knowledge. Only a few enzymes could be measured, and the various metabolic pathways were just being put together. Many of the assays were bioassay-linked; for example, hormones were determined by what they did to plant growth physiology.

Heath (1980, Table 1) postulated that oxidants' entry must lead to membrane impairment and osmotic imbalance, leading to altered biochemical pathways. The interaction of these two processes leads to selective permeability loss, which forces a decline in transpiration. Photosynthesis inhibition is forced by these events leading to altered water potential and a lack of biochemical intermediates. The collapse of normal cell processes will lead to cellular injury. Furthermore, weak acids formed by the exposure of plants to either HF or SO_2 may lead to cytoplasmic poisons, including osmotically active agents, which then become entangled with the above processes. Many of the functions were suspected but not yet proven as there were few or no connections for events. Olszyk and Tibbitts (1981) had shown that SO_2 and O_3 did interact in an interesting but challenging to understand manner. The line of how varied toxic elements of a generalized atmosphere can interact to cause more significant or sometimes lesser injury has not been well studied as yet.

Nonetheless, many reports were moving towards an understanding of how O_3 altered plant productivity. Some systems were more sensitive, and some had no relation to O_3 exposure. For a time, the physiology was decoupled from both genetics and biochemistry (see above). It was towards the 1990s that real progress was made, and a link with a physiological understanding about biochemistry and especially genetic control was the driving force.

1.15.1 Model Systems

The concept of a model biological system was discussed at length by Heath (1994a). These model systems were simpler systems of unicellular cells or organelles, which, it was thought, could provide insight to more complex whole plant systems. This was the case for chloroplasts subjected to O_3 (Coulson and Heath 1974). Ozone bubbled into a suspension of isolated chloroplasts inhibited electron transport in both photosystems without uncoupling ATP production. Photosystem I was found to be a little more sensitive than photosystem II. O_3 was found not to act as an energy transfer inhibitor since the drop in ATP production and high energy intermediate (measured by amine induced swelling) was nearly parallel to the decline in electron transport. O_3 did not seem to penetrate the grana region through the outer membrane of intact plastids. Simultaneously, it inhibited the bicarbonate-supported O_2 evolution, and the rate of ferricyanide-reduction in osmotically ruptured intact plastids was unaffected.

The gassing system for the green alga, *Chlorella sorokiniana*, was similar to that used for chloroplasts, with most of the O_3 passing through the solution unreacted, until quite late in the fumigation process (Heath et al. 1974, Heath 1987b). The fumigation dose was varied by time of exposure. After fumigation of the cells, they were removed to another vessel to probe the viability of cells, loss of varied ions, or photosynthesis. For example, the cellular viability (as measured by a cell's ability to form a plaque on agar plates) was reduced by 60% after 50 minutes of O_3 exposure (2.6 μmoles O_3/min). A dramatic increase in TBAR (a test for malondialdehyde, a product of lipid peroxidation) formation with an 8-9% loss of the unsaturated fatty acids was noted (Frederick and Heath 1975). These kinetics were mirrored by the loss of K+ from the cells measured by ion-specific electrodes (Chimiklis and Heath 1975).

As measured by O_2-evolution and the fluorescence kinetics, photosynthesis was completely inhibited by 20 minutes of O_3 exposure (total introduced O_3 = 3.7 μmoles, Heath et al. 1982). Under these conditions, the kinetics of chlorophyll fluorescence (in the dark-adapted alga, measured as yield excited by one actinic light; with the level before actinic light exposure equal to Fo) were also examined. After 2 minutes of O_3 exposure, the initial peak of fluorescence became higher (Fv/Fo = 3.0-3.2) than that initially (Fv/Fo = 2.2) but was significantly inhibited (Fv/Fo

= 1.4) by more prolonged exposure (4x). The value of Fo rose by about 10% during the first 5 minutes, but then fell to less than 0.4 after 20 minutes. The decline in Fo indicated a general disruption of the cell and was consistent with the dramatic decrease in the ability to hold metabolites within the cell and pump ions into the cell (Heath 1984a). Furthermore, the cell's ultra-structure underwent a dramatic alteration at this extended time of exposure, suggesting a general cellular collapse (Swanson et al. 1982).

The rise and subsequent decline in Fv/Fo suggest a change in electron flow or energy transduction. Higher Fv/Fo indicates a blockage in electron flow out of PS II (similar to the addition of DCMU or a lack of CO_2) and is consistent with the rise in Fo. The later decline suggests that either energy is being used more efficiently in photosynthesis (which is not supported by the oxygen evolution data) or, more probably, energy transduction is being altered.

In this period, the permeability and membrane properties of the *Chlorella sorokiniana* are being changed such that ion distribution is no longer normal (Heath 1987b, Heath and Castillo 1987). It can be argued that in this period (or equivalent dose), the ionic imbalance of the cell is beginning to affect the normal processes of photosynthesis. In this system, no studies of any of the photosynthetic enzymes were undertaken, but the data support the concept that initially (within 2 minutes) Ribulose Bisphosphate Carboxylase/Oxygenase (Rubisco) is being inhibited (Pell et al. 1994b).

Thus, the data collected on *Chlorella sorokiniana* argue that there are three phases of O_3 disruption: (1) an ion leakage and transport impairment of the plasmalemma induced by short exposure to O_3, temporarily leading to a rise in Fv/Fo and a slight inhibition of Rubisco and photosynthesis; (2) a loss of membrane permeability and depolarization of the plasmalemma leading to a fall in variable fluorescence with an apparent blockage of electron flow within the chloroplast; and (3) a general disruption of the integrity of the cell concurrent with a decline in dark level of the chlorophyll fluorescence yield (Fo). In retrospect, these data suggest that changes in fluorescence patterns would occur only upon extreme disruption of cells within leaves, corresponding to high O_3 levels in the atmosphere and gross visible injury. Therefore, many of these model studies must be equated with acute fumigations of plants, rather than chronic, except for very low doses at early time scales (Heath 1987a).

1.15.2 Lipid Alterations and Membranes

From studies on model systems, it seemed that the membranes of the cell were the primary target of O_3 for three reasons: 1) the plasma membrane was the first limiting region of the cell and so must be "hit" first; 2) electron microscopy showed that membranes were often altered or the internal structures of organelles were being changed, and so membranes must be altered to shift the internal structures; and 3) unsaturated fatty acids of membranes were highly reactive with O_3. One might expect that lipids within the membrane would protect enzymes within them, but Banerjee and Mudd (1992) showed in a model system that enzymes within lipid vesicles could be altered more rapidly than outside.

Certainly, in chemical systems, O_3 rapidly attacks double bond systems (Bailey 1958). One of the more studied chemical reactions of O_3 with lipid is the Criegee mechanism listed below, in which the O_3 molecule adds to the double bond with an oxygen bridge and a dioxygen bridge. This intermediate breaks down into a wide range of oxidative products (Fig. 1.21 and see Heath 1987b, for more details).

It was thus expected that fatty acids of plants, which have many double bonds, would be attacked by O_3 with breakage of the double bonds. Since these lipids play a significant role in the formation of membranes, that breakage would be expected to play an important role in altering membrane function, leading to a loss of membrane permeability. That alteration of membrane permeability would lead to gross instability and the death of the cell. Furthermore, some early studies found a compound of malondialdehyde, a marker for the oxidation of fatty acids (Thiobarbituric acid (TBA) reaction; see Heath and Packer 1965; Heath 1978). That linkage was not very well proven because sucrose itself can generate an orange color with the TBA reaction.

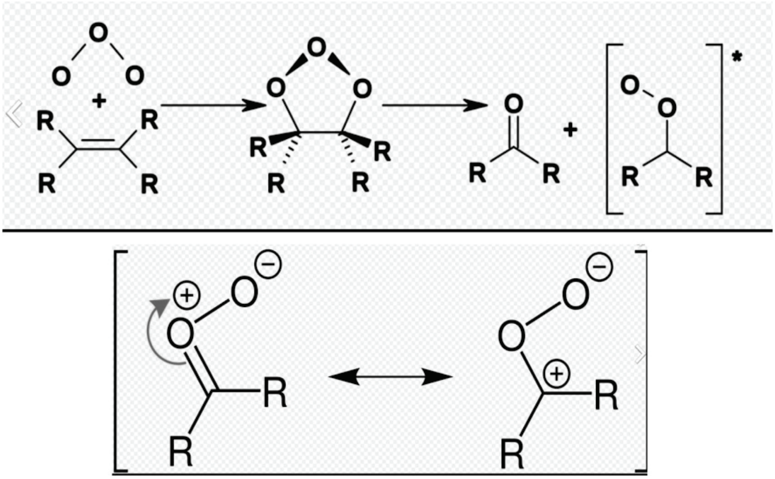

Fig. 1.21. Ozonation of a double bond within a fatty acid by the Criegee mechanism. For a complete diagram, see Figure 7 in Heath, 1989. There are 8 different units, mostly fragmentation from the carbon double bond. From Ozonolysis in Wikipedia Commons, the free media repository

Yet, it is difficult to study lipids in leaves since there are many pools of a great diversity of lipid types. Also, similar lipids are used in several of the membranes of organelles and the plasma membrane; the loss of one kind of fatty acid would not show a site of that loss. If one expected to have O_3 attack the plasma membrane lipids, they would have to be separated from lipids within each organelle: a difficult task. Thus, it was thought to approach lipid injury/damage by other methods, see Fong and Heath 1981. The concept was to label the fatty acids within the lipids with radioactive acetate: acetate on the leaf's surface was taken up by the cells by a passive transport and then entered into fatty acid synthesis via the acetyl-CoA molecule. Following a pulse of acetate, the leaf would then show changes in lipid turnover. This interesting idea was tried, but the acetate's entry was not as constant as it needed to be, leading to uncertainty in the turnover rates. Furthermore, in the 1970s, analysis of lipids was by TLC plates leading to difficulties in analysis.

To understand these lipids' turnover, Sakaki (1998) fed acetate directly to the leaves by applying a small drop of the solution before fumigation (two days). This allowed the acetate to enter the leaf with partial equilibration with the acetate pools within the leaf. The experiments of Fong and Heath (1981) were frustrated by variability when the labeling was done just before fumigation. Evidently, under those conditions, permeability and other O_3-induced metabolic changes altered the flow of acetate, and so the specific activity within the leaf was uneven. In the data collected by Sakaki et al. (1990), this did not seem to be a problem since the related amounts of fatty acids within the lipids measured by radioactivity were uniform for both fumigated and non-fumigated cases. Unfortunately, the fatty acid's specific activity was not stated in the publication to allow a complete evaluation.

In a similar experiment, Sakaki et al. (1985) followed the ratio of Diglycoside Diglyceride (DGDG) to Monoglycoside Diglyceride (MGMG) in spinach plants exposed to 0.5 ppm O_3 for up to 6 hours and found that the ratio declined from 1.82 to 1.37. These diglycerides are generally found in the thylakoid membranes of the chloroplast and, as such, would indicate changes in those membranes (Heath 1987b). There was no discussion of the amount of visible injury observed, which can be expected to be sizeable since spinach is generally very sensitive to O_3.

1.15.3 Transport of materials through the membrane

Early physiological studies by the Ting group regarding how O_3 exposure alters leaf discs' ability to take up sugars (Evans and Ting 1973, Perchorowicz and Ting 1974) or the tracer Rb for K (Evans and Ting 1974) demonstrated that uptake was stimulated by O_3 exposure suggesting an increase in permeability, and disc uptake is not the same as transport within the mesophyll cell structure. Most uptake is through the cut edge of the disc. Yet O_3 does change something within the leaf leading to faster uptake.

Primary studies by Thomson and his colleagues (Swanson et al. 1973) showed that after exposure of plants to O_3, the membranes in chloroplasts seemed to be disorganized as observed by electron microscopy and formed unusual EM structures. Furthermore, lipid bodies were more pronounced in chloroplasts in plants exposed to O_3, suggesting that the lipid metabolism or membranes were being altered. Certainly, membrane functions, such as membrane fluidity (Pauls and Thompson 1981), permeability by potassium exchange via ATPase reactions (Dominy and Heath 1985), and calcium exclusion (Castillo and Heath 1990), are rapidly altered by O_3 exposure. The similarity of O_3-induced membrane disruption to wounding responses (Langebartels et al. 1991; Kangasjarvi et al. 1994) would suggest a membrane trigger, perhaps a change in Ca^{2+} movements (Heath and Taylor 1997), leading to the induction of normal wound-regulated genes (Mehlhorn et al. 1991; Ecker and Davis 1987; Karenlampi et al. 1994).

Mudd et al. (1969) reported on the susceptibility of amino acids and proteins to O_3. Cysteine was the most susceptible amino acid, followed by methionine, tryptophan, tyrosine, histidine, cystine, and phenylalanine, listed in decreasing order of susceptibility. Proteins are associated with membranes and span the membranes as transport agents. If exposed to O_3, these membrane proteins would be expected to have sulfhydryls oxidized to -S-S- or even into $-SO_3$, as Mudd found. Indeed, significant alterations of both transport units and fragility in red blood cells are found (Mudd et al. 1996; Koontz and Heath 1979). Yet Mudd et al. (1997) found that O_3 did not react with the RBC membrane's lipids.

These observations lead to the hypothesis that O_3 induces a loss of membrane permeability and the inhibition of transport of some ions by membrane components' oxidation. In particular, a change in membrane function leading to a rise of intracellular Ca^{2+} would lead to the alteration of all sorts of intracellular metabolism, similar to that observed in the wounding of plant tissue. Castillo and Heath (1990) demonstrated that plasma membrane Ca^{2+} transport systems were *in vivo* altered by O_3 exposure, assayed after isolation from exposed plants with the inhibition of an efflux pump and stimulation of influx permeability.

Heath (1987b) also argued that the breakdown of O_3 in water could produce H^+ and alter the wall space pH. The loss of K^+ from and the gain of Ca^{2+} into the cell's interior coupled with an increased amount of K^+ outside and a shift in apoplastic pH could increase the loss from the cell of a wide range of metabolites, induce enzyme activation and alter normal gene transcription. Peroxidases (Castillo et al. 1984) and superoxide dismutase (Castillo et al. 1987) were stimulated, yet diamino oxidase (Peters et al. 1989) was inhibited by O_3 fumigation *in vivo*. Further, O_3 exposure lowers the amount of ascorbate acid within the wall (Castillo et al. 1987) and stimulates ethylene production, probably via a membrane wounding response (Tingey et al. 1976a, Wang et al. 1990). If the membrane potential depolarizes, the cell will likewise react as in wounding and respond to a pathogen attack (Boller 1991). Depolarization would then lead to the production of ethylene and the activation of extracellular peroxidases. Those sequential events could trigger a series of other metabolic responses, summarized in Heath (1994b), with experimental evidence given in a series of excellent papers (Langebartels et al. 1991, Schraudner et al. 1992). One should note the possible relation between wound-ethylene release and the ethylene-induced closure of the stomata (Taylor et al. 1988, see above). Thus, the two types of injury – "inhibition of photosynthesis" and "visible" – may be the interactions between an ethylene presence and the pathways that stimulate ethylene release.

1.15.4 Antioxidants

The interaction of ozone and plant tissues is mainly driven by three distinct processes: changes in external O_3 concentration, O_3 uptake into the leaf, and O_3 detoxification within the leaf. The diurnal pattern of detoxification does not necessarily match the diurnal patterns of external O_3 concentration and uptake (Heath et al. 2009; Wang et al. 2015), responsible for injury/damage to vegetation (Musselman et al. 2006; Mishra and Agrawal 2015).

As stated above, oxygen and its ultimate reaction compounds are very toxic to life. Often those toxicants are localized in specific regions or structures to limit further reactions. This limitation method may not always be successful, and thus, several sequences can stop various oxygen-derived compounds (see Table 1.5). The detoxification of varied radicals generated by O_3 within plants was discussed by Heath (1987b, 1999) and has been linked to several antioxidant systems with plants. In general, these reactions are noted in very humid air and with aqueous systems, much like inside cells. Those possible reactions involve hydroxyl radicals, superoxide, peroxyl radicals, and other postulated radicals of O_3. One potential antioxidant for lipid hydroperoxides is tocopherol, a lipid antioxidant (see Heath 1996c). While much work has been done on tocopherol reactions, little has been demonstrated in plants that tocopherol is critical to protect lipids from O_3 (compare Csallany et al. 1970 and Abbasi et al. 2007). In fact, in a pure chemical demonstration, Giamalva et al. (1986) showed that O_3 attacks tocopherol very poorly, and so a secondary type of reaction with an O_3 produced hydroperoxide must be expected to react with tocopherol.

Table 1.5. Various enzyme systems drive antioxidant reactions. Adapted from Heath 1999, Table 2. GSH and GSSG are glutathione (reduced and oxidized). Of note is that peroxidase reactions have often been measured using substrates linked to a specific NADH reaction (see Castillo and Heath 1990). Many of these enzymes are isozymes, similar reactions but differing protein structures found in various organelles. The latter three reactions are linked so that ascorbate levels can remain virtually intact by speeding the production of H_2O_2 and using GSH to detoxify it, through varied GSSG reductases

Catalase	$2\ H_2O_2 \rightarrow O_2 + 2\ H_2O$
Peroxidase*	$2\ H_2O_2 + NADH \rightarrow NAD + O_2^- + H^+$
Superoxide Dismutase	$2\ O_2^- + 2\ H^+ \rightarrow O_2 + H_2O$
Ascorbate Peroxidase	$2\ H_2O_2$ + Ascorbate → Dehydroascorbate + $2\ H_2O$
Glutathione Peroxidase	$2\ H_2O_2 + GSH \rightarrow GSSG + 2\ H_2O$
Ascorbate Reductase	Dehydroascorbate + 2 GSH → GSSG + Ascorbate

Early experiments showed a protective role of ascorbic acid (AsA) against O_3 injury in pinto bean plants (Freebairn 1960). Since then, several papers have supported the view of a direct correlation between AsA content and O_3 tolerance (Mächler et al. 1995). Over the years and decades, it was admitted that O_3 tolerance in a given plant species or cultivar could only be somewhat predicted based on its AsA content.

The apoplastic ascorbate appears to be a good candidate to react with O_3 and Reactive Oxygen Species (ROS) (Polle et al. 1995, Luwe et al. 1993, Plöchl et al. 2000, Turcsanyi et al. 2000, Wang et al. 2015). The apoplastic ascorbate accounts for less than 10% of the total leaf ascorbate amount but plays important roles in antioxidant defense, perhaps due to its position within the membrane and the entry of toxic species (Noctor and Foyer 1998, Pignocchi and Foyer 2003, Dumont et al. 2014, Yendrek et al. 2015). However, many studies showed that a higher AsA content is not the only possible mechanism involved in O_3 tolerance (Ranieri et al. 1999, Conklin and Barth 2004, D'Haese et al. 2005, Dizengremel et al. 2013; Grantzet al. 2013, Dai et al. 2018). The problem of regenerating oxidized ascorbate via the ascorbate-glutathione (AsA-GSH) cycle located in cytosol and organelles was raised, pointing out a possible better role of glutathione as the main antioxidant (Dizengremel et al. 2013, Dusart et al. 2019a, b).

Guzy and Heath (1993) examined enzyme activation in a slightly different method using 12 cultivars of the bean, differing in their sensitivity to O_3, fumigated with O_3 in a growth chamber. Each fumigation was ended as soon as the most sensitive variety began to show waterlogging at the leaf margins, so fumigation duration varied from 75 to 135 minutes. O_3 concentration remained constant but high during the fumigations at approximately 0.4 ppm. Assays were done on stomatal conductance, photosynthetic rate, total ascorbate levels, free sulfhydryl concentration, and a non-specific peroxidase.

The major conclusions (Guzy and Heath 1994) were:

> *Some O_3-tolerant varieties appeared to lower chlorophyll loss solely by blocking O_3 entry through decreased stomatal conductance, whereas other O_3-tolerant varieties appeared to mitigate O_3 damage through the action of the antioxidants, ascorbate, and non-protein sulfhydryl. Similarly, O_3-sensitive varieties showed possible alternative mechanisms of sensitivity. Damage appeared to derive from either comparatively high stomatal conductance or comparatively low levels of antioxidants despite low stomatal conductance.*

In other words, O_3 toleration can be accomplished in different ways. Moreover, a ranking of chlorophyll loss versus the varied processes did not correlate with much of anything measured. This study shows how difficult it is to "dose" the plant with a known and constant O_3 level and then examine processes that are complex and difficult to measure.

1.15.5 Phytohormones

Current concepts of oxidant-induced alterations of plants involve major systems (reviewed by Heath 1999, see above), including varied ROS species able to generate wounding proteins and activate ethylene production and lead to premature senescence. The activation of some or all of these systems rapidly leads to an early loss of productivity combined with the inability to remobilize nutrients elsewhere in the plant. The involvement of phytohormones in plants' response to O_3 was largely admitted early on, even though they were not considered responsible for the primary response but rather as signals of stress (Meyer et al. 1987).

1.15.5.1 Auxin

Frits Went, a Dutch botanist, discovered auxin's role as a plant growth substance (Went 1926) and proposed the oat coleoptile curvature test for a bioassay of auxin (Went, 1928). The first compounds considered as auxins (auxin a and b, heteroauxin) were isolated from human urine by Kögl and Haagen-Smit (Kögl and Haagen-Smit 1931; Kögl et al. 1934). In 1933 Went moved to the USA, working at the California Institute of Technology, where Haagen-Smit joined him in 1937. A few years later, Haagen-Smit isolated Indole-3-acetic acid (IAA or heteroauxin) from higher plants (Haagen-Smit et al. 1941). At the beginning of the 1950s, when the damages caused to plants by smog were worrying, Went's group discovered that smog decreased the Avena test's coleoptile sensitivity and proposed this bioassay to quantify low amounts of photooxidants in the smog (Hull et al. 1954).

Interestingly, it was recently observed that ROS production induced by oxidative stress, such as O_3, can alter auxin gradients and auxin-mediated signaling in plants (Blomster et al. 2011; Choudhury et al. 2017). Auxin is probably implicated in the adaptive strategy to withstand the stressful O_3 impact through redox-dependent ROS-auxin crosstalk (Tognetti et al. 2012). Sixty years separate the first observations of auxin inhibition under smog from the current research on altering hormone action under oxidative stress. Moreover, these relations show that plants are an integrative system, where one incidence of stress can provoke changes in many, seemingly unrelated pathways.

1.15.5.2 Ethylene

In the early 1990s, it was clear that multiple independent pathways did interact after O_3 exposure (see Heath 1994b). An attempt was made to link variable pathways to a membrane injury's initial event (Fig. 1.22A). Early studies showed that ethylene was involved in O_3 injury: however, the cause and effect were poorly linked in these experiments (Craker 1971, Tingey et al. 1973, 1976a). Due to its known link with the wounding of plants, ethylene was soon reported to be produced by plants fumigated with O_3 (Craker 1971). Ozone was delivered at a relatively high concentration (250 ppb), and, after only 2 hours of fumigation, the production of ethylene was accompanied by progressive leaf damage. With similar high concentrations of O_3 delivered to a series of plants, a good correlation was observed between ethylene production and a concentration of O_3 up to 750 ppb (Tingey et al. 1976a). Interestingly, in this study, wheat was largely more sensitive to O_3 than corn (see Fig. 1.19). Ethylene, produced by oxidative impact before a visible injury occurs, was associated with the further development of early senescence and plant tissue injury (Craker 1971).

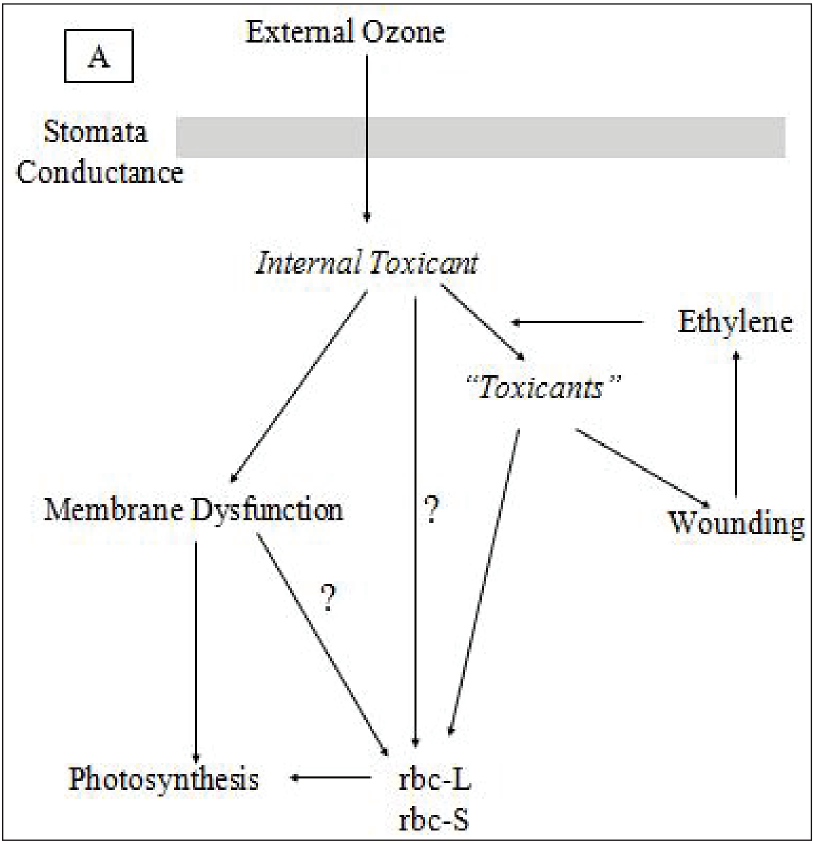

Fig. 1.22.A. Multiple pathways and branching of varied toxic species. A toxicant produced by O_3 can have multiple effects. Redrawn from Heath 1994b,

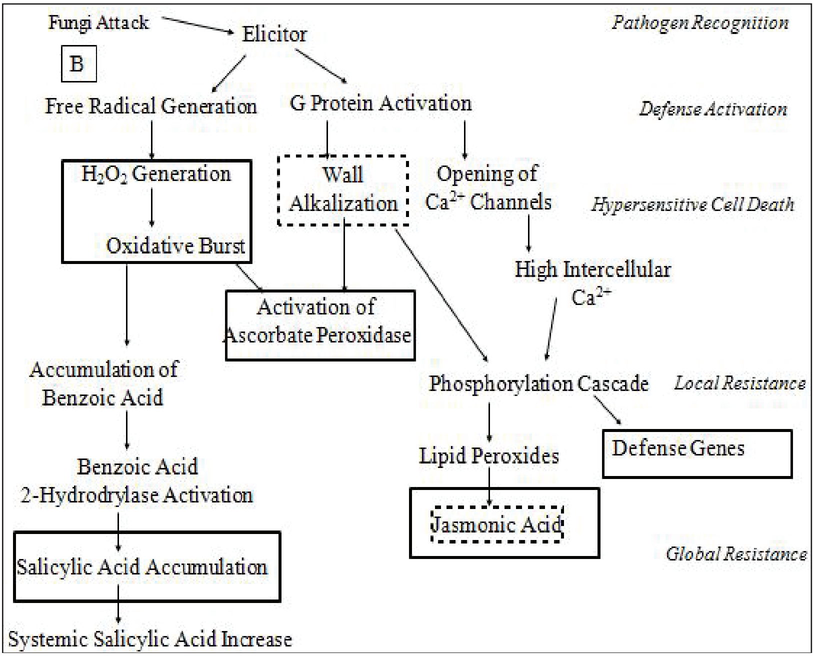

Fig.1.22. B. Branched pathways generated by a fungi attack leading to varied wounding species and activation of defensive genes. Adapted from Ward et al. 1991, Lamb and Dixon 1997, Simon-Plas et al. 1997

As research continued, it was clear that plants' pathogen attack activated similar pathways, and alterations of membrane function were the primary stimulus of the remaining systems (see Fig. 1.22B for a more modern diagram of how antioxidants, internally generated H_2O_2, and wounding were linked). Most importantly, defense genes were stimulated and produced more globally compounds used to activate regions of the plant that are not immediately involved, such as salicylic acid and jasmonic acid. No doubt, we are just beginning to understand these relations and how genetic stimulation is carried out.

Polyamines were also implicated in O_3 toxicity as there was an activation of some of the polyamine pathways (Bors et al. 1989; Ormrod and Beckerson 1986). Furthermore, it was known that polyamines could eliminate some radical reactions (see Drolet et al. 1986) linked to O_3 entry into the plant (see above). The linkage of the activation of ethylene formation and polyamines was ultimately shown by the pioneering studies of Langebartels of Sandermann's team (see Langebartels et al. 1991). One can understand the difficulty of these studies by examining the known pathways of ethylene and polyamines (Fig. 1.23). They are interlinked but activated by different systems, including other phytohormones. Furthermore, while the activation of the pathways may be somewhat rapid, the actual formation of ethylene is slightly delayed due to the need for other metabolic conditions to be present, the gas of CO_2 and auxin.

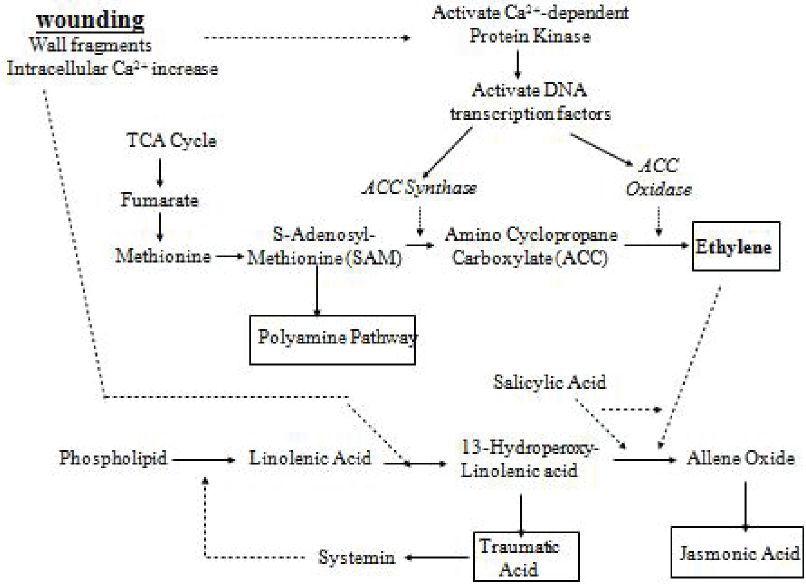

Fig. 1.23. Involvement of the polyamine and ethylene pathways with cell wall wounding and activation of defense pathways. Compare with Fig. 1.22B. Synthesis of newer work by authors

Twenty years later, it was shown that a significantly lower O_3 concentration (80 ppb) delivered for 5 hours a day to potato induced ethylene production after 3 days of exposure (Glick et al. 1995). The increase in ethylene level correlated with a decrease in the rbcS mRNA transcript (a small subunit of Rubisco). These changes preceded a decline in Rubisco and accelerated senescence with visible damages on leaves (Glick et al. 1995).

1.15.5.3 Secondary metabolism

As mentioned above (paragraph 1.1.3.2), PEP was increasingly used under O_3 exposure to produce secondary compounds. In isoprenoid-emitting plants, the synthesis of isoprenoids, which play an antioxidant role, was increased (Ormrod et al. 1995, Valkama et al. 2007, Vickers et al. 2009). As early as the mid-1970s, it was shown that O_3 exposure caused an accumulation of flavonoids in the leaves of soybean (Keen et al. 1975) and of phenolics in loblolly pine seedlings (Tingey et al. 1976b). Ozone is now well known to cause increases in activities and gene transcription of a series of enzymes of the shikimate pathway and the phenylpropanoid pathway in leaves, leading to increased production of phenolic compounds and monolignols (Rosemann et al. 1991, Tuomainen et al. 1996, Booker and Miller 1998, Dizengremel 2001, Cabané et al. 2004, Betz et al. 2009). In poplars exposed to O_3, an increase in lignin in the wood was observed, counterbalanced by a decrease in other cell wall components (Richet et al. 2012). In particular, there is an increase of the lignin to cellulose ratio, which would allow the tree to maintain radial growth while minimizing carbon cost (Richet et al. 2011).

It is well defined that O_3 exposure can shift the normal homeostasis within the plant and that the effects are multiplied within the varied pathways (Heath 2007). Undoubtedly, some of the differences in observations of various species and varied fumigation regimes are the varied activations of these multiplicity pathways. We expect that much more will be clarified as the interrelations between pathways and activation processes become better understood.

1.16 Steps of control of O_3 uptake

It would be delightful to say that research on O_3 interaction with plants forced governments to crack down on the air; however, the driving force of the issue was O_3 being unhealthy for humans. This research led to the "primary" standard for air quality (in the USA) or, what was the highest level of O_3 that induced no changes in human health? The "secondary" standard was set by the levels of O_3 that induced "no alteration in plants' productivity."

In California, state standards were set by an early committee that turned into the California Air Resource Board with a clear mandate to set urban air basin standards. It became clear that the reaching of those standards would require a national effort. Cars sold in the US had to be made to release the various toxicants that internal engines made; the oxides of nitrogen emitted (see equation 1-1) had to be reduced to near zero. Ultimately, in the mid-1970s, the United States' federal government, passed a complex law called "The Clean Air Act," which continues to be modified. This law generated the concept of primary and secondary standards and required the Environmental Protection Agency (EPA) of the US to set the standards based upon reports generated by scientific research and written by scientists. Each decade the oxidant standard (used O_3 primarily) had to be re-thought and have a revised report.

The current US secondary standard (see National Ambient Air Quality Standards for O_3, 2015, including the primary standard) is 0.070 ppm averaging time over 8 hours linked to an annual fourth-highest daily maximum, averaged over 3 years.

Similarly, the European scientific community drew the policymaker's attention to the problems caused by air pollution to the health of human people and vegetation. Thirty-two countries in the pan-European region decided to cooperate to reduce air pollution. In 1979, they signed the Convention on Long-range Transboundary Air Pollution (LRTAP), negotiated under the auspices of the UNECE (United Nations Economic Commission for Europe), creating the first international treaty to deal with air pollution on a broad regional basis. The LRTAP Convention's objective is to limit and, as far as possible, gradually reduce and prevent air pollution over a long range and trans-boundaries. Like the US, the success of the Convention in air pollution abatement is linked to its solid scientific underpinning. This was established by developing a common knowledge base, including an extensive international network of scientists of various disciplines aimed at joint monitoring and modeling programs. Besides, the Convention has provided a platform for scientists and policymakers to exchange information. Initially mainly centered on acid rain and sulfur and nitrogen oxides, the Convention's work later included O_3. The Gothenburg protocol signed in 1999 was set up to abate acidification, eutrophication, and ground-level O_3. It entered into force in 2005, and a revised protocol was adopted in May 2012.

For the protection of vegetation, from May to July each year, the target value would be (AOT40 based, calculated from 1h values) 18,000 µg/m3·h averaged over five years.

1.17 Metrics: a tool for determining responses

Unfortunately, the science of air quality and its interactions with plants was too good. Enough information was gathered to show that the air quality of urban regions had to be improved to prevent damage within many regions and would not alter important ecological areas "down-wind' from where it was produced. Political forces demanded that "we develop a metric that could be used region-wide to regulate the amount of O_3 that could be in the atmosphere but would not harm plants".

Yet plants vary in their response to O_3 because many bioprocesses interact. Those interactions must be taken into account and averaged such that one level of O_3 was at the tipping point between normal growth and injury. A simple threshold would not do since environmental conditions vary in each region and with each growing plant. The scene was set for a long and quite active battle between varied metrics that could be expressed: how to weight conditions of O_3 according to how plants expressed injury (Musselman et al. 2006). In other words, the thresholds improve dose-response relationships and might account for the detoxifying capacity of leaf tissues. Some of the attempts are shown below in Table 1.6.

It was not satisfying to express the effect of O_3 as a function of the time plants are exposed to the pollutant. The first indexes for estimating the O_3 impact were using the cumulative concentration of atmospheric O_3 near the plant. Several indexes have been developed over the past few decades, using different metrics to relate O_3 concentrations to the pollutant's measured factor. Mainly in the USA, the first metrics in the 1980s were the average O_3 concentration delivered during the growing season and daylight (7h = M7 or 12h = M12). The accumulated daylight O_3 concentration (sum over 8 hours) was used, and a threshold for injury was chosen. This was the case for SUM06, which is the sum of hourly concentrations greater than 0.06 ppm and, primarily in Europe, AOT 40, the accumulated amount of O_3 over a threshold value of 40 ppb. The W126 standard, also used in the USA, is the sum of hourly concentrations during a growing season weighted by a sigmoidal function, such that higher concentrations are given more weight.

Table 1.6. Successive modes of expression of the threshold for O_3 toxicity. The SUM00 index is the sum of all mean hourly concentrations (in ppb.h). AOT40 is the Accumulated amount of O_3 over a Threshold value of 40 ppb. D represents the detoxifying cellular power. CUO: Cumulative Uptake of O_3. POD_Y (Phytotoxic O_3 Dose) is the accumulated stomatal O_3 flux above a threshold of Y nmol m^{-2} s^{-1} below which it is assumed that any O_3 molecule absorbed by the plant will be detoxified

index	measurement	parameter	unit
atmospheric concentration-based	$[O_3]$	SUM00 AOT40	ppb.h or ppm.h
stomatal flux-based	O_3 uptake	ΣO_3 Flux CUO	mmol O_3 m^{-2}
integrated flux	effective *phytotoxic* flux ?	$\Sigma(O_3$ Flux-D) PODy	mmol O_3 m^{-2} *with y in nmol.$m^{-2}s^{-1}$*

At the end of the 1990s, the scientific community estimated that the concentration of O_3 outside the plant was not a useful index since it only considers the concentration of O_3 around the leaf. An improvement was thus made by taking into account the uptake of O_3 through the stomata (CUO or cumulative uptake of O_3), and a further step was recently made (around 2009) by trying to integrate the detoxifying capacity (POD or phytotoxic O_3 dose). PODY, is the accumulated stomatal O_3 flux above a threshold flux of Y nmol m^{-2} s^{-1}, i.e.:

PODY = $\sum$ max (Fst − Y, 0) dt, where stomatal flux Fst, and threshold, Y, are in nmol m^{-2} s^{-1}. This sum is evaluated over time, from the start to the end of the growing season. The estimated accumulated stomatal flux, POD (in mmol m^{-2}) was determined by summing hourly O_3 uptakes. The hourly O_3 uptake (in mmol.m^{-2}.h^{-1}) was estimated by the integration of rapid uptake (Fst, in nmol m^{-2} s^{-1}) over an hour with Fst = 0.663 gs $[O_3]_{atm.}$ Fst is O_3 flux entering the leaf; gs is the stomatal conductance to water vapor, and $[O_3]_{atm}$ is the O_3 concentration around the leaf. The leaf boundary layer resistance was considered negligible compared to the stomatal resistance and was not considered in the calculation of O_3 fluxes.

PODy, derived from flux-based methods, integrates instantaneous O_3 flux thresholds to determine the cumulative stomatal O_3 uptake. Such thresholds improve dose-response relationships and may account, in part, for the detoxifying capacity of leaf tissues. The problem of integrating the detoxification power of leaf cells as a parameter in the indexes for O_3 risk assessment is still debated (Dusart et al. 2019a). A major sticking point is how to obtain good values for stomata responses and the detoxifying capacity of each cultivar and species within a controlled region.

However, to evaluate the current and predict the long-term effects of O_3 on plants at larger scale than the cell or individual plant, the concentration-based metrics, either seasonal mean daytime O_3 concentration (M7, M12), accumulated daytime hourly O_3 concentration above a threshold (SUM06, AOT40) or continuously weighted growing season averages (W126) are still in use (Wang and Mauzerall 2004; Van Dingenen et al. 2009; Avnery et al. 2011; Anav et al. 2016).

A leap in experimental evidence of O_3-derived injury patterns was made with better fumigation facilities, open-topped chambers (see above). This "final" exposure chamber was used to formulate the above varied metrics. As argued above, these chambers were a great system, but because of the mixing of O_3 with ambient air and the enclosure, they should not be an endpoint; however currently, possible improvements, which will be expensive, seem to be unsupportable.

Initially, the plants used were of obvious agricultural importance: those plants that exhibited visible injury lowered the economic value and production index. After many observations, it was equally clear that plants in an ecological setting were likewise altered, often those of low economic value, and deemed unimportant to political consideration. These alterations tend to weaken the plant, generally, trees, such that other diseases or insect pests could kill the tree, eliminating all economic value. Now all countries could see that urban air, moving into important ecological areas for tourists and the harvesting of trees, would be critical and needed studies. Enough information was gathered to show that urban regions' air quality had to be improved to prevent damage within the region and ecologically important areas "down wind" of where it was produced.

Changes in the political environment, specifically funding, made scientists trained in plant production move into other related fields – such as relating to Earth's rising CO_2 level. In our opinion, that shift virtually eliminated research in oxidant air components and plants. No funding meant fewer students were entering the area. Thus, with the aging and ultimate retirement of the current researchers who are coupled to the improvement in techniques, they will be permanently lost. It will take a significant effect to rebuild these downwind resources.

Perhaps this loss is much like the loss of buggy whip manufacturing – not important. But we wish to point out that while O_3 has been studied well and its interaction is partly understood, the urban air contains many more potentially-toxic compounds, and we know virtually nothing about those interactions with plants or humans.

1.18 Concluding Remarks

The research history on the impact of O_3 upon plants is only around seventy years old. During the first half of this period, two landmark papers can be distinguished. In 1952, Haagen-Smit demonstrated that O_3, a secondary product of the photochemical smog, was responsible for smog's visible damage (Haagen-Smit et al. 1952). Thirty-five years later, Reich debated in a premonitory paper about most of the problems linked to the mode of action of O_3 on crops, trees, and ecosystems, even though the differences between species were thought to be restricted to differences in the stomatal conductance (Reich 1987). The next improvement came from utilizing the "omics" techniques to better identify pathways and genes implied in response to O_3 (Heath 2008, Renaut et al. 2009, Soltani et al. 2020).

It appears now that multiple parameters are implied in the toxicity of the O_3 flux into the plant (Heath et al. 2009). This kind of scientific dialogue reappears in the research on O_3-induced injury. As early as 1958, Todd clearly described plants' dual response to O_3 in Los Angeles, showing either visible symptoms of injury on leaves or reduction of growth without visible damage. It was recently proposed that visible foliar symptoms or growth reduction would be plants' strategies to counteract the O_3 attack (Marzuoli et al. 2019), even though the two processes could concomitantly or successively occur. Besides, there is a time-dependency of the response of parameters such as visible injury, impairment of leaf gas exchange, and tree-ring width when determining the O_3 risk of different tree species exposed at differing phenological stages (Novak et al. 2007).

The impact of the damages caused by O_3 on cultivated plants was linked early to economic losses. As soon as 1949, in the Los Angeles basin, the monetary losses for direct injury on eleven crops were estimated to exceed US$480,000, reaching US$3,000,000[10] four years later (Middleton 1956). Half a century later, the estimated global crop yield

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[10] Inflation may increase this number from 15-20x.

losses due to current O_3 concentrations range from 3% to 5% for maize, 6% to 16% for soybean, and 7% to 12% for wheat, representing economic losses of \$14 billion to \$26 billion (van Dingenen et al. 2009; Ainsworth et al. 2012). For rice, the estimation varied from 4% loss (van Dingenen et al. 2009) to 17% loss (Feng and Kobayashi 2009). In the United States, agronomic crop loss to O_3 is estimated to range from 5% to 15%, with an approximate cost of \$3 to \$5 billion per year, whereas in Europe, for 23 crops in 47 countries, the economic cost is estimated to be \$9.6 billion per year (Ainsworth et al. 2012). The negative effect of O_3 accounts for a 40% loss in Asia (primarily, China and India), which may be due to the higher sensitivity of Asian cultivars to the pollutant (Emberson et al. 2009). Due to O_3 concentrations in 1990, estimated losses in China, Japan, and South Korea were 1–9% of yield for wheat, rice, and corn and 23–27% for soybean, with an associated economic cost of US\$ 3.5, 1.2, and 0.24 billion, respectively, for those three countries (Wang and Mauzerall 2004). Ozone remains at potentially phytotoxic levels in Asia (Sicard et al. 2017). By contrast, there is a gap in knowledge about the effects of O_3 on crop production in Africa (Ainsworth et al. 2012) even though recent studies (Hayes et al. 2019) show a strong sensitivity of wheat and bean to O_3 while finger and pearl millet appear relatively tolerant. The two millet species belong to the C4 type, which could at least partly explain the result. It was early shown in forests that O_3 was causing severe damages to ponderosa pine in southern California (Parmeter et al. 1962; Miller et al. 1963). The extent of conifer forest damaged by photochemical pollution increased from 25,000 acres in 1962 to 100,000 acres in 1969, with Jeffrey pine also injured (Miller and Millecan 1971). Similar symptoms of O_3-caused foliar injury were observed for white pine in the eastern USA (Berry and Ripperton 1963). Fifty years later, it was estimated that current ambient O_3 concentration could decrease tree biomass by 7% (Wittig et al. 2009), knowing that the results obtained with young trees could hardly be extrapolated to mature trees growing in forests (Ainsworth et al. 2012).

A synoptic scheme can easily represent the timing of the main discoveries in the field of O_3 and plants (Table 1.7). Most of the discoveries made after 1990 have resulted from the improvement of technological tools since the fundamental ideas were already present in the discussion part of some landmark papers.

While the main purpose of this chapter is to focus on the story of O_3 and plants over decades, we propose some paths for future research:

[1] The parameters for offering reliable O_3 risk assessment models must be improved. Better consideration of phenology must also be taken into account (Anav et al. 2018). Visible injury as an index must be made

into a digital/computer-driven photo index to eliminate human-based estimates. The role of primary production of nutrients and secondary products needs to be better integrated. Measurements of amounts of flows of antioxidants need to be made more precise, and any metric index must use those findings rationally. Also, productivity should be better expressed on a leaf mass basis rather than a leaf surface area (Feng et al. 2018). In relation to the above point, it should be attempted to reconcile the individual plant's changes to be integrated into plant growth and survival at the ecosystem level. Physiological and meteorological models must be reconciled (Li et al. 2016, Emberson et al. 2018). These problematic tasks are challenging but necessary to help to deliver recommendations to stakeholders.

[2] In the future, the O_3 level will continue to increase in Asia, potentially damaging crops and forests. In the USA and Europe, O_3 will still be dangerous for ecosystems, especially in the southern part of Europe and the Eastern part of the US, due to normal airflow. In this context, the current indexes for risk assessment are not operational and must be improved: the detoxifying barrier remains challenging for improving the metrics (Dusart et al. 2019b). Moreover, stomatal uptake metrics (POD) may not necessarily be better than the old concentration-based AOT40 (Anav et al. 2016).

[3] Bring Large Data Techniques and Artificial Intelligence (AI) technology forward. It may be that the use of large data (e.g., the extensive monitoring of fields with their plants) may be able to bring out better relations between the exposure of O_3 and the final effects of O_3 on various plants. In most cases in the past, only a few parameters have been measured simultaneously. AI has been used to sort out relationships in complex systems[11] and clearly, plant scientists need to use these concepts.

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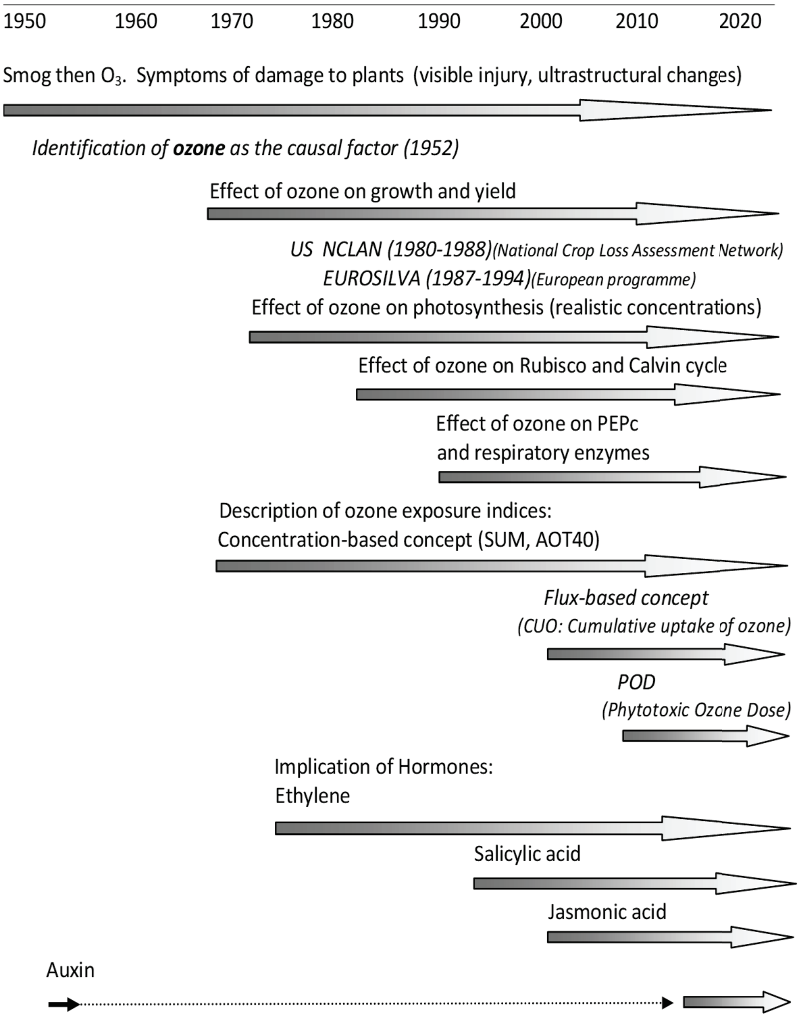
[11]. See Artificial intelligence, Section 7—health care, under Wikipedia, the free encyclopedia.

Table 1.7. Synoptic table of the evolution of research on O_3 and plants



[4] With the improvement of the metrics used to measure the impact of O_3 on leaf tissue, a scheme with the different steps following O_3 entering the leaf through stomata must emerge: avoidance linked to a possible stomatal closure and resistance linked to a variable ability of the antioxidant systems should be related to counteracting the deleterious oxidative damage of membrane constituents and enzyme proteins

(Dizengremel et al. 2008, Matyssek et al. 2012; Dizengremel et al. 2013). The role of signaling pathways, characterized by intricated relationships between ROS and hormones, remains to be clarified to better understand the switch from successful physiology towards programmed cell death.

[5] It is still necessary to combine relatively short experiments in controlled conditions and longer site-driven experimentations with individual or mixed species (Cailleret et al. 2018). An effort is needed to do upscaling through collaborative research to integrate biochemical damages at the cell level to multi-annual effects on growth and productivity in forest stands, crop fields, and ecosystems (Felzer et al. 2007, Jolivet et al. 2016, Emberson et al. 2018). Another examination of which model system could be successfully used to obtain insightful hypotheses of plant effects must be undertaken.

[6] Studies on the combination of O_3 and drought, increasing CO_2, temperature, and nitrogen "fertilization" must be continued to understand their combined impacts on plant growth and productivity. There also needs to be an understanding of how O_3 pollution contributes, via the differential sensitivity of plant species and cultivars, to shifts in where agricultural and ecological plants will be forced to move by the global climate changes. This leads to a remark of Cailleret et al. (2018, 1378), who interestingly pointed out the need to move from an "air pollution" perspective to a "forest growth" perspective. Last but not least, the possible implication of O_3 in current land-use changes and assisted migration of plant species must be planned and carried out (Vitt et al. 2010, Alfaro et al. 2014).

Great strides have been made over the last 70 years to understand how O_3, released in the atmosphere through human activities, will alter plant growth and productivity, but we cannot rest upon what we now know. That limited knowledge is only a start due to how complex biological systems function. The release of greenhouse gases, among them O_3, affects climate on a global scale since these gases modify radiative transfer and thus change the energy balance of Earth, which is entering a new geologic era, the Anthropocene (Barnosky et al. 2012). In this context, the atmosphere is polluted not just with O_3, but many other oxidants, which may be uncontrolled and certainly have not been studied. While useful, our current knowledge will not be enough to prevent a global collapse due to humankind's interference.

Acknowledgments

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CHAPTER TWO

TROPOSPHERIC OZONE, FORMATION, DISTRIBUTION, AND TRENDS OVER TIME

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Abstract

Tropospheric ozone (O_3) is the third most important greenhouse gas that is transported from the stratosphere or formed locally by photochemical reactions among precursors such as nitrogen oxides (NOx), carbon monoxide (CO), methane (CH_4), volatile organic compounds (VOCs), and peroxyacetyl nitrate (PAN). The lifetime of tropospheric O_3 is long enough, i.e., a few days in the boundary layer to a few months in the free troposphere, to allow its (and its precursors) long-range transport from the regional to hemispheric scale through trans-Atlantic, trans-Pacific, and trans-Eurasian transport. Therefore, remote areas such as the Arctic region can be affected. The current annual mean background O_3 concentration at the surface layer is 35–50 ppb in the Northern Hemisphere and 15–25 ppb in the Southern Hemisphere. At regional and local scales, O_3 production depends on the VOCs/NOx ratio. Since the 1990s, anthropogenic O_3 precursor emissions have decreased in North America and Europe, while eastern Asian emissions have increased. Therefore, both mean and peak O_3 concentrations decreased in North America and Europe, and were raised in East Asia. The reduction in O_3 means that concentrations and higher percentiles have been associated with reductions in NOx and VOC emissions in the European Union and North America since the 1990s. The increase in lower concentrations and percentiles can be attributed to a reduction in local NOx emissions, due to, e.g., vehicle emission controls, resulting in lower O_3 titration by NO. Generally, the global background O_3 increase can be driven by the net impacts of climate change, i.e., increased stratospheric O_3 inputs, higher CH_4 emissions, changing lightning NOx emissions, weakened NO titration, and impacting reaction rates, through sea surface temperatures and relative humidity changes.

Keywords: Greenhouse gas; Photochemical reactions; Climate Change; Temperature; Humidity

2.1 Ozone formation and spatial distribution

Tropospheric ozone (O_3) is a secondary air pollutant formed by photochemical reactions among precursors emitted by anthropogenic and natural sources such as nitrogen oxides (NOx), carbon monoxide (CO), methane (CH_4), and volatile organic compounds (VOCs) as well as peroxyacetyl nitrate (PAN), principal tropospheric reservoir species for NOx and hydroxyl OH radicals (Milford et al. 1994; Fischer et al. 2014; Monks et al. 2015). Ozone is the third most important greenhouse gas resulting in a direct radiative forcing of 0.35–0.37 W m^{-2}, contributing to climate change (Unger et al. 2006; Shindell et al. 2009; Stevenson et al. 2013; Kulkarni et al. 2015). To date, tropospheric O_3 pollution is a significant air quality issue for human health, biodiversity, vegetation, and climate over large regions of the globe (Proietti et al. 2016; Ochoa-Hueso et al. 2017; Sicard et al. 2017; Fleming et al. 2018; Lefohn et al. 2018; Mills et al. 2018; WMO 2018; Agathokleous et al. 2020; Sicard et al. 2020a; Sicard et al. 2021; Sicard 2021).

2.1.1 At the regional scale

According to the literature, the current annual mean background O_3 concentrations at mid-latitudes of the Northern Hemisphere range between 35 and 50 ppb at the surface layer (IPCC 2014), with the highest values occurring over Greenland and the Tibetan plateau and in the latitude band 15–45°N, particularly in summer (>50 ppb), while the lowest O_3 burden (<20 ppb) is recorded in central Europe and Southeastern Asia, particularly during the cold period.

Higher O_3 mean concentrations (>40 ppb) are observed at high-elevation areas, e.g., in the Rocky and Appalachian Mountains and over the Tibetan plateau (Fig.2.1). At high elevation, O_3 destruction by deposition and NO levels are lower (Chevalier et al. 2007), and solar radiation, biogenic VOCs emissions, the exchange between the free troposphere and boundary layer, and stratospheric O_3 inputs are more abundant compared to the surface (Kulkarni et al. 2011; Lefohn et al. 2012). In summer, air masses remain colder and dryer leading to lower O_3 losses by photolysis (Helmig et al. 2007).

The high O_3 mean concentrations (>50 ppb in summer) observed over the **Tibetan plateau** are attributed to the combined effects of i) the high-elevation surface (mean elevation of 4,000 m a.s.l.), ii) the strong thermal and dynamic forcing of the Tibetan plateau on regional climate associated with the large-scale circulation, and iii) the *in-situ* photochemical production due to the significant amount of O_3 precursors from road traffic, biofuel energy source, coalmines and trash burning (Tian et al. 2008; Liu et al. 2010; Chen et al. 2011; Guo et al. 2015; Wang et al. 2015; Sicard et al. 2017).

Low dry deposition rates for O_3 (<0.05 cm s^{-1}) over snow and ice surfaces and deserts (Wesely and Hicks 2000), higher insolation, downward transport of stratospheric O_3, local photochemical production, and large-scale transport are known factors to explain higher O_3 pollution over Greenland and deserts (e.g., Near East, Colorado Desert).

Over Greenland, seasonal mean surface-layer O_3 concentrations ranged from 25 to 45 ppb in agreement with observations ranging from 25 to 50 ppb (Rasmussen et al. 1997; Helmig et al. 2007). *In-situ,* O_3 production is the primary source of O_3 in the lower troposphere at high latitudes (Thomas et al. 2013). It can be attributed to local sources, e.g., NOx enhancement from snowpack emissions, thermal decomposition of PAN, summertime boreal forest fires, and ship emissions (Stohl et al. 2007; Law and Stohl 2007; Legrand et al. 2009; Walker et al. 2012). The PAN decomposition is an important source of NOx and O_3 at latitudes over 60°N (Walker et al. 2012), and the PAN to NOx ratio increases with altitude and latitude (Singh et al. 1992; Thomas et al. 2013). Long-range transport of O_3 and its precursors from Southeastern Asia and North America and stratospheric O_3 inputs contribute to background O_3 levels and peaks over Greenland (Helmig et al. 2007; Law and Stohl 2007; Derwent et al. 2010; Thomas et al. 2013). The anthropogenic and biomass-burning pollution from North America contributed to observed surface O_3 concentrations in Greenland (18% and 5.2%, respectively) in summer 2008 (Thomas et al. 2013); a 10% contribution from stratospheric air masses was found between 3 and 5km of altitude (Law and Stohl 2007).

For latitudes over 60°N, Arctic haze contains high levels of O_3 precursors such as NOx and VOCs (Solberg et al. 1996; Law and Stohl 2007) due to volcanic emissions, e.g., in Alaska, anthropogenic emissions from urban areas, oil industries, and shipping, in particular in northern Russia (Law and Stohl 2007).

In the Northern Hemisphere, the highest surface O_3 concentrations are observed in the latitude band 15–45°N. The O_3 transport by the large-scale Brewer-Dobson overturning circulation, i.e., an upward motion from the tropics and downward motion at higher latitudes, leads to higher O_3 concentrations in the extra tropics (Hudson et al. 2006; Parrish et al. 2012).

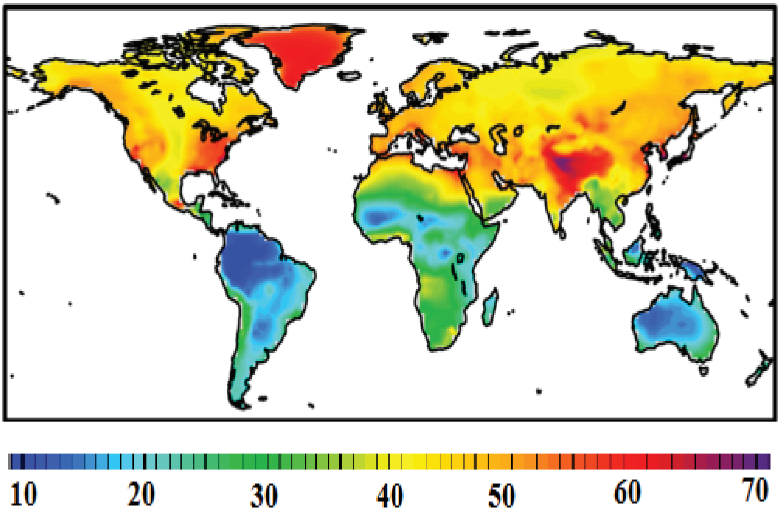

Fig. 2.1. Surface ozone average concentrations (in ppb) at the lower model layer for the GISS–E2–R model over the historical period (2000–2004). Extracted from Sicard et al. 2017

2.1.2 At the local scale

The local O_3 production depends on the VOC to NOx ratio (Kang et al. 2004; Toro et al. 2006; Pusede and Cohen 2012; Sicard et al. 2020b). Higher surface O_3 concentrations are measured in rural and remote areas compared to urban areas (Monks et al. 2015; Sicard et al. 2016, 2020b). The urban areas are characterized by a low ratio due to high NOx concentrations, where NOx tends to inhibit O_3 formation (Markakis et al. 2014). In urban areas, a reduction in NOx emission increases the O_3 formation (Kang et al. 2004). The highest O_3 concentrations observed at rural sites are due to the combined effects of i) O_3 and/or precursors' transport from urban areas, ii) higher biogenic VOC emission (mainly isoprene), and iii) low O_3 titration by NO (Sicard et al. 2016).

2.2 Temporal ozone variability

2.2.1 Daily

The typical diurnal O_3 variation coincides with the intensity of solar radiation and higher air temperature, i.e., during the morning and early afternoon. Ozone concentrations grow due to the photo-oxidation of O_3 precursors by OH° radicals and downward O_3 transport by convective heating (Pusede et al. 2015; Derwent et al. 2015). At night, ground-level O_3 concentrations remain low because there is no photolysis of NO_2 and photo-oxidation of O_3 precursors (Mavrakis et al. 2010), and coincide with the rapid destruction of O_3 by NO and no downward O_3 transport due to a stable air temperature profile (Mavrakis et al. 2010; Monks et al. 2015). In urban areas, the nighttime O_3 minimum is more pronounced than in rural areas due to the lack of O_3 titration by NO (Mavrakis et al. 2010; Im et al. 2013; Sicard et al. 2016).

A weaker diurnal O_3 variation, or nighttime maxima, can be observed at high-elevation sites, above 1500 m a.s.l., where the nocturnal mixing layer height is minimum, leading to higher O_3 concentrations by containment (Chevalier et al. 2007; Woodman 2010; Lin et al. 2017). During the nighttime, VOCs' oxidation is dominated by reactions with nitrate radicals and affects O_3 formation (Stutz et al. 2010).

2.2.2 Seasonal

During spring (March-April-May), the mean surface O_3 concentration experiences a peak (>40 ppb) over Greenland, the Tibetan plateau, and regions over latitude 60°N, e.g., Alaska and Siberia, whereas the regions in central Europe, eastern US and south-eastern Asia display a minimum (<30 ppb). Higher O_3 mean values (35–40 ppb) were already observed over Greenland in spring (Rasmussen et al. 1997; Helmig et al. 2007). Springtime O_3 maxima are observed at high-elevation sites (>1500 m a.s.l.) and remote continental areas, typically representing baseline conditions with little influence from fresh local pollution (De Leeuw 2000; Monks 2000; Chevalier et al. 2007; Derwent et al. 2018). Springtime O_3 maxima are modulated by the stratosphere-troposphere O_3 exchange (Zhao et al. 2010) and hemisphere-wide photochemical production (Monks 2000). The stratospheric O_3 inputs and biogenic, biomass burning, and lightning NOx emissions are maximum during springtime and coincide with the intertropical convergence zone (Tang et al. 2011; Han et al. 2018). For latitudes over 60°N, air pollutants are transported to the Arctic, primarily from Eurasia, leading to higher O_3 concentrations (namely Arctic haze) in early spring when the dry deposition is low (Law and Stohl 2007). Over the last two decades, an increase in Asian emissions' contribution to surface O_3 levels in Europe has been observed during the springtime (Wild et al. 2012; Cooper et al. 2014).

In summer (June-July-August), the highest surface O_3 concentrations are observed over the Tibetan plateau (>45 ppb) and Greenland, western and eastern US and, in latitude band 15–45°N (>40 ppb). In comparison, lower O_3 concentrations (<30 ppb) are found over Canada, south Asia, and regions over latitude 60°N. At northern mid-latitudes, surface O_3 exhibits a well-known summertime maximum attributed to the peak in local and regional photochemical O_3 production due to maximum sunlight, more extended daylight, and long-range transport, e.g., East Asian monsoon (Mavrakis et al. 2010; Parrish et al. 2013; Li et al. 2014) and maximum forest fire emissions emitting mainly NO (Andreae and Merlet 2001). The seasonal swings of the Hadley cell and Northern Hemisphere subtropical westerlies play an important role in determining the seasonality of the transport pathway (Han et al. 2018).

During autumn (September-October-November), results show higher mean O_3 concentrations (>30 ppb) over Greenland, the Tibetan plateau, and regions with desert and ice, i.e., Alaska, the Arabian Peninsula, and the western US. The minimum concentrations (<20 ppb) are found in central Europe and south-eastern Asia. During the cold period with limited sunlight, the O_3 titration occurs under high NOx levels, in particular in winter (no photolysis reactions of NO_2), and thus the freshly emitted NO reacts rapidly with O_3 to produce NO_2 (Doherty et al. 2005; Bloomer et al. 2010; Monks et al. 2015). Besides, low dry deposition rates for O_3 over snow and ice surfaces and deserts (Wesely and Hicks 2000) and the *in-situ* photochemical production explain higher O_3 concentrations over these areas in winter. Over Africa, biomass burnings are active in winter between 0 and 10°N and 15 and 40°E (Sauvage et al. 2005; Aghedo et al. 2007; Han et al. 2018), and regional CO emissions peak during winter in North Africa (Han et al. 2018). At northern mid-latitudes, a shift of the seasonal cycle has been detected over the past decades, i.e., the observed peak O_3 concentrations appear earlier in the year (about 8.5 days per decade at Mace Head over the time period 1987-2017) in response to climate change combined with changes in emissions (e.g., increased O_3

production in Asia) and an increasing contribution from the stratosphere (Parrish et al. 2013; Derwent et al. 2018). Climate change may have caused changes in; i) large-scale transport pathways of O_3 and its precursors in the troposphere, ii) stratosphere-to-troposphere O_3 transport, iii) natural emissions (e.g., lightning, forest fires), and iv) O_3 photochemistry by variations in air temperature, water vapor, and natural emissions (Pozzoli et al. 2011; Stevenson et al. 2012; Wild et al. 2012; Parrish et al. 2013). Over the past two decades, the spatial distribution of anthropogenic emissions of O_3 precursors has changed significantly.

2.3 Factors affecting ozone distribution

Ground-level O_3 concentrations are determined by global-to-local O_3 precursors' emissions, long-range transport of O_3 and its precursors, stratosphere-to-troposphere exchange, meteorology associated with inter-annual variability, climate change, and loss processes such as reactions with water vapor and OH° radicals and dry deposition at the surface (Wild et al. 2004; Zhang et al. 2011; Li et al. 2014; Monks et al. 2015; Lin et al. 2017; Hogrefe et al. 2018; Lefohn et al. 2018).The dominant source of tropospheric O_3 is by photochemical reactions; VOCs and CO are oxidized in the presence of NOx (Lelieveld and Dentener 2000). The natural emissions of O_3 precursors are mainly from biogenic (e.g., isoprene), biomass burning (mainly NO), CO from oceans, and lightning NOx sources (Guenther et al. 1995; Monks et al. 2015; Albrecht et al. 2016) while anthropogenic NOx, CO and VOCs emissions are mainly from the road and non-road transport traffic (automobiles, ships, and aircraft), fossil fuel combustion and industry (Olivier et al.1998; Bond et al. 2001; Granier et al. 2003; Miyazaki et al. 2012; Wang et al. 2015). For instance, the contribution of ocean shipping emissions to European annual mean O_3 concentrations is 3–5 ppb, i.e., around 10% (Jonson et al. 2018), and wild fire emissions can result in 2–8 ppb enhancements to mean O_3 at individual sites in summer in the US (Lin et al. 2017).

On a global scale, the anthropogenic NOx emissions are dominated by fossil fuel combustion (~50%, in particular by road transport and power plants) and biomass burning (~20%), representing more than 70% of total emissions (Granier et al. 2003). Natural sources represent about 30%, mainly lightning and soil processes (Olivier et al. 1999; Bond et al. 2001). Globally, the most important sources of anthropogenic VOC emissions are on-road traffic, industry, gasoline evaporation, and solvent use (Borbon et al. 2013). In the EU, the most significant anthropogenic VOC emissions are "solvent and product use" (~40%) followed by "mobile sources" (~40%) in 2011 (EEA 2015). Nationally, biogenic emissions were estimated to contribute approximately 74% to total VOCs in 2011 in the US, for instance (US EPA 2015). Biogenic VOC emissions include emissions from wildfires and plants, mainly isoprene and monoterpenes, affecting hydroxyl OH° radical concentrations (Atkinson2000; Kulmala et al. 2004). Worldwide, the largest source of CO is natural from, e.g., oceans, volcanoes, and forest fires, while nationally, biogenic CO emissions were estimated at 26% of the total CO emissions in 2011 in the US (US EPA 2015). The main anthropogenic CO emissions are from road and non-road transport, for instance, 84% of the anthropogenic emissions in the US and 95% in the EU in 2011 (Hudman et al. 2008; EEA 2015; US EPA 2015).

The lifetime of tropospheric O_3 is long enough to allow long-range transport of O_3 and its precursors from the regional to hemispheric scale through trans-Atlantic, trans-Pacific, and trans-Eurasian transport simulated in particular during Hemispheric Transport of Air Pollution (HTAP) experiments by applying a global 3-D chemistry-transport model e.g., MOZART-4 and GEOS Chem (Jaffe et al. 2003; Wild et al. 2004; Wang et al. 2009; Emmons et al. 2010; Wilson et al. 2012; Li et al. 2014; Derwent et al. 2015; Nopmongcol et al. 2017; Huang et al.2017; Lin et al. 2017; Han et al. 2018). Therefore, remote areas such as the Arctic region can be affected (Langner et al. 2012; Thomas et al. 2013).

The intercontinental transport from North America and Asia to Europe is maximal in summer and autumn (Derwent et al. 2004; Lefohn and Cooper 2015). The contribution to annual mean daily O_3 concentrations recorded at 21 monitoring stations across Europe in 1998 (40.3 ± 4.1 ppb, on average) can be attributed to production over Europe (48.5 ± 10.8%), North America (20.4 ± 4.4%), and Asia (11.5 ± 2.6%) and due to stratospheric inputs (18.4 ± 3.8%), showing that O_3 sources outside Europe are significant at any locations (Derwent et al. 2004; Jonson et al. 2018). The rest of the world's contributions are larger than the effects of European emissions alone (Jonson et al. 2018).

By trans-Pacific transport, Asian emissions enhance monthly mean O_3 concentrations by 2–6 ppb in the Western US and by 1–3 ppb in the Eastern US (Brown-Steiner and Hess 2011; Verstraeten et al. 2015; Huang et al. 2017; Lin et al. 2017) and can contribute up to 15 ppb during pollution episodes at rural sites (Lin et al. 2012). The North American emissions contribute to 1–3 ppb of surface O_3 over China (Ni et al. 2018).

By trans-European transport, European air pollution adversely impacts air quality in Asia and can even reach Eastern Siberia (Pochanart et al. 2003). The contribution of European emissions reaches 2–3 ppb for surface monthly O_3 over North China and 1–2 ppb over central China and 0.5–1 ppb over South China during the springtime (Li et al. 2014; Ni et al. 2018) while in summer, influences from Europe are approximately one ppb lower over China (Li et al. 2014). Trans-Eurasian transport of O_3 and its precursors enhances monthly mean O_3 concentrations by 0.5–3.5 ppb over Siberia and 0.2–2.5 ppb over Japan (Wild et al. 2004). Emissions from the Middle East and India increase surface O_3 concentrations by 1–4 ppb and 1–5 ppb, respectively, over Western China, depending on the location and season, and by <1 ppb in Eastern China (Li et al. 2014). Siberia has a small impact on surface O_3 (<1 ppb) in China (Li et al. 2014). Emissions from all Eurasian regions contribute to surface O_3 by 10–15 ppb over Western China, superimposed upon a 35–40 ppb natural background (Li et al. 2014).

Compared to other continents, Africa has the most frequent lightning (Albrecht et al. 2016) and the largest burned areas (Giglio et al. 2013). About 70% of tropospheric O_3 produced over Africa is exported worldwide, contributing to the global tropospheric O_3 budget (Aghedo et al. 2007; Zare et al. 2014; Han et al. 2018). The Hadley cell connects the subtropical westerlies to form a transport route from Africa to South Asia (Han et al. 2018). The transport of O_3 from Africa to the South Asian lower troposphere is most extensive in winter (4 ppb, 8% on average) and lowest in autumn (2 ppb, 5% on average) in the latitude band 5–40°N (Han et al. 2018). The influence of African O_3 in South Asia is more considerable than that of European and North American O_3 (Han et al. 2018).

The downward O_3 transport from the stratosphere is an important source of tropospheric O_3 (Tang et al. 2011; Akritidis et al. 2016). For instance, stratospheric inputs can episodically increase the daily 8–h mean O_3 by 20–40 ppb at high-elevation Western US stations (Lin et al. 2017). Several studies report an increased exchange with the free troposphere or stratosphere in response to a warming climate (e.g., Hegglin and Shepherd 2009; Zeng et al. 2010), in particular at the high-elevation Himalayan Plateau (Lefohn et al. 2012; Schnell et al. 2016).

2.4 Surface ozone trends over time

Since the 1990s, anthropogenic O_3 precursor emissions have decreased in North America (NOx, -3.7% year^{-1}; VOCs, -3.3% year^{-1}) and Europe (NOx, -2.5% year^{-1}; VOCs, -34% year^{-1}), while eastern Asian (NOx, +4.3% year^{-1}; VOCs, +2.3% year^{-1}) emissions have increased (Jonson et al. 2006; Xing et al. 2013; Hidy and Blanchard 2015; Xing et al. 2015; Sicard et al. 2016; Zhang et al. 2016; Lin et al. 2017). Therefore, both mean and peak O_3 concentrations decreased in North America and Europe (Sicard et al. 2021) and increased in East Asia (Chang et al. 2017). Since early 1990s, the emissions control policies of O_3 precursors around the world are effective in rural areas (on average: - 0.23 ppb year^{-1} since 1990s), in particular from 2005 onwards with a reduction of 0.24 ppb year^{-1} in the North America and of 0.41 ppb year^{-1} in Europe between 2005 and 2014 (Sicard 2021). From the mid-1990s to 2000s, increases of 1–2 ppb year^{-1} are observed in spring to sum mer ground-level O_3 in China (Ding et al. 2008; Ma et al. 2016; Sun et al. 2016). In East Asia, an increase of 0.21 ppb year^{-1} was reported at 39 rural stations over the time period 2000-2010, however slight decreases were recently reported at 2 rural stations nearby Beijing in China (Sicard 2021). The O_3 concentrations increased in most cities (on average: + 0.31 ppb year^{-1} since 1990s), in particular since 2005 with + 0.33 ppb year^{-1} in North America, + 0.68 ppb year^{-1} in East Asia and + 0.27 ppb year^{-1} in Europe between 2005 and 2014 (Sicard 2021). The background O_3 concentrations increased by, on average, +0.16 ± 0.22 ppb year^{-1} at 89% of 319 urban stations and decreased by -0.05 ± 0.21 ppb year^{-1} at 65% of 306 rural stations globally over the period 1995–2014 (Sicard et al. 2018). The background O_3 increase in cities was confirmed by other studies covering global trends from 2000 onwards in Europe and the US (Wilson et al. 2012; Sicard et al. 2013; Simon et al. 2015; Sicard et al. 2016; Lefohn et al. 2018). These increasing O_3 background levels in the cities can be mainly attributed to a lower O_3 degradation by NO due to the reduction in local NO_x emissions (Sicard et al. 2016; Lefohn et al. 2018).

Generally, a reduction in surface O_3 mean concentrations and high percentiles was observed during the warm period (Parrish et al. 2013; Jhun et al. 2015; Sicard et al. 2016) in line with decreasing O_3 precursor emissions within Europe and North America since the 1990s (Cooper et al. 2014). During the cold period, the increase in O_3 mean concentrations at European and North American stations can be attributed to the lower loss of O_3 via NO titration, as a consequence of the reduced NOx emissions within Europe and North America, and to the intercontinental transport from the Asia maximal in autumn (Jonson et al. 2006; Wilson et al. 2012; Parrish et al. 2013; Cooper et al. 2014; Sicard et al. 2016).The net impacts of climate change can drive the global background O_3 increase, i.e., an increase in stratospheric O_3 inputs, higher CH_4 emissions, changing lightning NOx emissions, weakened NO titration by reducing NOx emissions and impacting reaction rates, through sea surface temperatures and relative humidity changes (Lau et al. 2006; Voulgarakis et al. 2013; Stevenson et al. 2013; Young et al. 2013).

2.5 Conclusions

At any geographic location, the O_3 distribution is determined by processes and changes occurring at hemispheric, regional, and local scales (Monks et al. 2015). Despite the reduction of O_3 precursor emissions in Europe and North America since the 1990s, non-attainment of the target value for O_3 can persist (Sicard 2021). The current background O_3 levels may thus still exceed target values to protect human health and annual and perennial vegetation (Sicard et al. 2017). Climate change, long-range transport, and increased emissions in Asia might reduce the benefits gained from regional O_3 control strategies by increasing background O_3 levels in the future (e.g., Lefohn and Cooper 2015). To meet O_3 air quality objectives, a combined global strategy, at least in both the Southern and Northern Hemispheres, is needed (Jonson et al. 2018).

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CHAPTER THREE

GLOBAL SURFACE OZONE LEVELS UNDER DIFFERENT EMISSION AND CLIMATE SCENARIOS

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Abstract

A few issues about tropospheric ozone (O_3), such as a better understanding of spatio-temporal changes, are still challenging. Recent studies display a mean global increase in background O_3 concentration from the current level of 35–50 ppb to 55–65 ppb and even up to 85 ppb at the Northern hemisphere mid-latitudes by 2100. Regional chemical transport models are often used to reproduce the surface O_3 levels worldwide. Projected changes in surface O_3 vary considerably among models and emission scenarios. In general, the models well reproduce the seasonal variability and spatial pattern in surface O_3 at a regional-to-global scale. The last emission scenarios, i.e., the Representative Concentration Pathways (RCPs), were developed as part of the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. These scenarios include different assumptions on climate, energy access policies, and land cover and land-use changes. In the *Atmospheric Chemistry and Climate*

Model Intercomparison Project framework, different simulations were performed from numerous global or regional chemistry models (e.g., MOCAGE, MOZART-4, and EMEP) under the new RCP scenarios. Compared to the early 2000s, changes in surface O_3 by 2100 worldwide range from about 4–5 ppb in the RCP8.5 scenario to reductions of about 2–10 ppb on average in the most optimistic scenario, RCP2.6. For RCP2.6 and RCP4.5, decreases are observed due to fewer precursor emissions. Simultaneously, for RCP8.5, all models show climate-driven increases in ground-level O_3, mainly attributed to a substantial increase in CH_4 emissions coupled with global warming and a weakened NO titration.

Keywords: Tropospheric ozone; Transport model; Global warming; NO titration

3.1 Introduction

Tropospheric ozone (O_3) pollution is still a significant air quality issue over large regions of the globe (Sicard et al. 2017). Long-range transport of O_3 and precursors of O_3, such as nitrogen oxides (NOx) and Volatile Organic Compounds (VOCs), can elevate the local and regional O_3 background concentrations (Wilson et al. 2012; Derwent et al. 2015). A better understanding of spatial changes of surface O_3 is still challenging. Projected changes in ground-level O_3 vary among emission scenarios and models (Stevenson et al. 2006; Lamarque et al. 2013, Sicard et al. 2017).

In the Atmospheric Chemistry and Climate Model Intercomparison Project (ACCMIP) framework, sixteen global chemistry models were deeply investigated (Lamarque et al. 2013; Young et al. 2013). ACCMIP models were used to evaluate projected changes in atmospheric chemistry and air quality under different emission and climate assumptions (e.g., Lamarque et al. 2010; Fiore et al. 2012; Voulgarakis et al. 2013). However, here we focus on six global models (Table 3.1). All models simulate gaseous tropospheric chemistry: from 16 species in CESM-CAM to 110 species in MOCAGE depending on the degree of representation of non-methane hydrocarbon chemistry in their chemical scheme. The aerosol indirect effects, including interactions between aerosols and gas-phase chemistry, are represented in GFDL-AM3, GISS-E2-R, and MIROC-CHEM (Table 3.1).

All models simulate the temporal and spatial evolution of anthropogenic and natural emissions at a global scale such as black carbon (BC), organic carbon (OC), secondary organic aerosols (SOA), non-methane VOCs, ammonia (NH_3), NOx from soils, and lightning, and carbon monoxide (CO) from oceans and vegetation (Stevenson et al. 2012). Surface methane (CH_4) concentrations, with spatial variation, are prescribed in all models using data from the database of the Coupled Model Intercomparison Project Phase 5 (CMIP5), except for GISS-E2-R, in which interactive wetlands emissions for the future are used (Shindell et al. 2012). Natural biogenic emissions are provided by the MEGAN v2.1 inventory (Model of Emissions of Gases and Aerosols from Nature). Biogenic VOCs depend on meteorological conditions and are responsible for ground-level O_3 changes (Sicard et al. 2009). Only GISS-E2-R incorporates climate-driven isoprene emissions, the most abundant and reactive biogenic VOC. The other models prescribe fixed biogenic emissions, e.g., based on constant present-day isoprene emissions for all simulations. GISS-E2-R has interactive isoprene but fixed soil NOx (Young et al. 2013). Possible changes in lightning activity with climate change are recognized to impact the lightning NOx emissions; therefore, most models use Price and Rind's (1992) parameterization based on the simulated convective activity (e.g., Lamarque et al. 2013). For stratospheric O_3, the models are grouped into two categories: 1) models with interactive or semi-offline chemistry, and 2) models with prescribed O_3. Some models (GFDL-AM3, GISS-E2-R, MIROC-CHEM, and MOCAGE) include full stratospheric chemistry schemes, while CESM-CAM is based on linearized O_3 chemistry, and UM-CAM uses the CMIP5dataset to prescribe offline O_3 in the stratosphere.

The last emission scenarios, i.e., the Representative Concentration Pathways (RCPs), were developed as part of the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (Myhre et al. 2013) and include different assumptions on climate, energy access policies, land cover and land-use changes (Kirtman et al. 2013). The three RCP scenarios are named for their nominal radiative forcing level by 2100. The rise in global mean surface temperature, by the end of the 21st century, is in the ranges 2.6–4.8°C (RCP8.5), 1.1–2.6°C (RCP4.5), and 0.3–1.7°C (RCP2.6) relative to the mean temperature over the pre-industrial period (Lamarque et al. 2013). The length of historical and RCP simulations varies between models, but for all models the historical runs cover a period centered around 2000, while the time-slice of RCPs is centered around 2100 (Table 3.1).

Table 3.1: Characteristics of the models, including simulation time slice, spatial resolution, simulated gas species, and associated bibliographic references. CCM is Chemistry Climate Model, CTM is Chemistry Transport Model, CGCM is Chemistry–General Circulation Model, BC is Black carbon, OC is Organic carbon, SOA is Secondary Organic Aerosols, DMS is Dimethyl sulfide, VOC is Volatile Organic Compounds (from Lamarque et al. 2013 and Young et al. 2013).

Models	**Type**	**Simulation length**	**Resolution (lat/lon)**	**Number of vertical pressure levels& top level**	**Species simulated**
CESM–CAM	CCM	2000–2009 &2100–2109	1.875/2.5	26 levels 3.5 hPa	**16 gas species**; constant present-day isoprene, soil NOx, DMS and volcanic sulfur, oceanic CO.
GFDL–AM3	CCM	2001–2010 &2101–2110	2.0/2.5	48 levels 0.017 hPa	**81 gas species**; SOx, BC, OC, SOA, NH_3, NO_3; constant pre-industrial soil NOx; constant present-day soil and oceanic CO, and biogenic VOC; climate-sensitive dust, sea salt, and DMS.
GISS–E2–R	CCM	2000–2004 &2101–2105	2.0/2.5	40 levels 0.14 hPa	**51 gas species**; interactive sulfate, BC, OC, sea salt, dust, NO_3, SOA, alkenes; constant present-day soil NOx; climate-sensitive dust, sea salt, and DMS; climate-sensitive isoprene based on present-day vegetation.
MIROC–CHEM	CCM	2000–2010 &2100–2104	2.8/2.8	80 levels 0.003 hPa	**58 gas species**; SO_4, BC, OC; constant present-day VOCs, soil NOx, oceanic CO; climate-sensitive dust, sea salt and DMS.
MOCAGE	CTM	2000–2003 &2100–2103	2.0/2.0	47 levels 6.9 hPa	**110 gas species**; constant present-day isoprene, other VOCs, oceanic CO and soil NOx.
UM–CAM	CGCM	2000–2005 &2094–2099	2.50/3.75	19 levels 4.6 hPa	**60 gas species**; constant present-day biogenic isoprene, soil NOx, biogenic and oceanic CO.

3.2 The ability of models in reproducing local/regional ozone patterns

We show the simulated global O_3 spatial pattern of mean annual O_3 concentration at the lower model layer in Fig.3.1. The averaged values (simulated percentage) of global, Northern Hemisphere (NH), and Southern Hemisphere (SH) mean surface O_3 are derived from averaging values over the global/NH/SH land areas only (Table 3.2).

The six models well reproduce the year-to-year variability and spatial pattern of mean O_3 concentrations at a regional-to-global scale (Sicard et al. 2017). The highest surface O_3 concentrations (Fig. 3.1) are found in the NH, highlighting a hemispheric asymmetry. The multi-model O_3 mean concentration averaged over the domain's land points, is 37.9 ± 4.3 ppb in the Northern Hemisphere and 22.9 ± 3.8 ppb in the Southern Hemisphere (Table 3.2). The historical ground-level O_3 mean concentrations were ranging between 35 and 50 ppb in the NH, with the highest values occurring over Greenland and in the latitude band 15–45°N, particularly around the Mediterranean basin, over deserts (e.g., Near East, Sierra Nevada) and at high-elevation areas, e.g., in the Rocky and Appalachian Mountains and over the Tibetan plateau (>50 ppb) while the lowest O_3 burden (15–30 ppb) was recorded in the SH, particularly over the Amazon, African and Indonesian rainforests (Fig. 3.1). Higher O_3 mean concentrations (>60 ppb) are observed in Southwestern and Northeastern US, and East Asia (e.g., Beijing). The higher O_3 levels in areas downwind of O_3 precursor sources are well simulated in the GISS-E2-R and MOCAGE models. All models, except MIROC-CHEM, well reproduce the high surface O_3 mean concentrations over Greenland and over deserts. The high-elevation areas, characterized by higher O_3 burdens, are well simulated in the GISS-E2-R and MOCAGE models. Over Greenland, mean O_3 concentrations during the historical runs ranged from 40 to 55 ppb except in MIROC-CHEM (20-25 ppb). Model-to-model differences in surface O_3 can be observed due to different natural emissions of O_3 precursors (e.g., lightning NOx, biogenic VOCs) as well as the complexity of chemical schemes used (Young et al.2013; Sicard et al. 2017) and the representation of land cover and the O_3 deposition sink to vegetation (Geddes et al. 2016; Travis et al. 2016).

Regarding validation from ground measurements (e.g., CASTNet and GAW sites), substantial discrepancies between observed and simulated O_3 concentrations can be observed (Parrish et al. 2014; Lin et al. 2015; Huang et al. 2017; Jonson et al. 2018; Sicard et al. 2021). The models well reproduce the day-to-day variability in surface O_3 in the US with correlation coefficients ranging from 0.40 to 0.80 for CASTNet sites (Lin et al. 2012). Compared to ground observations, the global models overestimate O_3 concentrations by 14% across Europe on average (Jonson et al. 2018). In Europe, based on Airbase stations, the reproduction of the daily temporal variability is very good both at 361 background rural ($r = 0.78$) and at 586 background urban sites ($r = 0.77$) with a Root Mean Square Error lower than 5ppb and a fractional bias of 15.8% in summer (Terrenoire et al. 2015).

The seasonal pattern of ground-level O_3 is well reproduced at inland and mountain sites (<10 ppb on average) while a large overestimation of O_3 concentrations (>10 ppb on average) is observed near the coastal areas (e.g., Parrish et al. 2014; Strode et al. 2015; Lin et al. 2017; Huang et al. 2017). The models generally underestimate mean O_3 concentrations (<10 ppb) during high O_3 seasons and overestimate mean O_3 concentrations (>10 ppb) during low O_3 seasons (Fiore et al. 2009; Huang et al. 2017). The bias is mainly attributed to bottom-up emissions, stratosphere-to-troposphere O_3 exchange, the low vertical and horizontal resolution of the model and the underestimated transboundary pollution and O_3 loss processes by NO titration (Ding and Wang 2006; Li et al. 2014; Parrish et al. 2014; Huang et al. 2017). Averaging the results from a larger number of models allows the canceling out of the positive or negative biases from individual models (Huang et al. 2017).

Recently, the high spatial resolution model Weather Research and Forecasting model with Chemistry (WRF-Chem) was used to simulate the spatial and seasonal variability of main physical and chemical variables over Asia for the year 2015 at 8-km horizontal resolution (De Marco et al. 2020; Sicard et al. 2021). The simulated atmospheric composition was evaluated against ground-based observations in China for the year 2015. Overall, WRF-Chem reproduced well the mean annual cycle of surface O_3concentrations in all seasons across China, with an over estimation of surface O_3 concentrations in winter (13%) and autumn (11%), in particular in South Asia and Eastern China, and a slight mean bias for spring 2015 (3%).

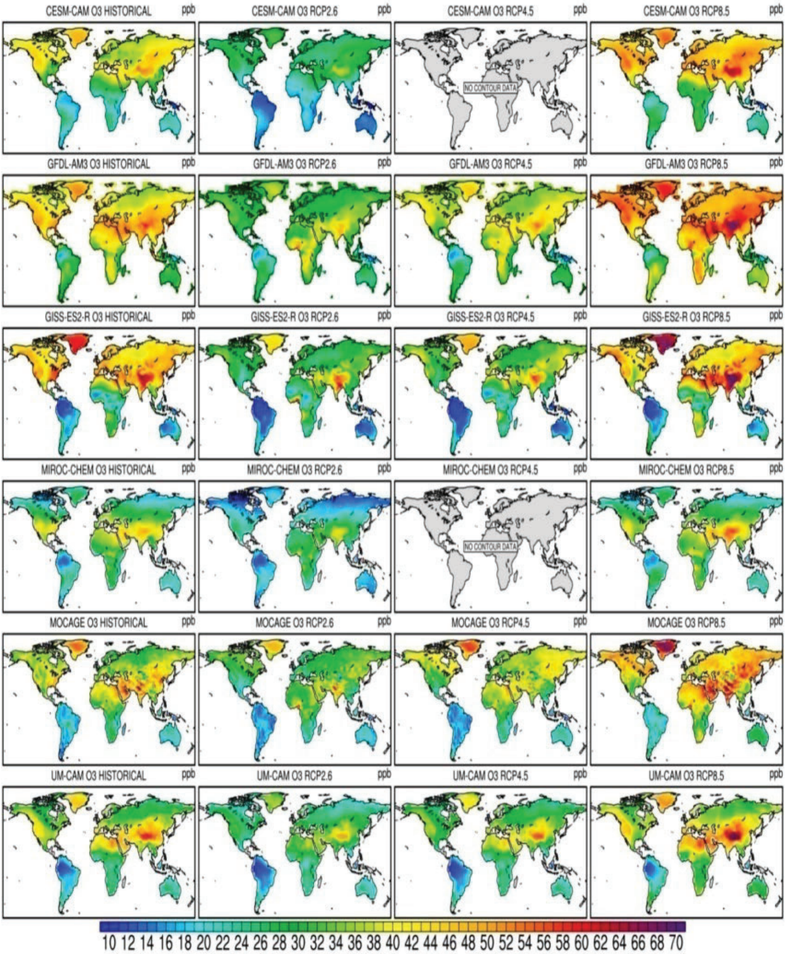

Fig. 3.1: Surface ozone average concentrations (in ppb) at the lower model layer for each ACCMIP model over the historical period and for RCP2.6, RCP4.5, and RCP8.5 simulations by 2100 (from Sicard et al. 2017). The data are missing for 2 models under RCP4.5 ("No contour data")

Table 3.2: Global and hemispheric (averaged over the land points of the domain) mean annual-average surface ozone concentrations (in ppb) for the historical simulations in each model (Northern and Southern Hemisphere, i.e., NH and SH) and simulated percentage changes (%) in mean annual-average surface ozone concentrations between 2100 and historical simulation for RCPs (from Sicard et al. 2017). Missing or not available data are identified (n.a). The last row shows averages and standard deviations (SD)

Models	**O_3 conc. global (ppb)**	**O_3 conc. SH (ppb)**	**O_3 conc. NH (ppb)**	**RCP2.6 global (%)**	**RCP2.6 SH (%)**	**RCP2.6 NH (%)**	**RCP4.5 global (%)**	**RCP4.5 SH (%)**	**RCP4.5 NH (%)**	**RCP8.5 global (%)**	**RCP8.5 SH (%)**	**RCP8.5 NH (%)**
CESM–CAM	31.3	20.9	36.4	- 29.1	- 20.6	- 31.3	n.a	n.a	n.a	+ 21.9	+ 22.5	+ 20.5
GFDL–AM3	38.6	30.6	42.9	- 20.5	- 10.8	- 24.5	- 11.7	- 6.9	- 13.5	+ 15.5	+ 18.6	+ 14.5
GISS–E2-R	35.8	22.3	42.3	- 23.5	- 5.8	- 27.9	- 20.4	- 6.3	- 23.9	+ 7.0	+ 19.3	+ 3.8
MIROC-CHEM	27.9	20.4	31.4	- 23.3	- 12.3	- 26.8	n.a	n.a	n.a	+ 3.9	+ 10.3	+ 2.2
MOCAGE	32.9	21.5	38.3	- 12.8	+ 7.4	- 18.5	- 1.8	+ 17.7	- 7.0	+ 20.1	+ 40.4	+ 16.7
UM–CAM	31.3	21.4	36.0	- 17.3	- 4.7	- 21.1	- 8.3	+ 0.9	- 10.8	+ 14.4	+ 24.3	+ 11.4
Mean ± SD	33.0 ± 3.8	22.9 ± 3.8	37.9 ± 4.3	- 21.1 ± 5.6	- 7.8 ± 9.4	- 25.0 ± 4.7	- 10.5 ± 7.7	+ 1.4 ± 11.5	- 13.8 ± 7.2	+ 13.8 ± 7.1	+ 22.6 ± 10.0	+ 11.5 ± 7.3

3.3 Projected changes in ozone concentration

The future projections of the mean surface O_3 concentrations vary considerably with the different scenarios and models (Fig. 3.1). Under the RCP2.6 scenario, all models predict that surface O_3 will decrease enormously worldwide, except in Equatorial Africa, where higher O_3 levels are observed in GFDL-AM3, GISS-E2-R, and MOCAGE. Under the RCP4.5 scenario, the surface O_3 mean concentrations are lower than the historical runs worldwide for all models except in MOCAGE, where deterioration is observed over Canada, Greenland, and East Asia (Fig.3.1). For all models, the surface O_3 levels are higher for RCP8.5 than the historical runs. The highest increase is found in Northwestern America, Greenland, the Mediterranean basin, the Near East, and East Asia. The averaged relative changes in surface O_3 concentrations for the different RCPs are (Table 3.2): -21% for RCP2.6, -10% for RCP4.5, and +14% for RCP8.5 with a strong disparity between both hemispheres, e.g., 8% in the SH and -25% in the NH for RCP2.6 (Sicard et al. 2017). RCP8.5 is the only scenario to show an increase in global background O_3 levels by 2100 (+23% in the SH and +11% in the NH). For all models and RCPs, the O_3 hotspots (>50 ppb) are over Greenland and South Asia (Fig.3.1). The highest increases are observed in Northwestern America, Greenland, the Near East, and South Asia (>65 ppb).

The surface-air-temperature increase in the RCP2.6 scenario is 2–3 times weaker than that of the RCP4.5 scenario and 4–5 times weaker than that of the RCP8.5 scenario, and the greatest changes (>5°C) are observed over the Arctic, above latitude 60°N, and in the latitude band 15–45°N (Seidel et al. 2008; Nazarenko et al. 2015) similar to the global pattern of surface O_3 levels. The models can reproduce the global pattern of the distribution of air temperature changes in agreement with surface O_3 concentration changes.

For RCP2.6 and RCP4.5, absolute decreases are observed in the NH due to fewer O_3 precursor emissions, e.g., in Europe (a reduction of 5–7 ppb). RCP8.5 has had relatively low NOx, CO, and VOCs emissions but very high CH_4 emissions throughout the 21st century (Voulgarakis et al. 2013; Sicard et al. 2017). Stronger increases are found over the high-elevation Himalayan Plateau reflecting an increased stratospheric O_3 influx in response to a warming climate (Zeng et al. 2010; Lefohn et al. 2012; Schnell et al. 2016). Under the RCP8.5 scenario, the increase in surface O_3 concentrations, by 14% on average and up to 10 ppb in South Asia, can be attributed to the higher CH_4 emissions coupled with strong global warming, exceeding 2°C, and a weakened NO titration by reducing

NOx emissions and a more significant stratospheric O_3 influx (Wild et al. 2012; Stevenson et al. 2013; Young et al. 2013; Sicard et al. 2017). The net impacts of climate change can also drive the O_3 increase, i.e., increased stratospheric O_3 intrusion, greater lightning NOx emissions, and impacting reaction rates through sea surface temperatures and relative humidity changes (Voulgarakis et al. 2013; Young et al. 2013). A reduction in surface O_3 concentrations is simulated over the equatorial region under RCP8.5, where the increased relative humidity, in a warmer climate, increases the O_3 loss rate (Zeng and Pyle 2003).

3.4 Conclusions

The global atmospheric chemistry transport models well reproduce the spatial pattern of historical O_3 concentration at a global scale; in particular, GISS-E2-R and MOCAGE can simulate the higher O_3 levels downwind of precursor sources and in the high-elevation areas. The model outputs emphasize the strong asymmetry in the tropospheric O_3 distribution between the NH and the SH. The natural emissions of O_3 precursors (e.g., lightning NOx, CO from oceans, isoprene) and the complexity of chemical schemes are significant sources of the model-to-model differences. Compared to the early 2000s, the results suggest changes in surface O_3 of -9.5 ± 2.0 ppb (NH) and -1.8 ± 2.1 ppb (SH) in the cleaner RCP2.6 scenario and of +4.4 ± 2.8 ppb (NH) and +5.1 ± 2.1 ppb (SH) in the most pessimistic RCP8.5 scenario. For RCP2.6 and RCP4.5, absolute decreases are observed in the NH due to fewer precursor emissions. For RCP8.5, all models show climate-driven increases in surface O_3 in particular over the Western US, Greenland, South Asia, and Northeast China, and the changes ranged from +1 to +5 ppb over North America and Europe. This O_3 increase can be mainly attributed to a substantial increase in CH_4 emissions coupled with global warming.

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CHAPTER FOUR

BIOLOGICAL MONITORING OF OZONE POLLUTION WITH VASCULAR PLANTS

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> *To the Philosopher, the Physician,*
> *the Meteorologist, and the Chemist, there is perhaps*
> *no subject more attractive than that of ozone.*
> C.B. Fox. Ozone and Antozone,
> Their History and Nature
> J. & A. Churchill, London, 1873

Abstract

Biological monitoring (BM) uses organisms to determine the presence, amounts, temporal/spatial changes, and effects of both abiotic and biotic factors in the environment. The huge difference between BM and traditional physico-chemical measures of air quality is that the latter measures take into account emissions and immissions but only indirectly measure the "health" of the atmosphere because they do not look directly at biological responses. Specific plants, and namely tobacco cv. Bel-W3 are considered to be reliable bioindicators of ambient ozone (O_3). Leaf injury on these plants is usually the first indication that a region has developed an O_3 problem. Bel-W3 tobacco is a "perfect" bioindicator of O_3 in the air, as it is highly sensitive. It develops easily recognizable and quantifiable peculiar foliar symptoms, and its vegetative period is coincident with the photo-smog season. Biomonitoring protocols are applied in community, local, regional, and nation-wide campaigns. The entire methodology, from plant cultivation to exposure, injury assessment, data treatment, statistical analysis, and presentation of results, is now standardized. Some sticking points are that: *(i)* BM does not replace modeling or conventional methods of direct measurement of air pollutants, it complements them by demonstrating the biological effect; *(ii)* bioindicator stations can make it possible to supplement at moderate costs the pre-existing network; and *(iii)* bioindicators raise public awareness by recourse to living organisms (*i.e.*, O_3-sensitive plants make invisible air pollution visible).

Keywords: Biological monitoring; Ozone; Bioindicator; Vascular Plants

4.1 Biological Monitoring, Basic Concepts and Definitions

According to the consensus, “biological” is defined as “the science of physical life, dealing with plants, animals and microbes, their morphology, physiology, origin and distributions”; and a “monitor” is “something that reminds or gives a warning.” So, “biological monitoring” (hereafter “BM”) may be defined as “the use of living organisms to determine the presence, amounts, temporal or spatial changes and effects of both abiotic and biotic factors in the environment” (Fath 2019).The huge difference between BM and traditional physical and chemical measures of air quality is that the latter measures take into account emissions (at the sources) and immissions (at the receptors), but only indirectly measure the “health” of the atmosphere, and do not look directly at biological responses to pollution.

Plants can be categorized according to the way they respond to pollutants. For example, metals such as cadmium and nickel can be absorbed into plant material and retained in the cytoplasm, vacuoles, and cell walls. The concentration of the pollutant can then be analyzed as *posteriori* via chemical protocols. The plant has *accumulated* the pollutant. Alternatively, aero dispersed (persistent, non-convertible) chemical species may be merely adsorbed onto the leaf surface, so plants act as “biodeposimeters” (Bargagli 1998). In addition (second response), pollution can affect the (relative) distribution of a species in a biological consortium. Environmental change in the medium- to long-term is signaled in a community either by expansion or by a recession of resistant and sensitive genotypes, respectively. For example, a plant may be inhibited by concentrations of a chemical above a certain threshold. The biological response can therefore control the presence/absence/abundance of a species at a location to allow the taxon to be used as a *presence indicator* of ambient pollution. Lichen communities are a paradigmatic study case of this issue (Ferry et al.1973; Nimis et al. 2002). The idea of (bio)monitoring the abundance of lichens as a measure for air pollution effects goes back to the 19th century when Nylander (1866) mapped some parks in Paris.

The third response to pollution is recognized, whereby visible evidence of injury (or reduction in biomass production) is exhibited (Falla et al. 2000). Proving that specific injury symptoms are useful indicators of air pollution dates back to early in the last century (Ruston 1921). Plants undergo physical changes when exposed to ambient concentrations of a pollutant above a specified "threshold × duration" combination (i.e., "dose"). The prominent (leaf) damage is a reaction to pollution and allows plants that exhibit this response to be categorized as *reactor* (or "effect") species. Symptoms should be considered to be diagnostic, provided that they are verified in exposure/response studies under experimental conditions. So, a "reaction bioindicator" can be defined as an organism (in this case, a plant) exhibiting a typical, quantitative and verifiable response when exposed to a specific stressor, such as excessive ozone (O_3) air pollution. These sensitive plants can be used to detect the presence of an air pollutant at a specific location or region and can provide unique information regarding changes in air quality. This is usually accomplished by observing the amount and severity of pollutant-induced foliar injury to a single specific plant genotype. In most cases, the assessment of bioindicators involves the subjective determination of the extent of evident injury symptoms. As a general rule, a "good" bioindicator plant should satisfy some essential requirements, such as, *(i)* to be very sensitive to a specific pollutant and then to have a low threshold for visible injury, the reaction must occur quickly when exposed to realistic pollutant concentrations, without being killed by the pollutant levels with which it comes into contact; *(ii)* the visible response should be precise, characteristic, possibly specific and definitively reproducible in every site and under any condition; *(iii)* the intensity of the visible response must be easily quantified and an appropriated calibration procedure against the actual pollutant doses must be possible; this allows the extrapolation of pollution levels from biological data; and *(iv)* the indicator should have an adequate period of vegetation (coincident with the significant presence of the target pollutant) and be easily cultivable, well adapted to the environment and tolerant to the other major stress factors, both of a biotic (pests, parasites) and abiotic (thermal extremes, water deficit, other chemical pollutants) nature (Nali et al. 2006). Bioindicators are primarily important for demonstration and educational projects. Visible effects of air pollutants on plants are very convincing and easy to understand by non-scientists and particularly by policy makers and representatives of the media. In some cases, instead, the relevant parameter is the ratio between biomass produced by a sensitive *vs.* a resistant genotype after exposure to ambient air in the presence of a pollutant agent with phytotoxic activity.

Bioindicators of air quality can be used in two ways; they can be either deliberately introduced, *i.e.*, translocated from one place to another ("active BM", with "sentinel" individuals), or be native/cultivated (already present) species ("passive BM", with "detector plants").Active and passive methods focus on two distinctive features, active monitoring performs regular checks, whereas passive monitoring concentrates on real open-air phenomena by "watching" what is happening (Falla et al. 2000). In the latter case, the interpretation of results must consider an essential number of criteria related to the heterogeneity of the living conditions, such as genetic variability, soil quality, climatic parameters, and the health status of selected plants.

Finally, automatic devices cannot evaluate unforeseen compounds (they are specific and only detect the compound(s) for which they are conceived, an-analyzer-for-a-pollutant), and the cumulative/synergistic biological effects of pollutants in the mixture. Of course, they only characterize conditions at the time of sampling, not allowing the integration of past environmental conditions. On the contrary, the use of BM can answer these issues. On the other hand, plants present the disadvantage of being unable to provide a response in a time lapse comparable with that of on-line analyzers, not to mention the myriad of chemical, physical and biological factors that influence the response of biota to stress factors.

The reciprocal relationships between conventional monitoring and BM of O_3 are depicted in Fig. 4.1. In a few words, BM does not replace modeling or the physico-chemical methods of direct measurement of air pollutants; it complements them by demonstrating the biological effect.

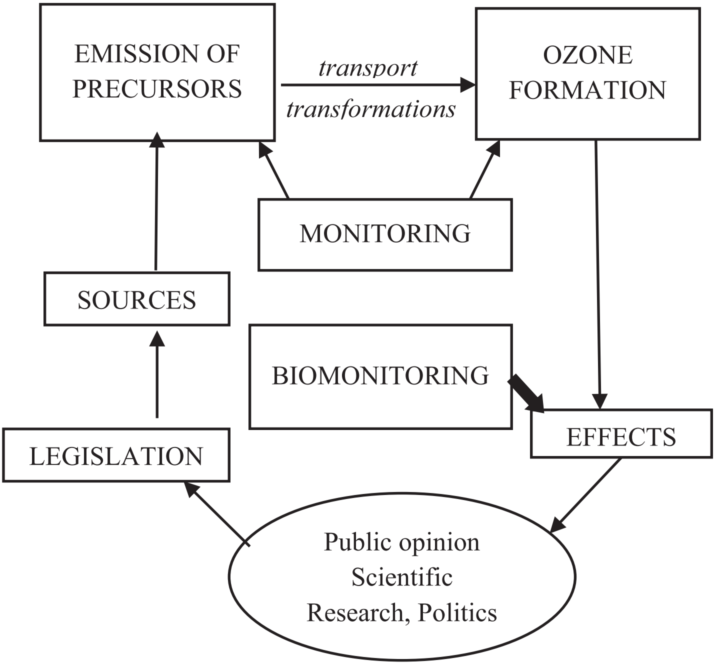

Fig. 4.1. The integrated "system" which connects ground-level O_3 formation/diffusion and its monitoring/biomonitoring. Ozone is a secondary pollutant, not released directly into the lower atmosphere, but instead is formed *in situ* by a complex series of chemical reactions involving sunlight, oxides of nitrogen and volatile organic compounds, including those emitted from both natural and manmade sources. Conventional monitoring is in charge of measuring these precursors at the source(s). The next step is represented by the transport and chemical transformations of these pollutants, to give rise to O_3 molecules in the photo-smog. Again, automatic analyzers are irreplaceable in giving detailed knowledge on the actual on time concentration of O_3. But the rule of conventional monitoring ends up here: no information is given. Concerning the biological impact of a certain pollution scenario. It is this the mission of biological monitoring. It is easily agreeable that conventional and biological monitoring of air quality are complementary/additionally partners.

4.2 Monitoring of Ground-Level Ozone, A Brief History

Hidy et al. (2017) reconstructed in detail the history of ambient-air O_3 measurements. Generally, the discovery of O_3 is ascribed to Christian Friedrich Schönbein in the year 1840. He developed a protocol for O_3 quantitative detection using an iodate-based oxidation method. Paper strips are coated with potassium iodide and starch; when placed in the presence of O_3, the potassium iodide oxidizes and is converted to potassium iodate (KIO_3). In the chemical process, the paper changes color to various hues of blue. The response of the strips also depends on a variety of factors, especially relative humidity, and thus only serves as a rough indicator of O_3 concentration. The first sustained, quantitative urban measurements appear to have been made in Paris in the 1870s. Systematic ground-level O_3 monitoring began in the Los Angeles area in the 1950s using the cracking rate of rubber bands exposed to polluted air. The next step was a potassium iodide wet chemical method. Measurements evolved from wet chemical to instrumental methods in the 1970s.

The method most commonly applied since then uses ultraviolet (UV) absorption at 254 nm (Beer's Law); a second method involves chemiluminescence from the reaction of O_3 with NO or with a reactive hydrocarbon. Nowadays, most continuous indoor and outdoor automatic air analyzers work on the principle of UV absorption measuring O_3 with detection limits as low as 0.2 ppb, or even less. They may be installed in stationary monitoring units or mounted on *ad-hoc* adapted vehicles. The principle consists of measuring absorption by O_3 molecules of UV light (provided in the past by mercury lamps, now replaced by LED technology). Ozone concentration is determined by the difference between UV absorption of the gas sample and the sample without O_3 after filtration performed by a high-quality catalytic converter. Both signals are detected by a photodiode, offering accurate and stable signal detection. These user-friendly electronic devices are provided with an internal O_3 generator for span check control and with settable alarm levels for concentration. A wide variety of internal diagnostics is usually available and instruments can gather information quickly and produce reliable data. Costs for the purchase and maintenance of analyzers are significant and may represent limiting factors for the set-up of diffused monitoring networks. Besides, these instruments require electrical power, and this could be a handicap in field campaigns in back-country sites, even if, recently, portable solar-powered continuous O_3 monitors have been used for real-time monitoring in remote locations (Burley et al. 2015).

Passive samplers complete the inventory of non-biological procedures of O_3 detection. They offer a simple, cost-effective means of measuring air pollutants in the many situations where integrated measurements (e.g., dose, exposure, time-averaged values) are more useful than short-time resolution (Sanz et al. 2007). Their key limitation is that the time-averaged data obtained do not describe diurnal patterns of pollutant exposure, which can be important when considering the stomatal uptake and biological effects of pollutants. However, statistical procedures have been successfully used to estimate biologically relevant O_3 exposure indices from passive samplers (Ferretti et al. 2012).

4.3 Ozone and Plant Life and Health

Ground-level ozone pollution is a significant health hazard and causes more damage to plants than all other common air pollutants combined in many regions of the world (Ainsworth 2017). Air pollution due to tropospheric O_3 has been known since the late 1950s to cause significant injury and economic losses to many agricultural crops, native plants, and forest tree species throughout many regions of North America and Europe (Middleton et al. 1950); more recently, Asian countries are experiencing similar problems (Royal Society 2008). Given projected trends in populations, economic outputs, and the associated increased demands for required energy supplies, not to mention climatic variations, O_3 air pollution impacts are very likely to increase (Ainsworth et al. 2020). The effects of O_3 on biological systems are attributed to its ability to spontaneously dismutate or react with cellular constituents to generate excess active oxygen species (Rao and Davis 2001). Ozone enters leaves through open stomata during normal gas exchange and does not accumulate in plant organs; it quickly reacts in the apoplast to produce reactive oxygen species (ROS), such as superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($\cdot OH$) and singlet oxygen ($^1O_2$) (Janků et al. 2019). The most common effect induced by long-term sublethal O_3 exposures on sensitive plants is the induction of premature leaf senescence, as a consequence of damage to cell membrane biochemistry and architecture (Miller 1999). These symptoms are specific and hard to detect under natural conditions. However, under more stressful conditions, foliar necrotic lesions in the form of interveinal stipple on the adaxial leaf surface have been documented on O_3 sensitive plant species in many countries under natural conditions (Flagler 1998). They appear as uniformly sized red to brown minute spots, but a wide symptomatic divergence has been reported. Of course, not all plant injury is O_3-induced, and mimicking symptoms may be due to a plethora of biotic and natural or man-made abiotic stress factors. Probably the most appropriate definition for such symptoms should be "ozone-like visible injury." A distinctive pattern of O_3 injury is the localization of the upper leaf surface. Still, field diagnosis of plant diseases only based on symptom observation is "more an art than a science" (Grogan 1981).

4.5 Active Biological Monitoring of Ozone with Vascular Plants

4.5.1 Tobacco

It was 1951 when growers of cigar-wrapper tobacco (*Nicotiana tabacum*) in Connecticut were first concerned about a devastating leaf spot disease of unknown origin (tentatively named "weather fleck") (Heggestad and Middleton 1959). As suggested by the name, the appearance of symptoms in the field was related to weather, especially hot sunny periods. Damaged cured leaves of tobacco had little or no value. Palisade cells were the first to show injury. It is noteworthy that the cigar wrapping tobacco types are considered entirely different genetically from tobaccos of other types, such as flue-cured (Heggestad 1991). An O_3-supersensitive germplasm (named "Bel-W3", "Bel" is for "Beltsville", MD, the location where the research was carried out) was identified during breeding activities, and it was 1962 when Bel-W3 was officially proposed as a biological indicator of O_3 exposure (Heggestad and Menser 1962). Since then, Bel-W3 plants have been used in all continents (Heggestad 1991).

The general pattern of injury on Bel-W3 varies somewhat, depending on the age of the plant, leaf position, O_3 level, and environmental factors such as nutrition, moisture, and light (Heggestad 1966). When atmospheric concentrations of O_3 exceed approximately 40–50 ppb for 4 h, or 30 ppb for 8 h (this is the lowest threshold for all species tested by the scientific community), symptoms arise on leaves of Bel-W3. They include initial, adaxial-surface water-soaking in the form of small (a few millimeters), greyish, roundish, well-defined flecks. In a couple of days, sharply defined permanent dot-like lesions appear on both adaxial and abaxial surfaces as a result of complete mesophyll collapse (Pasqualini et al. 2003 – Fig. 4.2).

Fig. 4.2. Transversal section of a foliar lesion induced by ambient O_3 on tobacco Bel-W3; please note the complete collapse of the mesophyll

This is a very peculiar response, due to the extreme sensitivity of this germplasm, because, as a general rule, O_3 injury on “normally” sensitive species/cultivars (including other tobacco cultivars) is limited to the death of groups of palisade cells, resulting in necrosis which is only visible on the adaxial leaf side. The reaction is related to leaf age; older leaves are more susceptible (and symptoms concentrate in the proximal districts), and younger leaves exhibit lesions only in the distal regions. Leaves having a length of less than 6–8 cm are almost insensitive to O_3. The final color of mature lesions is light ivory. So, it is possible to separate the new O_3 injury from the old injury by its darker color. Lesions can coalesce to form larger necrotic areas causing the lamina to dry out, but because of the absence of an abscission layer, the dead leaves remain attached to the stem (Fig. 4.3).

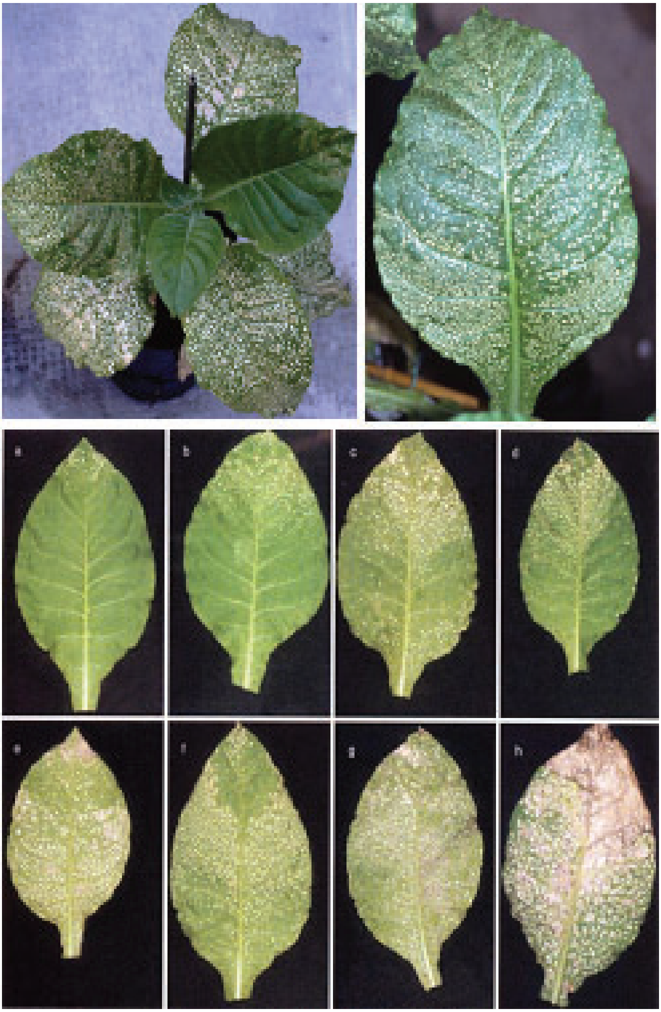

Fig. 4.3. *Top left*: Effects of a 7-day exposition to ambient O_3 of a tobacco Bel-W3 plant showing the peculiar distribution of O_3-induced fleck. Sensitivity is inversely related to leaf maturity: very young leaves are unaffected, young ones are only sensitive in their distal portions; and mature leaves are affected on the whole surface. *Top right*: Close-up of a mature leaf of tobacco Bel-W3 showing necrotic injuries due to O_3 after exposure of a week to ambient air. *Bottom*: Increasing levels of O_3-induced fleck on leaves of tobacco Bel-W3 due to a 7-day exposure to ambient air

Biomonitoring protocols are applied in community, local, regional, and nation-wide campaigns. The entire methodology, from plant cultivation to exposure, injury assessment, data treatment, statistical analysis and presentation of results, is now standardized and guidelines have been published first by the Association of German Engineers (VDI 2003) and more recently by the European Committee for Standardization (CEN 2016). Ozone-resistant tobacco plants (namely, the cv. Bel-B, whose sensitivity threshold in terms of visible injury, for 2-h exposures is 220 ppb, *vs* 100 ppb of Bel-W3, Heggestad 1991) are routinely inserted in the plots, so the appearance of injury on Bel-W3 but not on Bel-B provides further confirmation that such injury is due to O_3. In any case, foliar injury in Bel-B is only represented by upper-surface flecking (Krupa et al. 1993).

The plants start in an O_3-free greenhouse/controlled environment chamber (air is filtered through active charcoal) following standard agronomic protocols in terms of fertilization and watering. When they have developed 5–6 expanded leaves, they are transplanted into cultivation containers provided with water dispensing devices and moved to the test sites. The exposure sites should be open and reasonably distant from any local source of pollutions. As Bel-W3 is shadow tobacco, a shading net (50%) is provided; this also guarantees (partial) protection from bad weather conditions and animal vandalism. All fully expanded leaves are individually labelled (and the same number is maintained all through the exposure time), and every week, a leaf injury index (LII) is calculated. The impracticability of counting and measuring individual lesions for assessing leaf spots in large plant populations motivated scientists to rely upon other more rapid visual methods. Visual assessment still represents the best way to rapidly quantify the extent of necrotic injury induced by O_3 on tobacco when dealing with many field plots, which is a critical step. Usually, the proportion of necrotic areas is estimated in relation to the whole leaf area. To keep the subjective estimation error as small as possible, reference photographs of sample leaves measured with digital image processing (Della Mea et al. 1997) (or, more simply, with the transparent grid method, Ashmore et al. 1980) can be consulted. LII is calculated for each week as the difference in the mean percentage of the leaf area injured. Only leaves that received a score lower than 10% of the necrotic area at the week *n-1* are considered when processing the database for the week *n*. This is because the auto-collapsing of tissue can occur at certain levels of leaf damage, causing an increase of leaf injury regardless of the exposure to O_3. Assessment should be performed under ideal lighting conditions and leaves. As a general rule, leaves should always be approached from the same side (*e.g.*, the tip towards the evaluator). Attention should be paid to avoid misinterpretation of O_3 injury on Bel-W3 with lesions caused by other confounding stress factors, as tobacco leaves can be affected by invertebrate pests and other pathogens, such as viruses (especially PVY, the Potato Virus Y), bacteria (*Pseudomonas syringae* pv. *tabaci*, the causal agent of "wild fire"), and especially fungi causing minute round necrotic spots. This is the case, for instance, of *Cercospora nicotianae* (etiological agent of "frog-eye") and brown spot (*Alternaria alternata*) (Shew and Lucas 1991). No differential response of Bel-W3 and Bel-B to such biotic agents has been reported, and canonical laboratory investigations easily pinpoint the actual culprit of symptoms in biotic agents.

Traditionally mature tobacco Bel-W3 plants have been used in all continents for local, regional and nation-wide campaigns (Heggestad 1991). The first nation-wide experience was developed in the Netherlands (Posthumus 1976). In Italy, the first campaigns date back to the 1980s (Lorenzini and Panattoni 1986). However, the use of adult tobacco plants implies remarkable logistical difficulties in establishing a lot of plots over extended areas, especially due to the fragility and brittleness of their large leaves, so that massive long-distance transport is difficult, not to mention that a wide space (and a long time) in filtered-air growing facilities is required. Visual assessment of leaf injury has to be performed by remote operators; all of them must be adequately formed. In addition, adult plants stay in the place for several weeks (usually a month) before substitution, and exposure to below injury threshold O_3 concentrations may reduce the concentration of O_3 necessary to produce new injury (Heagle and Heck 1974; Steinberger and Naveh 1982). All of this may represent a limiting factor to the full application of the methodology over a large geographical scale.

To overcome such restrictions, a miniaturized kit for very young plants was developed by Giacomo Lorenzini at the University of Pisa, Italy (Lorenzini 1994 – Figs. 4.4, 4.5).

Fig. 4.4. The miniaturized kit based on seedlings of tobacco Bel-W3 for biomonitoring O_3. Please note the presence of fleck on cotyledons and first true leaves

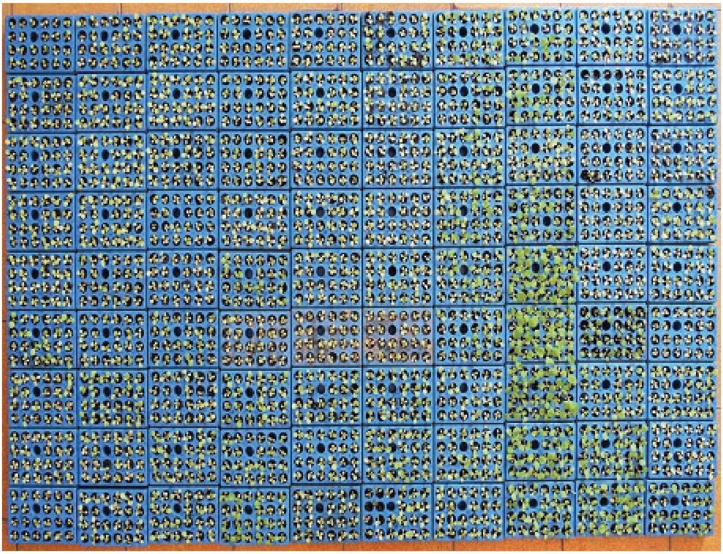

Fig.4.5. The final “harvest” of a typical field campaign based on the miniaturized kit based on seedlings of tobacco Bel-W3

The device is easily transportable (it may be shipped by mail) and, therefore, ideal for assessing spatial patterns over wide areas. The kit is based on a commercial polystyrene 24-well culture plate (12.5 × 8 cm) modified with a water reservoir. Each well (16 × 20 mm depth) is perforated, allowing the water in the base to hydrate the root system. Seedlings of the two tobacco cultivars (O_3-resistant and -sensitive, respectively) are germinated in trays in a peat/organic compost in a controlled environment growth chamber ventilated with charcoal-filtered air. Nineteen Bel-W3 plantlets and four Bel-B plantlets are transplanted into each tray, and the final well is left free for watering. When the first true leaf measures *ca.* 1 cm, the seedlings are individually transferred to the wells in the plate. After a couple of days, they are transported to the site and placed under a cloche with a 50% shading factor. The typical exposure time is one week; then, the kit is substituted with a new one. Symptoms evaluation is performed the following day (to allow the ripening of the last produced lesions) on cotyledons and the first true leaf using a hand lens. This allows the kits to be shipped to the home station, so that lectures of symptoms may be performed by a single, experienced team. The percentage of necrotic tissue is related to a pathometric scale. Correlation studies among the cotyledonar and foliar injury index and actual O_3 levels monitored through automatic analyzers emphasized that the sensitivity and accuracy of this methodology are comparable with those employing adult plants (Toncelli and Lorenzini 1999). The protocol is officialized by the Italian Environmental Agency (ANPA) (Lorenzini 1999). Several successful field applications have been reported (e.g., Nali et al. 2006 and references herein reported for Italy; Davies et al. 1998 for the UK; Gombert et al. 2006 for France; Cheng and Sun 2013 for China).

Handling, processing, and restitution of information gathered in BM campaigns follow the conventional steps of environmental data management. Usually, final results are communicated in graphical form (is pollution charts) using dedicated contouring or 3D surface mapping software packages (Fig. 4.6).

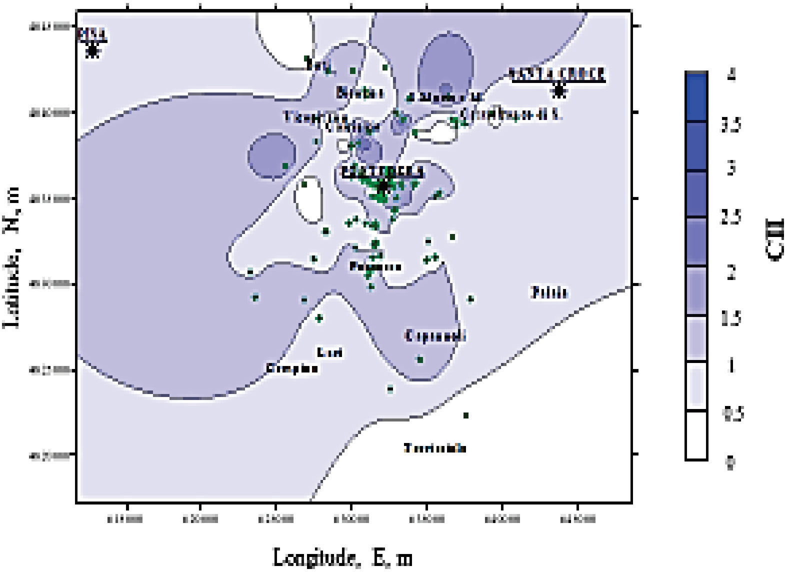

Fig. 4.6. Spatial distribution of the Cotyledonar Injury Index (CII, on a 0–4 scale) on tobacco Bel-W3 seedlings (“minikit”) in 14 municipalities of Valdera District (Province of Pisa, Central Italy) at the end of a typical 7-day O_3 exposure. Dots and asterisks indicate locations of biomonitoring points and of automatic O_3 analyzers, respectively. Geographical coordinates are expressed in the UTM/WGS84 System

Uncertainty is much more inherent in most biological data and, in particular, environmental biology, than in the physical and chemical systems. Quality assurance (QA) procedures are a fundamental part of BM activities. This means that all steps of the program should be correctly addressed, from the design of the locations of experimental plots to data collection, processing, and reporting. Large-scale campaigns involve several field observers and are exposed to risks of estimation errors in evaluating the intensity of O_3 induced lesions. Visual assessment of the intensity of foliar necrotic symptoms is the core activity of BM with Bel-W3. Shape, orientation, shading, surrounding figures, and personal traits enter into perception. Because disease scoring follows the Weber-Fechner law, which states that the response of an organism to a stimulus is typically encoded in a non-linear manner but instead is a linear function of the logarithm of the stimulus magnitude (Zimbardo et al. 2006), there is a trend to overestimate lower classes of injury and to assign too few values to higher classes (Lorenzini et al. 2000). As a rule, when lesions occupy less than 50% of the total leaf area the eye focuses on the diseased tissue, and *vice versa* (Horsfall and Cowling 1978). In addition, many leaves show an asymmetric distribution of lesions, mainly in terms of proximal/distal areas (this is a function of ontogenic factors), but also in terms of “right-and-left”. The role of operator training is discussed in detail by Francini et al. (2009).

The final step is the statistical correlation between and the extent of foliar injury on Bel-W3 tobaccos and mathematical descriptors of the actual ambient O_3 exposure. Ozone holds an undeniable peculiarity in atmospheric science, not being directly emitted by point sources (i.e., it is a “secondary” pollutant). So, the daily and annual variations in O_3 concentration at any given spatial scale depend on several factors, such as the proximity to large sources of O_3 precursors, the relative ratio(s) between precursors, geographical location, and the prevailing meteorological conditions. Sunlight is the driving force, but other factors such as wind prevalence, temperature, and relative humidity/vapor pressure deficit play crucial roles (Kley et al. 1999). As a consequence, daily and seasonal profiles of O_3 levels show huge differences, for instance, between day and night, summer and winter, and so on. In addition, effects of O_3 on vegetation are dependent on O_3 entry into the leaf, and this only happens during daylight hours; therefore, specific O_3 metric descriptors, different from those useful in human medicine, have been developed for vegetation on time, such as M12, W126 and AOT40 (see Mills et al. 2018 for details). After more than half a century of field and lab activities, the prevailing position in the scientific community is that the overall results suggest that the visible foliar response of tobacco Bel-W3 can be used as a qualitative, but not necessarily a quantitative indicator of relative ambient O_3 pollution on a generalized temporal or spatial scale (Krupa et al. 1993).

4.5.2 Other herbaceous species

Several reports describe the experimental selection of other plant genotypes as candidates for active ozone biomonitoring under different climatic conditions. This is the case, for instance, of the Sugar Baby cultivar of watermelon (*Citrullus lanatus*) (Gimeno et al. 1995), the local Egyptian cultivars of jute (*Corchorus olitorius*) (Madkour and Laurence 2002), the wild currant tomato species *Lycopersicon pimpinellifolium* (Iriti et al. 2006), and morning glory, *Ipomoea nil* (Lamano Ferreira et al. 2012). However, none of these candidates may successfully compete with tobacco Bel-W3 in terms of sensitivity, readiness of response, and appearance of pathognomonic foliar symptoms.

4.5.3 Citizen science applied to the biomonitoring of ozone, The “ozone garden projects”

During the last decade(s) in the USA, tens of “ozone garden projects” have been launched (Fishman et al. 2014). These are featured activities within visitor centers for environmental sciences, botanical gardens, science museum venues, community gardens, high schools, and similar locations. An O_3 garden is simply a collection of plants that are known to be sensitive to O_3. When exposed to O_3 over time, these individuals are expected to develop more or less characteristic signs of foliar injury. Usually, these plots are based on four plant species known to be easily established and maintained, the snap bean (*Phaseolus vulgaris*, see beneath), common (*Asclepias syriaca*) and tall milkweed (*A. exaltata*), and the cut leaf coneflower (*Rudbeckia laciniata*). Soybean (*Glycine max*) and potato (*Solanum tuberosum*) are also often present. Local genotypes may be involved as well. An O_3 garden serves as an outdoor laboratory for citizens, educators, and students to collect and analyze information on air pollution’s effects on O_3 sensitive plants. The garden demonstrates first-hand the observable and detrimental impacts of O_3 on living organisms and acts as a visual tool representative of O_3, which cannot otherwise be seen by the naked eye. Visitors interact with the garden to learn how to identify O_3 injury, which fosters a curiosity to look for O_3 effects on sensitive plants in their local area. Many of the existing O_3 gardens follow a common layout and detailed field guide and accompanying training mechanisms and handouts provided by NASA (Ladd et al. 2011). This approach focuses on how science, technology and climate change influence the efficiency of food production.

4.5.4 The use of EDU for biomonitoring purposes

The chemical N-[2-(2-oxo-l-imidazolidinyl) ethyl]-N’-phenylurea (abbreviated as EDU, for ethylenediurea) is a molecule with a significant ability to protect plants from injury by atmospheric O_3 (see Zhaozhong et al. 2020, for a review). The nature and modes of action (biophysical and biochemical) of EDU have been detailed by Singh et al. (2015). This compound is useful as a survey tool to determine the location and magnitude of crop losses due to O_3. In addition, it can be used to verify the response of an O_3 bioindicator in the field. Usually, half of the indicator plants are treated with a soil drench or foliar spray, before out planting. In the presence of significant O_3 pollution, the treated plants will not develop symptoms of foliar injury while the non-treated individuals will show varying degrees of typical O_3 injury symptoms, verifying that O_3 is the cause. For instance, weekly EDU applications have been recommended as a tool for confirming the deciduous shrub *Hibiscus syriacus* in diagnosing O_3 presence (Paoletti et al. 2009).

4.5.5 White Clover

As visual estimates of the intensity of foliar injury involve subjectivity, there may be some limitations to the tobacco protocols above described. But, even more important, visible injury induced by O_3 does not always translate into yield loss (Heagle and Letchworth 1982), a parameter which instead may be relevant for policymakers. So, bioindicator systems have been developed that relate biomass loss to ambient O_3 concentrations, based on non-subjective measures–, they use the shoot biomass response from sequential harvests as a quantitative descriptor of O_3 effects. This is the case of Heagle et al. (1994) who have developed a protocol by using a sensitive (NC-S) and a resistant (NC-R) clone of white clover (*Trifolium repens*). Plants are container-grown in a standardized pot culture and the total epigeous forage biomass (leaves, stems, flowers) is removed at 28-day intervals. Dry weights are obtained and the O_3 impact is determined by calculating the ratios of NC-S to NC-R. A ratio of less than one indicates that ambient O_3 has had an adverse effect of shoot biomass on NC-S (Figs. 4.7, 4. 8). The virus-free clones are maintained by vegetative propagation and are currently used in a number of research programs in the United States and Europe (Nali et al. 2006). Intimate bases of this differential behavior in the presence of O_3 have been elucidated (Francini et al. 2007).

Fig. 4.7. Biomass production of white clover (*Trifolium repens*) cv. Regal NC-R clone (left) compared to that of the NC-R clone (right) in a typical summer monitoring campaign

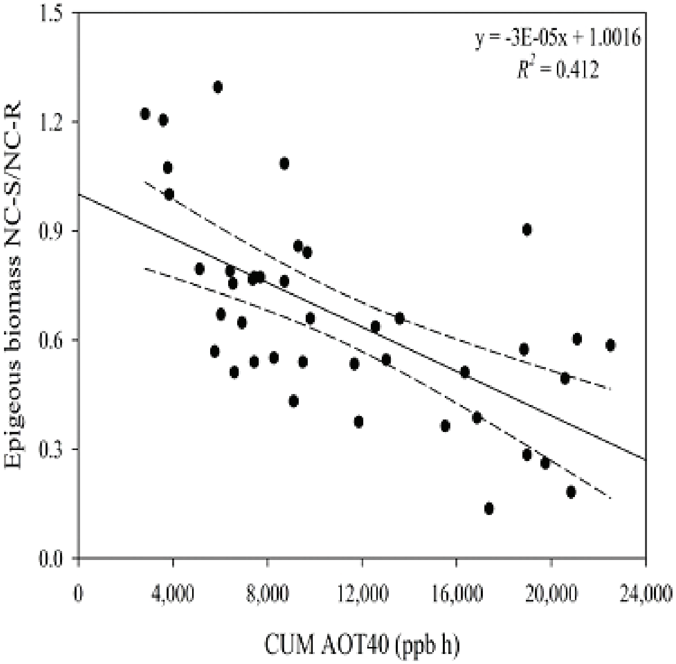

Fig. 4.8. Response of the NC-S/NC-R biomass ratio of white clover clones to AOT40 accumulated over the previous 4-week periods between 1997 and 2007 (only summer months) in Pisa (Italy); broken lines represent the confidence interval (P=0.05). Data from Nali et al. (2009). AOT40 represents the sum of the differences between the hourly O^3 concentrations (in ppb) and a threshold value of 40 ppb, for each hour in the interval 08:00–20:00 h (solar time)

4.5.6 Snap Bean

Similar to the above-described clover system, a protocol based on O_3-sensitive and -resistant snap bean (*Phaseolus vulgaris*) genotypes (S156, sensitive and R123 resistant) that produce similar biomass under low O_3 conditions, but are differentially affected at elevated O_3 levels typical of present-day pollution levels has been developed by Kent et al. (2005) (Fig. 4.9). An S156 to R123 pod yield ratio of approximately one is observed under charcoal-filtered air conditions, to decline towards lower values under the O_3 challenge, and the lower the ratio, the more damage is caused by O_3. A limiting factor is that multiple planting dates are required to obtain a series of snap bean harvests during a growing season available from a single clover planting.

Fig. 4.9. Ozone-resistant (R123, left) and O_3-sensitive (S156) snap bean plants after three months of ambient air exposure in the Castelporziano Presidential Estate (Rome). Photo credit: Elisabetta Salvatori, Sapienza Università di Roma, Italy

4.6 Passive Biological Monitoring of Ozone with Vascular Plants

Many native sensitive plants have been evaluated for their potential use as detector species capable of detecting the presence of O_3 air pollution through the development of specific and distinctive symptoms. The typical symptom of O_3 foliar injury on dicots is an adaxial surface interveinal discoloration of small groups of cells ("stipple"), appearing as uniformly sized red to purple to brown spots, but – as previously reported – several confounding biotic and abiotic stress agents may induce similar effects. Key diagnostic evidence is that O_3 injury symptoms do not appear on the veins of a leaf and usually only occur on the upper leaf surface, leaving the lower leaf surface symptom-free. Guides to the identification of an O_3-induced foliar injury area are available (Flagler 1998; Innes et al. 2001; Ladd et al. 2011). Forest surveys and open-top chamber studies indicate that black cherry (*Prunus serotina*) may be a reliable bioindicator of foliar injury due to ambient O_3 (Chappelka et al. 1999). Similar approaches do not identify specific levels of O_3 present in ambient air but rather identify whether conditions are favorable for O_3 injury to occur, integrating existing environmental conditions (relative humidity, soil moisture, temperature, etc.) that determine actual O_3 flux (Coulston et al. 2003).

Recently, a new way of recording visible ozone injury on vegetation has been created by the ICP Vegetation team (http, //icpvegetation.ceh.ac.uk). A smart-phone App allows users to upload photographs of ozone injury and location co-ordinates as soon as damage is observed. An expert team can confirm (or not) the reports to adjourn distribution maps.

4.7 Conclusive Remarks

Natural means of assessing spatial and temporal patterns of airborne pollutants at very low cost offer a suitable method of monitoring which many believe could provide a valuable adjunct to the scientific based techniques currently in place. Some sticking points are that *(i)* BM does not replace modeling or physico-chemical methods of direct measurement of air pollutants; it complements them by demonstrating the biological effect; *(ii)* bioindicators are a means of monitoring in case of a lack, or insufficiency, of the conventional means of analyzers to follow the aerial dispersion of ozone (as well as of other pollutants) and to map the polluted areas; *(iii)* bioindicator stations are practical for at least two reasons, they are adaptable to space and time limits. Moreover they are mobile and are easily constructed, as well as being more readily operational than the traditional network stations; *(iv)* bioindicator stations can make it possible to supplement at moderate costs the pre-existing network; *(v)* bioindication can orientate the location of physico-chemical stations by providing preliminary information about pollution levels; and *(vi)* bioindicators raise public awareness by recourse to living organisms (*i.e.*, O_3-sensitive plants make invisible air pollution visible). Visual assessments are quickly made and do not require expensive equipment, chemical analysis, or highly trained personnel, but, as their subjective nature creates concern, rigorous quality control criteria and procedures should be adopted to qualify BM activity. Caution must be applied in presenting the results from BM activities, as the response of supersensitive material, such as tobacco Bel-W3 cannot in any way be directly translated into impact on native plants and crops.

Specific plants, and namely tobacco cv. Bel-W3, are considered to be reliable bioindicators of ambient O_3. Injury on these plants is usually the first indication that a region has developed an O_3 problem. Bel-W3 tobacco as a "perfect" bioindicator, is highly sensitive and develops easily recognizable and quantifiable peculiar foliar symptoms and its vegetative period is coincident with the photo-smog season. A critical point is the establishment of robust exposure-response relationships, as the reaction of plants to O_3 depends amongst others on external growth factors that include edaphic and climatic conditions.

With BM, the impact of the effective O_3 dose on a living organism is directly measured. Information provided by indicator plants such as tobacco has incomparable value from the didactic and educational points of view and to raise environmental awareness (Fig. 4.10), the vision of the severe macroscopic injury which ambient air causes to sensitive organisms may stimulate in the citizen an unmatchable involvement in environmental issues. Standardization of methods is crucial to develop air quality standards based on effective monitoring, and the whole protocol based on Bel-W3 tobacco is defined in detail. Limiting factors and bottlenecks still endure, for instance; a world-wide seed bank is necessary to ensure genetic homogeneity of germplasm; used for field campaigns. Environmental protection agencies, usually ruled by non-biology-trained managers, often manifest a scarce enthusiasm towards BM, also supported by the poor commercial inclination of the BM advocates. The legislative context of the BM of O_3 is still to come.

A final recommendation, do not forget that to prevent air pollution effects on plants means to prevent negative implications on humans.

Fig. 4.10. Biological monitoring of ground-level O_3 is a formidable exercise of environmental education and problem-solving for pupils, students, teachers (and families). The picture is the result of an educational activity with children of a primary school

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CHAPTER FIVE

ADJUSTING THE BIOMONITORING MODEL WITH TOBACCO BEL-W3 FOR ESTIMATING OZONE LEVELS UNDER THE SUBTROPICAL CLIMATE

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Abstract

Numerous plant species that develop a visible injury in response to phytotoxic levels of ozone (O_3) may theoretically be applied in O_3 biomonitoring programs. Among them, the O_3-sensitive *Nicotiana tabacum* cv. Bel-W3 has been the most used worldwide. However, meteorological conditions may limit the relationship between leaf injury variations in such cultivar and ambient O_3 concentrations, restricting its bioindicator potential. Many studies have shown that the bioindicator efficiency of tobacco plants reduces from the Atlantic coast to the interior of São Paulo State in SE Brazil because of the weather conditions. We elaborated this chapter intending to adjust the protocol for tobacco Bel-W3 to improve the efficiency of O_3 biomonitoring in subtropical regions with dry winters and hot summers. The Metropolitan Region of Campinas

(MRC) in São Paulo State was chosen as the model region for such purposes because it is typically characterized by a seasonal climate and phytotoxic O_3 levels. The biomonitoring using tobacco Bel-W3 was performed in fourteen sites within the MRC, categorized according to their major neighboring land uses. The plants were exposed to each site for consecutive periods of 14 days for three years. After each exposure, the percentage of foliar injury was estimated, aiming at estimating the Leaf Injury Index (LII) in each plant. The leaf area (LA), leaf biomass (LM), and leaf mass/area ratio (LMA) were measured in the 5th, 6th, and 7th oldest leaves of each plant. O_3 concentrations, air temperature (T), relative humidity (RH), rainfall (P), and solar radiation (SR) were also recorded during the experimental period. A series of multiple linear regression analyses was performed among different O_3 descriptors and meteorological variables (vapor pressure deficit, T, RH, P, SR) and leaf traits (LII, LA, LM, LMA), aiming at proposing the most appropriate biomonitoring model for estimating the O_3 levels using tobacco Bel-W3. The most explicative multilinear model ($R^2 = 0.78$; $p <0.001$) was: O_3(24h average) = 6.386 – (0.0727*LA) + (0.32*SR), which was used for estimating average daily concentrations of O_3 during 14-day periods in the biomonitoring sites. The estimated O_3 concentrations varied between 40 and 100 mg/m^3 during dry exposure periods and between 60 and 160 mg/m^3 during wet periods. This adjusted model also indicated that crops and forest remnants are at higher risk imposed by O_3 than human populations in urban areas.

Keywords: O_3 biomonitoring; *Nicotiana tabacum* cv. Bel-W3; Protocol adjustment; Subtropical seasonal climate; Leaf area, Solar radiation; Risk prognosis

5.1 An overview of ozone effects on forest ecosystems and crops

Ozone (O_3) is the most abundant tropospheric oxidant and an important component of photochemical pollution (Ryerson et al. 2001). This air pollutant is formed in the troposphere under bright sunlight through the oxidation of the primary pollutants, such as nitrogen oxides (NOx) and volatile organic compounds (VOCs) (Percy and Ferretti 2004). These precursors originate from natural sources, including wildfires, downward intrusions of naturally produced O_3 from the stratosphere, biogenic hydrocarbon emissions, lightning NOx, and biogenic NOx emitted from soils, and also from anthropogenic fossil fuel and biofuel combustion or crop burning (Cooper et al. 2014). During the 20th century, the O_3 levels more than doubled due to industrialization and traffic developments (Vingarzan 2004). Data have indicated that O_3 concentrations in the 21st century will be higher than during the 1970s and 1980s. Nowadays, O_3 concentrations can reach 60 nmol.mol^{-1} during the summer in locations like the western USA, southern Europe, China, South Korea, and Japan (Gaudel et al. 2018).

Fowler et al. (1999) estimated the global forested area that is at risk of being affected by O_3 concentrations > 60 ppb (concentration likely to be phytotoxic to sensitive forest species), using a 3D chemistry-transport model (CTM). It simulated the atmospheric transport and chemistry of the pollutant emissions using scenarios for 1860, 1950, 1970, 1990, and 2100. The model showed no forest areas exposed to such O_3 levels in 1860 and 6% of global forests in 1950; 75% of these forests were located in temperate latitudes and 25% in the tropics. By 1990, 24% of global forests were exposed to O_3 concentrations >60 ppb, and this percentage will increase to almost 50% of global forests by 2100 (Fowler et al. 1999).

The accurate projections of current regional trends or future O_3 concentrations are complicated by its temporal and spatial heterogeneity, depending on the distance from sources of pollutant precursors, time of day, and time of year (Wittig et al. 2009). In rural areas of industrialized countries, for example, with moderate NOx levels, O_3 formation dominates (Ainsworth et al. 2012). In these remote areas, non-urban surface O_3 in the recent past (2010–2014) was mostly found in North America, Europe, and East Asia (Korea and Japan), indicating that the Southern Hemisphere has much lower levels of O_3 than the Northern Hemisphere (Gaudel et al. 2018). However, most of the maps' trends from satellites in the tropics have shown O_3 increases since 2003 above the Amazon (Gaudel et al. 2018). Another point of attention is that O_3 concentrations showed little yearly variation and no clear O_3 seasonality in Brazil's sub-tropical humid climate, contrasting with the findings at higher latitudes in the Northern Hemisphere (Moura et al. 2014).

Despite any O_3 uptake having the potential to affect plants, non-linear responses and lag times are expected, affecting how O_3 triggers them from the toxicological perspective (Musselman et al. 2006). Indeed, studies have extensively shown a hormetic-biphasic dose-response induced by a variety of chemical agents and environmental factors, including O_3, in a wide spectrum of endpoints and biological models (see Agathokleous et al. 2019a, for an overview). The hormetic responses to O_3 have been observed not only at the individual but also at community levels, including key ecological endpoints such as biodiversity, soil respiration, emission of leaf volatile organic compounds (VOCs), gas fluxes from soils, the demography of pests of agricultural crops, and reproduction of terrestrial and aquatic organisms (Agathokleous et al. 2019b). Taking into account this concept, the USA's EPA has recently considered the use of non-linear biphasic dose-response models, which could enhance the quality of both risk estimates and subsequent risk management decisions that guide the derivation of environmental standards and cost-benefit assessments (Agathokleous et al. 2019b). Non-linear responses should thus be taken into account when assessing the O_3 effects on forest ecosystems.

Besides stomatal uptake, many interacting factors, such as time between episodes, temporal variation, phenology, canopy structure, physiological and biochemical processes, environmental conditions, genetics, and soil and nutrient conditions, are involved in plant responses to O_3 (Musselman et al. 2006). Following transient exposure to high levels of O_3 (often exceeding 150 ppb and termed as acute in the literature), perception of stress involves ROS, hormones, Ca^{2+}, and mitogen-activated protein kinase (MAPK) signaling cascades (Ainsworth et al. 2012). The magnitude of the plant response is determined by the actual amount of the pollutant reaching the target site and the ability of the plant to restore homeostatic equilibrium (Musselman et al. 2006). Suppose O_3 is considered as a relatively new "stressor" (due to the anthropogenic increases over the last decades); in that case, adaptive responses induced by O_3 in the context of hormesis suggest that plants use biochemical pathways that evolved to cope with and adapt to other oxidative stresses that also protect against O_3 (Agathokleous et al. 2019a).

Injuries caused by O_3 toxicity include changes in stomatal conductance, a decrease in CO_2 assimilation, light-saturated photosynthesis (A_{sat}), and nonstructural carbohydrates, and an increase in the rates of mitochondrial respiration and senescence. These metabolic changes can alter source-sink relationships, reduce root biomass, and contribute to the overall decrease in growth and biomass (see Ainsworth et al. 2012, for an overview). In consequence, forests and crops can be significantly affected by O_3.

A meta-analysis was performed by Wittig et al. (2009) to estimate the magnitude of the impacts of the current $[O_3]$ and future $[O_3]$ on the biomass, growth, physiology, and biochemistry of trees representative of northern hemisphere forests. The authors showed that current ambient O_3 (40 ppb on average) significantly reduced the total biomass of trees by 7%; moderately elevated O_3 (64 ppb) reduced the total biomass by 11%, and elevated O_3 (97 ppb) reduced the total biomass of trees by 17% compared with controls. Besides, the root-to-shoot ratio was significantly reduced by elevated O_3 as well as the leaf area, Rubisco activity, and chlorophyll content. Trees also had lower transpiration rates and were shorter in height, and had a reduced diameter when grown at elevated O_3. In Brazil, some forest tree species showed visible symptoms and microscopical markers typically induced by O_3 (Moura et al. 2014; Pina and Moraes 2007).

Regarding crops, O_3 can reduce agricultural yields by (1) inducing acute visible injury, which can cause an obvious and immediate loss of economic value in crop species whose market value depends on their visible appearance; or (2) reducing photosynthetic rates and accelerating leaf senescence (Ashmore et al. 2005). Avnery et al. (2011) projected the global relative yield loss due to O_3 exposure by 2030. The loss might range between 4.0 and 26% for wheat, 9.5 and 19% for soybean, and 2.5 and 8.7% for maize, depending on the scenario considered. The authors also estimated that Brazil is expected to lose $500 million each year because of reduced productivity in crops exposed to O_3 (Avnery et al. 2011).

5.2 Ozone biomonitoring using tobacco Bel-W3

Bioindicator plants have been a standard tool for monitoring environmental quality, diagnosis of air pollution effects on vegetation, and risk prognosis in Europe (Klumpp et al. 1996; Nali et al. 2006; Ferreti et al. 2008; Fernández et al. 2015; Agathokleous et al. 2020). In developing countries, the use of indicator species is especially important for obtaining information on air quality because physical-chemical monitoring networks are limited by the high cost of monitors (Assis et al. 2018). This is Brazil's case, where only 12 among the 26 States have some private or public air pollution monitoring stations (Instituto de Energia e Meio Ambiente 2014). According to this report, the few existing air quality stations that were recently created do not monitor all the important pollutants and generally disrupt data.

Biomonitoring using plants is a powerful tool for assessing environmental health, particularly in locations impacted by pollution. It is based on the concept that living organisms always react to external stimuli. A living organism can be used as a bioindicator if it responds to changes in air quality in a specific, observable, and measurable way. Conclusions can be drawn about the pollutant, its source, and intensity through detailed and particular observations and measurements in bioindicator plants (VDI 2004; Klumpp et al. 2001). The standardization of biomonitoring techniques, from the plant's cultivation and exposure until the measurement and evaluation of results, is a fundamental requirement to validate and apply the results obtained in the field (Klumpp et al. 2001).

Basically, biomonitoring using plants has been conducted by two techniques: 1) active biomonitoring, which is performed by introducing sensitive or accumulating indicator plants into the environment to be monitored under standardized conditions, and 2) passive biomonitoring, which consists of using plant species that occur naturally in the ecosystem to evaluate the current state of the vegetation and the effects of air pollution on the vitality of the plants, and to detect resistance mechanisms (Nakazato et al. 2018).

Since the 1990s, both mentioned biomonitoring techniques have been employed in Brazil aiming to indicate the phytotoxicity levels of air pollutants around the air pollution sources of Cubatão (Domingos et al. 1998; Esposito et al. 2016; Esposito and Domingos 2014; Klumpp et al. 1994; Klumpp et al. 1996a, b; Moraes et al. 2000; Nakazato et al. 2016; Pedroso et al. 2016; Silva and Moraes 2013; Silva et al. 2013), the Metropolitan Region of São Paulo (Dafré-Martinelli et al. 2011; Ferreira and Domingos 2012; Sant'Anna et al. 2008; Savóia et al. 2009) and the Metropolitan Region of Campinas (Aguiar-Silva et al. 2016; Bulbovas et al. 2015; Dias et al. 2016; Domingos et al. 2015; Giampaoli et al. 2015; Sandrin et al. 2018).

Regarding O_3, several plant species that develop visible injury have been used as bioindicators. Among them, O_3-sensitive tobacco cv. Bel-W3 has been the most used worldwide, and its response to O_3 is the best described (Calatayud et al. 2007). However, meteorological conditions (temperature, relative humidity, vapor pressure deficit, and wind speed) may limit the relationship between leaf injury variations and ambient O_3 concentrations. In fact, these meteorological variables substantially affect the effective O_3 dose taken by tobacco plants and, consequently, on leaf injury (Ribas et al. 1998; Calatayud et al. 2007). In such a case, this bioindicator plant gives only qualitative information about O_3 pollution.

5.3 Efficiency of tobacco Bel-W3 for ozone biomonitoring in São Paulo State, Southeast Brazil

The efficiency of tobacco Bel-W3 as an O_3 bioindicator in SE Brazil varies depending on the local climate (Fig. 5.1). In sites near the Atlantic coast (Cubatão city), where the climate is tropical without a dry season (Af according to Köeppen´s classification), the cultivar Bel-W3 of tobacco offered sufficient background information about O_3 contaminations and potential phytotoxicity to plant species of the Atlantic Rainforest (Klumpp et al. 1994; Assis et al. 2018). The cultivar was even able to indicate a change in the O_3 phytotoxic profile due to the start of a new natural-gas-powered thermoelectric plant that provides power to an oil refinery at the industrial complex of Cubatão (Assis et al. 2018).

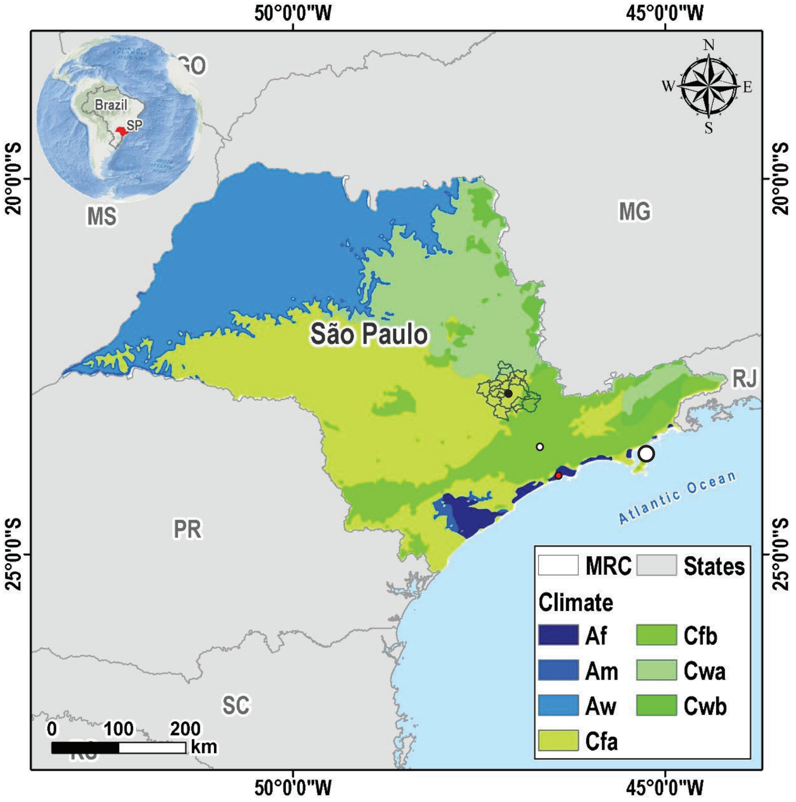


Fig. 5.1. Map of São Paulo State showing the climate types according to Köeppen's classification (source: Wikipedia 2020). Af= Tropical without a dry season; Am = Tropical monsoon; Aw = Tropical with a dry winter; Cfa = Humid subtropical oceanic without a dry season and with a hot summer; Cfb = Humid subtropical oceanic without a dry season and with a temperate summer; Cwa= Humid subtropical with a dry winter and a hot summer; Cwb= Humid subtropical with a dry winter and a temperate summer (Alvares et al. 2014).(🔴)Studies developed in Cubatão city by Klumpp et al. (1994) and Assis et al. (2018);(○) in the Metropolitan Region of São Pauloby Dias et al. (2011), Esposito et al. (2009); and Sant'Anna et al. (2008) and (●) in the Metropolitan Region of Campinas (current study).

Studies developed in the Metropolitan Region of São Paulo, the sixth-largest metropolitan area of the world (Alvim et al. 2017), where the climate is humid subtropical without a dry season and with a temperate summer (Cf according to Köeppen´s classification), have shown that the bioindicator efficiency of tobacco did not diminish under chronic doses of O_3 (Dias et al. 2011); however, it was diminished under higher O_3 doses (Sant'Anna et al. 2008). In the last case, a weak relationship between leaf necroses and O_3 would be one probable practical result, restricting the use of this bioindicator plant for estimating O_3 levels in polluted areas. Esposito et al. (2009) also indicated that leaf antioxidant responses could restrict the use of *N. tabacum* Bel-W3 as an O_3 bioindicator to both the atmospheric contamination and weather conditions of São Paulo.

The results obtained in the above-mentioned studies led us to assume that the bioindicator efficiency of tobacco plants reduces from the coast to São Paulo State's interior because of the weather conditions. They also stimulated us to raise the hypothesis that the protocol of tobacco Bel-W3 is adjustable in order to improve the efficiency of O_3 biomonitoring in subtropical regions with a dry winter and a hot summer. According to Käffer et al. (2019), the feasible adjustments may involve new leaf traits that are affected by weather conditions and are easily measurable in tobacco plants (such as leaf biomass and leaf area) and relevant meteorological factors (such as solar radiation, temperature, relative humidity). The improved biomonitoring protocol would enhance the explicability of the dose-response relationships between O_3 levels and bioindicator responses of tobacco Bel-W3, as shown by Käffer et al. (2019) for some cities of Rio Grande do Sul State (South Brazil).

The adjusted biomonitoring model would permit the estimation of the spatial and seasonal variations in the O_3 concentrations, expanding the use of such bioindicator plants, especially in regions where there is no O_3 monitoring by instruments. This expansion will be treated in detail in the next section. We chose the Metropolitan Region of Campinas (Fig. 5.1) as the model region in São Paulo State for evaluating the veracity of this further hypothesis because it is typically characterized by the Cwa climate according to Köeppen's classification (humid subtropical with a dry winter and a hot summer) and theoretically by phytotoxic O_3 levels.

5.4 Adjusting the ozone biomonitoring protocol for subtropical regions with dry winters and hot summers

5.4.1 Air pollution and climate in the Metropolitan Region of Campinas

Recently, studies conducted in the Metropolitan Region of Campinas (MRC), located in the central-east region of São Paulo State, have shown that both crops (Sandrin et al. 2018) and forests can be affected by the current levels of O_3 (Moura et al. 2014). Moura et al. (2018) found that more than 30% of *A. graveolens* and *P. gonoacantha* trees assessed in the forest fragments of MRC developed characteristic O_3-induced injury in the foliage. Besides, biomarkers of oxidative stress were observed in those species (Aguiar-Silva et al. 2016). Together with other morphological and chemical leaf traits, these biomarkers helped to indicate the potential of these native trees from the Atlantic semi-deciduous forest for air pollution biomonitoring (Domingos et al. 2015).

The MRC is characterized by a diversity of pollution emission sources, including urban settlements, highways, industries, and extensive agriculture dedicated to the cultivation of sugarcane, oranges, and ornamental plants (Bulbovas et al. 2015; Nakazato et al. 2018). It has also been affected by air pollution emissions from the Metropolitan Region of São Paulo due to the predominantly south and southwest winds in the region (Aguiar-Silva et al. 2016; Boian and Andrade 2012). The predominant climate in the MRC is Cwa (humid subtropical with a dry winter and a hot summer), according to Köeppen's classification, and it is characterized by one hot and rainy season between October and March and one dry season between April and September (Alvares et al. 2013).

Although the average prevailing wind direction is southeast in the MRC, the wind direction typically changes during a single day, and the predominant wind directions are SSE and SE in the early morning and evening; SSE, SE, and NE in the morning; and NNE, SSE, N, SSW, and S in the afternoon (Boian and Andrade 2012; Bulbovas et al. 2015).

The concentration of atmospheric pollutants in the MRC is influenced by the predominant weather conditions and pollution emission diversity. The average hourly concentrations of primary pollutants tend to be higher during the dry seasons than during the wet seasons; in contrast, O_3 tends to increase during rainy seasons compared to the average hourly levels during the dry months (Domingos et al. 2015).

5.4.2 The biomonitoring program

Biomonitoring using tobacco Bel-W3 was performed between June 2010 and September 2013 in fourteen sites within the MRC. These sites were categorized according to their major neighboring land uses: I1, I2, and I3 were located near an industrial pole; I/A was placed near the industrial pole and agricultural crops; crops, mainly sugarcane plantation predominantly surrounded A1, A2, A3, A4, A5, A6, and A7; A/U was located near crops and an urban area; and, U1 and U2 were close to urban areas (Fig. 5.2). All procedures adopted for cultivation followed the methodology proposed by the VDI (2003). The plants were exposed in each site for consecutive periods of 14 days, following the recommendations available in the VDI (2003).

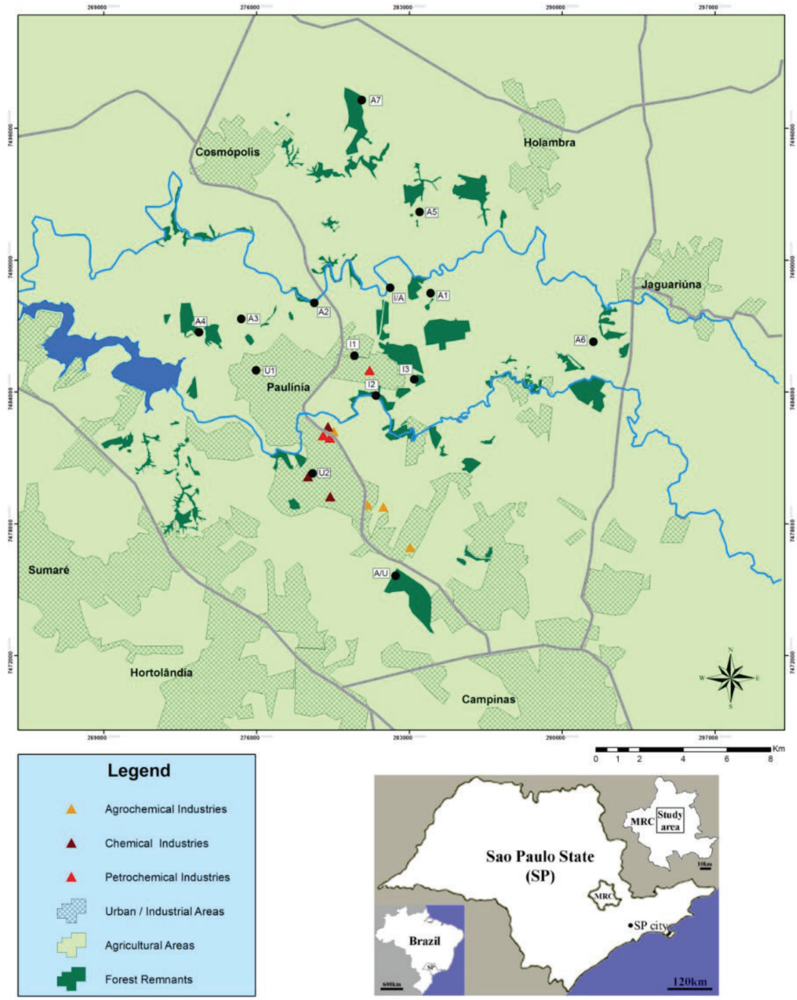


Fig. 5.2. Map of the Metropolitan Region of Campinas with the location of the exposure sites. I1, I2, and I3 – industrial areas; I/A – an industrial/agricultural area; A1, A2, A3, A4, A5, A6, and A7 – agricultural areas; A/U – agricultural/urban area; and U1 and U2 – urban

After each exposure, the plants were evaluated for the presence of O_3-induced foliar injury, aiming at estimating the Leaf Injury Index (LII). LII was estimated for the whole plant based on the percentage of leaf area covered by typical necrosis calculated in intervals of 5% and the number of leaves with and without necroses. The leaves with symptoms were grouped according to the affected leaf area's percentage into five classes: 1–6%, 7–25%, 26–50%, 51–75%, and 76–100%. LII was then estimated according to the following formula (Furlan et al. 2007):

$$\mathrm{LII}\ (\%) = \frac{(\mathrm{N}_1 \times 1)(\mathrm{N}_2\ \times 2)(\mathrm{N}_3 \text{ x } 3)(\mathrm{N}_4 \text{ x } 4)(\mathrm{N}_5 \text{ x } 5) \text{ x } 100}{(\mathrm{N}_0 +\ \mathrm{N}_1 +\ \mathrm{N}_2 +\ \mathrm{N}_3 + \mathrm{N}_4 + \mathrm{N}_5) \text{ x } 5}$$

where N_1, N_2, N_3, N_4, and N_5 are the number of leaves in classes 1, 2, 3, 4, and 5, respectively, and N_0 is the number of leaves without necroses.

We also measured the leaf area and biomass and estimated the leaf mass/area ratio of the 5th, 6th, and 7th oldest leaves of each plant because they were generally the most affected by necroses and contributed the most to the resulting LII in each tobacco plant.

5.4.3 Monitoring of relevant environmental variables

O_3 in the MRC was analyzed based on data from the monitoring station of Paulínia managed by the Environmental Company of São Paulo State (CETESB) that publishes hourly averages online by means of open-access datasets (http://www.cetesb.sp.gov.br/ar/qualidade-do-ar/32-qualar). This station is next toU2, the biomonitoring site (Fig. 5.2). The hourly O_3 concentrations were used to calculate 24h averages, maximum hourly values, averages during sunlight ($O_3$11h), and the accumulated dose over a threshold of 40 ppb (AOT40) or 78.4 μg m^{-3} (1 ppb is 1.96 μg m^{-3} at standard temperature and pressure). All these O_3 descriptors were calculated for each 14-day exposure period of tobacco plants (Fig. 5.3). In the study period, the O_3 average values during 24h and 11h, maximum hourly values, and AOT40 were higher during wet periods (mainly spring seasons; from September to December) than dry periods (Fig. 5.3).

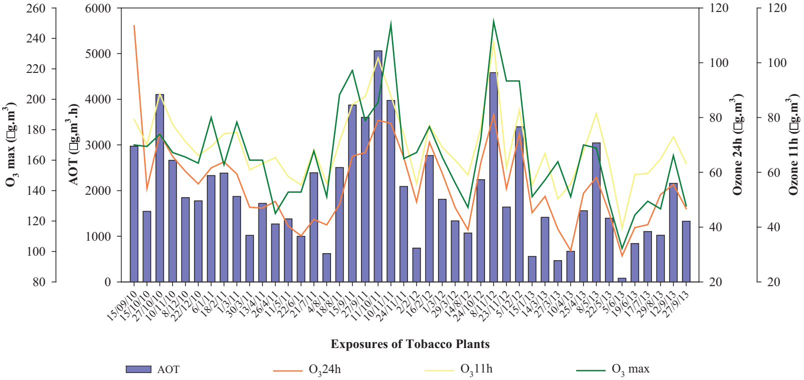


Fig. 5.3. Mean values of hourly O3 concentrations calculated for periods of 24h (O3 24h) and during sunlight (O311h), maximum hourly O3 concentration values (O3 max), and the accumulated dose over a threshold of 78.4 µg.m^3 (AOT) for 14-day exposure periods of tobacco Bel-W3 at the U2 site. Source: CETESB website (http://www.cetesb.sp.gov.br/ar/qualidade-do-ar/32-qualar)

Several meteorological parameters, including air temperature and humidity, rainfall, and solar radiation, were also recorded. We used data from five meteorological stations located in Paulínia, Americana, Campinas, Jaguariúna, and Nova Odessa (Fig. 5.4). Higher values of these parameters were registered during spring and summer seasons (from September to February) coinciding with wet periods, compared to those reported during autumn and winter seasons (from March to August, coinciding with dry periods), evidencing the climatic seasonality in the region of study (Fig. 5.4). The vapor pressure deficit (VPD) was also calculated based on temperature and relative humidity estimates and by means of the automatic calculator of Autogrow System Ltd. (http://www.autogrow.com/downloads/download-software-and-drivers).

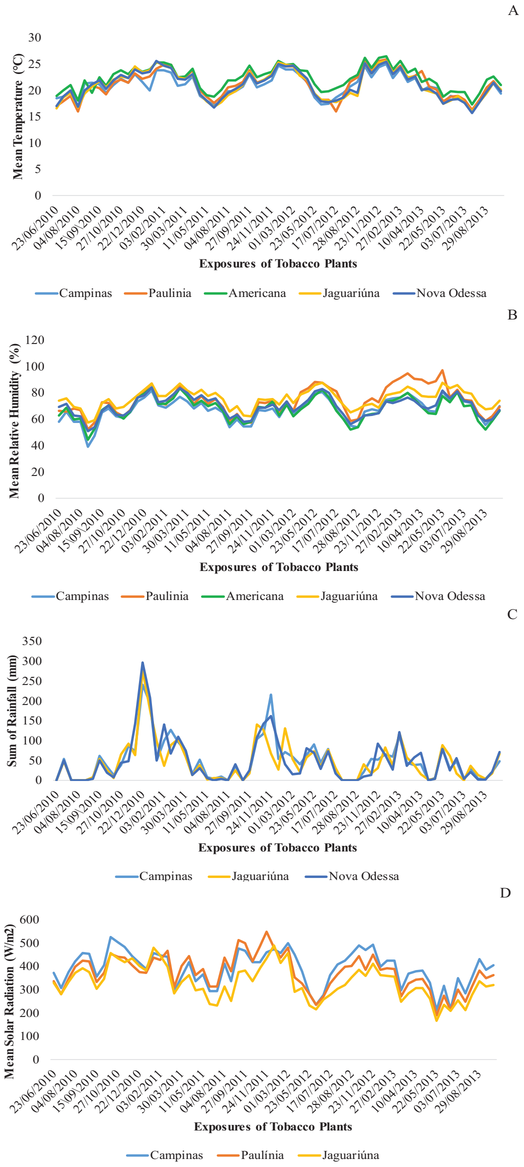
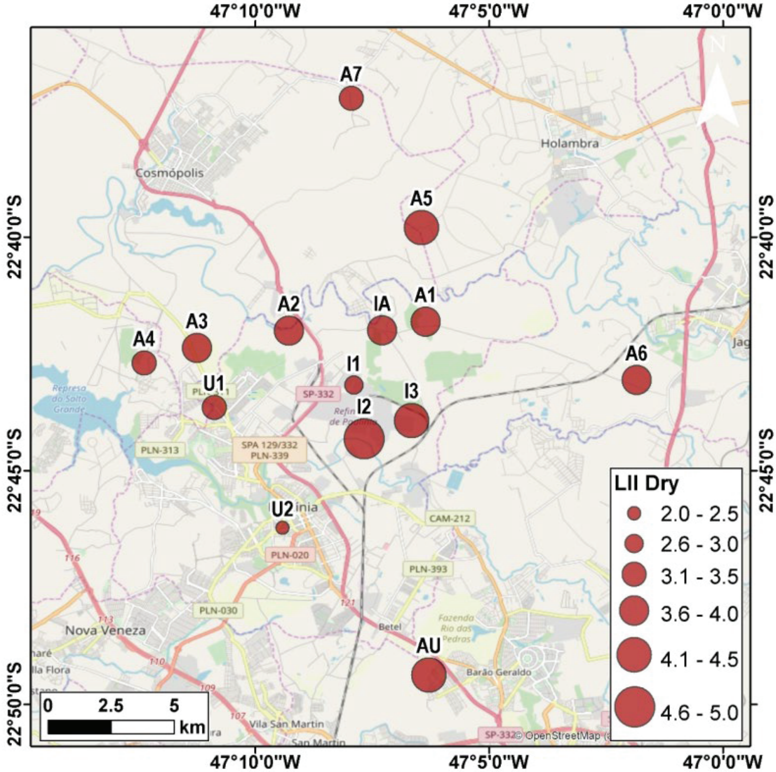


Fig. 5.4. Mean values of temperature and relative humidity and solar radiation and accumulated rainfall for 14-day exposure periods of tobacco Bel-W3 at the U2 site. Sources: CETESB website (http://www.cetesb.sp.gov.br/ar/qualidade-do-ar/32-qualar) and Campinas Agronomy Institute

5.4.4 Adjusting the biomonitoring model

Initially, the leaf injury index (LII) calculated for tobacco plants exposed in all sites was plotted on two maps, one for the average LII during the dry periods and the other for the average LII during the wet periods (Fig. 5.5). The maps were processed and generated using ArcGIS v. 9.2, Geographic Projection, and datum WGS 1984, and presented in the scale 1:150,000. The distribution of results on maps showed little variation between study sites and dry (between 2 and 5%) and rainy periods (between 1.5 and 4.5%) (Fig. 5.5). The absence of seasonal variation in the LII values between dry and rainy periods was unexpected, once O_3 concentration seasonality was evident during the study period at the U2 site, where the monitoring station is located, as shown in Figure 5.3. Therefore, the question that arises is whether or not the small spatial variation indicated by LII corresponds to reality.



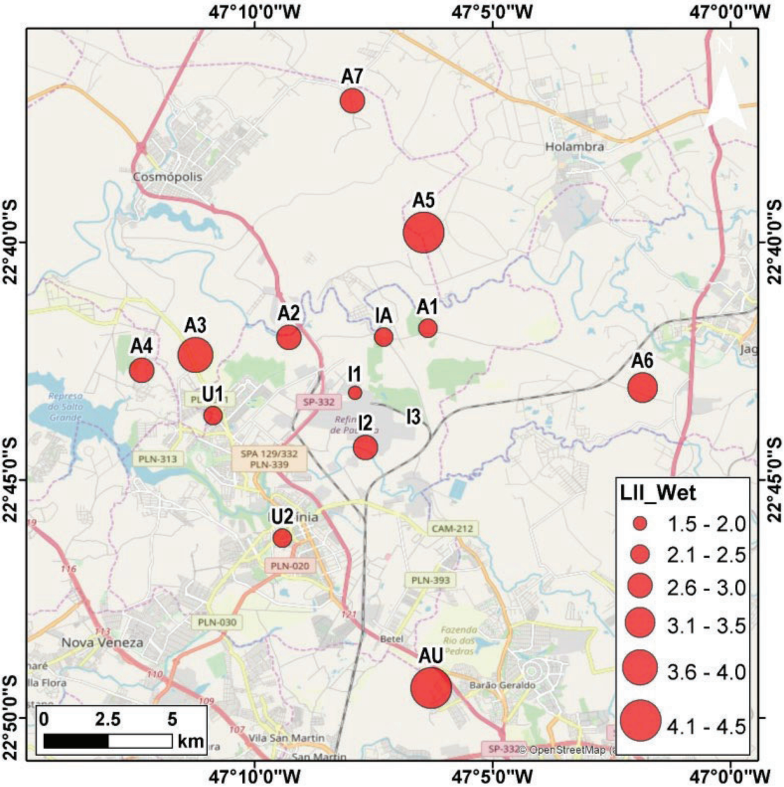


Fig. 5.5. Distribution of Leaf Injury Index (LII; %) values of tobacco Bel-W3 at the biomonitoring sites during dry and wet periods

Besides, the LII in tobacco plants exposed at the U2 site tended to correlate negatively with O_3 concentrations. This linear regression curve indicates that the higher the average O_3 concentrations, the lower was the LII (Fig. 5.6).

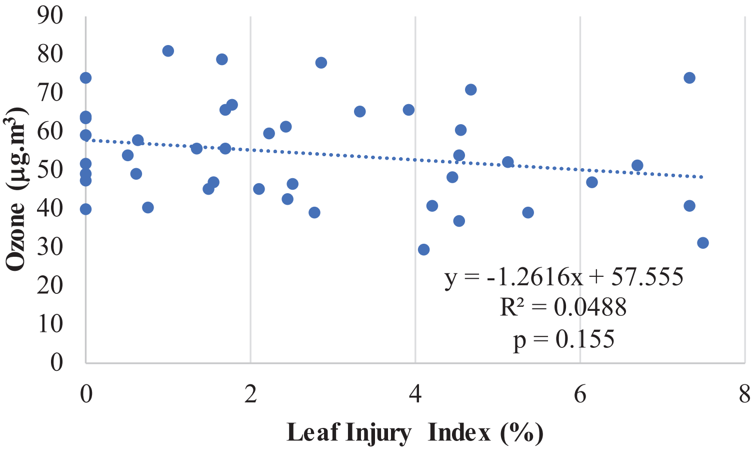


Fig. 5.6. Linear regression between the leaf injury index in tobacco Bel-W3 and O_3 concentrations at the U2 site. Source of O_3 data: CETESB website (http://www.cetesb.sp.gov.br/ar/qualidade-do-ar/32-qualar)

The average LII in tobacco plants obtained during the whole biomonitoring period was then plotted together with average O_3 concentrations and the vapor pressure deficit (VPD) was registered in the same 14-day periods (Fig. 5.7). Several 14-day exposure periods of high O_3 concentrations coincided with high VPD values and low LII values. The possibly unfavorable climatic conditions in these periods, pointed out by high VPD values, caused stomatal closure in the tobacco plants, restricting O_3 uptake (Alves et al. 2011; Calatayud et al. 2007; Laffray et al. 2007; Pina and Moraes 2010) and preventing the manifestation of injury. These results indicate that the climate of the MRC negatively influences the bioindicator response of tobacco as observed by other authors around the world (Alves et al. 2011; Biondi et al. 1999; Calatayud et al. 2007; Esposito et al. 2009; Laffray et al. 2007). The combination of high temperature and radiation, with low relative humidity, leads to high VPD, increasing the hydric stress to plants (Biondi et al. 1999; Laffray et al. 2007).

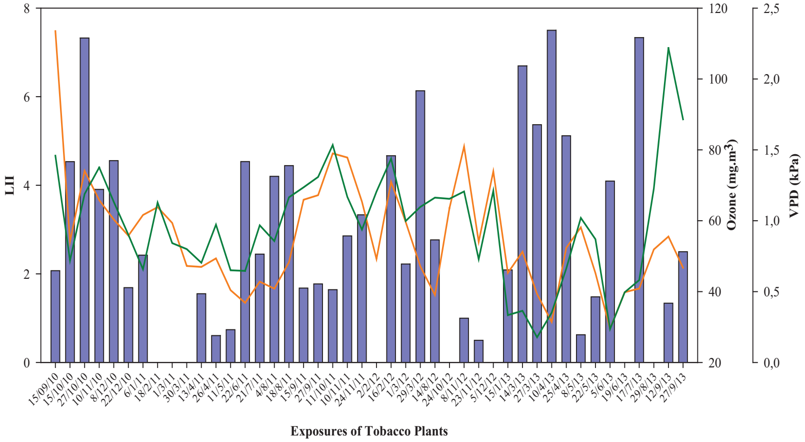


Fig. 5.7. Average values of the Leaf Injury Index (LII; bars) in tobacco Bel-W3 plants and 24h average values during 14-day periods of O_3 concentrations (mg.m^3, orange line) and VPD (kPa, green line) exposed at the U2 site during 14-dayperiods along with the entire study. Source of O_3 and meteorological data: CETESB website (http://www.cetesb.sp.gov.br/ar/qualidade-do-ar/32-qualar)

Therefore, a series of multiple linear regression analyses among the different O_3 descriptors and meteorological variables [VPD, temperature (T), relative humidity (RH), solar radiation (SR), accumulated rainfall (P)] and leaf traits [leaf mass (LM), leaf area (LA), leaf mass/area ratio (LMA) and LII] was performed aiming at proposing the most appropriate biomonitoring model for estimating the O_3 levels using tobacco Bel-W3 under the humid subtropical climate characterized by dry winters and hot summers. These regression analyses were applied to data from 44 exposure periods performed at the U2 site. Ozone descriptors were always taken as dependent variables and meteorological factors and leaf traits as independent variables. The multiple linear analyses were performed using the stepwise backward method. The adjustment procedure started from the saturated models (with all the independent variables included), removing the variable with the smallest effect to explain the O_3 variations. New adjustments were successively performed. At the end, only variables that significantly (*P<0.05*) contributed to the explanation of these variations remained in the resulting linear equations.

The most explicative multilinear model ($R^2 = 0.78$; $p < 0.001$) was the following:

$$O_3 = 6.386 - (0.0727*LA) + (0.32*SR)$$

where, O_3 = O_3 expressed as 24h average values during 14-day periods; LA is the average leaf area of the 5th, 6th and, 7th oldest leaves of each plant; and SR is the solar radiation expressed as 24h average values during 14-day periods.

As shown by this equation, the 24h average values during 14-day periods of O_3 can be predicted by reductions in leaf area of the 5th, 6th, and 7th oldest leaves of each tobacco plant and increases in average values for 14-day periods of solar radiation. The negative correlation between leaf area and O_3 was also observed by Kaffer et al. (2019) and Saitanis and Karandinos (2002), who related the reduction in LA with an increase in necrotic areas caused by O_3. However, the positive correlation between O_3 and SR was not found by Kaffer et al. (2019). The percentage of leaf necroses, usually used in biomonitoring programs (Assis et al. 2018), was shownto be inadequate for O_3 biomonitoring in the subtropical region studied since LII was removed from the multilinear model proposed. In fact, solar radiation seemed to be a key meteorological factor in the region studied, more than VPD, because it regulates both the O_3 formation and leaf expansion of tobacco plants. The reduction in LA of plants exposed to the high intensity of solar radiation may restrict the O_3 uptake and then the LII.

In addition, the model was considered to be valid for the studied region based on the significant positive correlation between estimated O_3 concentrations for 14-day periods applying the equation as mentioned earlier and 24h average values during 14-day periods of O_3 effectively measured in the monitoring station settled at the U2 site (Fig. 5.8).

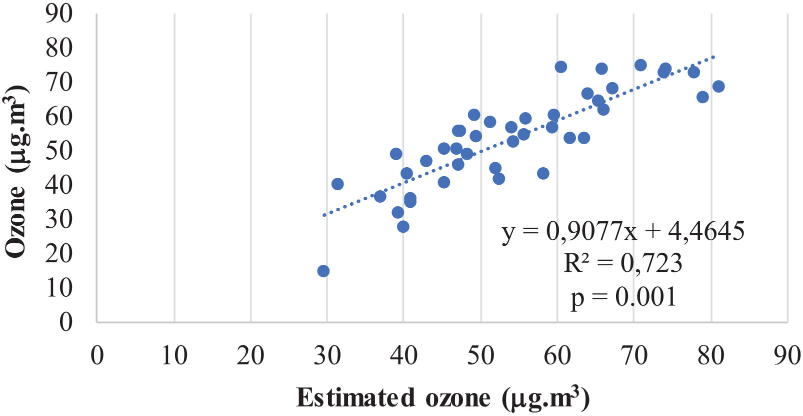
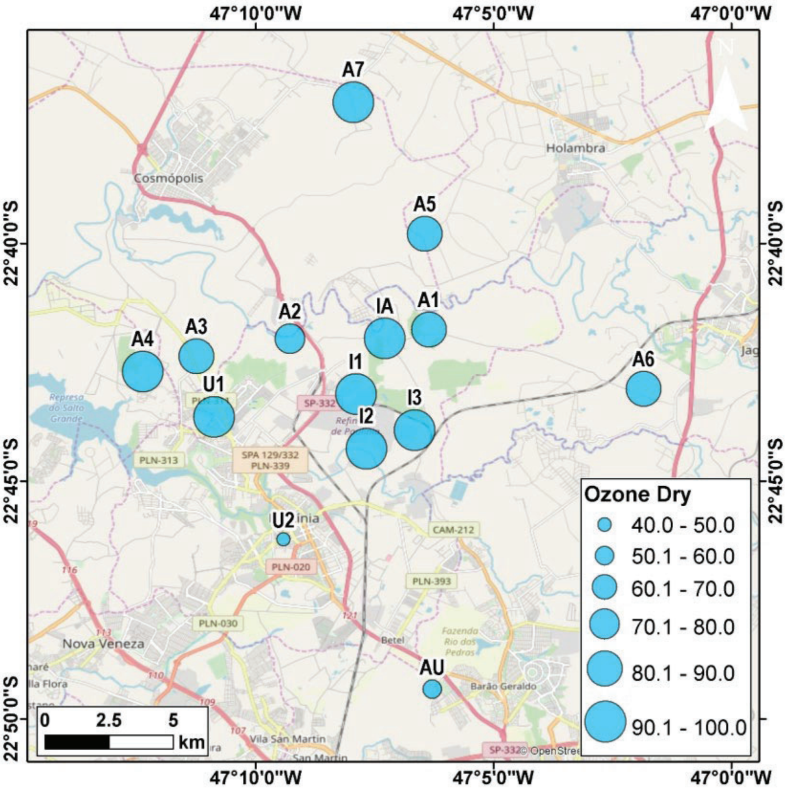


Fig. 5.8. Linear regression between O_3 concentrations measured in the monitoring station settled at the U2 site and O_3 concentrations estimated through the most explicative multilinear model [O_3 = 6.386 – (0.0727*LA) + (0.32*SR)]. Both measured and estimated O_3 concentrations were expressed as 24h average values during 14-day tobacco Bel-W3 exposure. Source of measured O_3 concentrations: CETESB website (http://www.cetesb.sp.gov.br/ar/qualidade-do-ar/32-qualar)

Therefore, this equation was used for estimating average daily concentrations of O_3 during 14-day periods in the biomonitoring sites during dry and wet periods, which were used for constructing two new maps using ArcGIS v. 9.2 (Fig. 5.9).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Cosmópolis
Holambra
Nova Veneza
Barão Geraldo
Betel
Vila San Martin
SP-332
PLN-313
SPA 129/332 PLN-339
PLN-020
PLN-030
CAM-212
PLN-393
Represa do Salto Grande
Fazenda Rio das Pedras
0 2.5 5 km
Ozone Dry
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70.1 - 80.0
80.1 - 90.0
90.1 - 100.0
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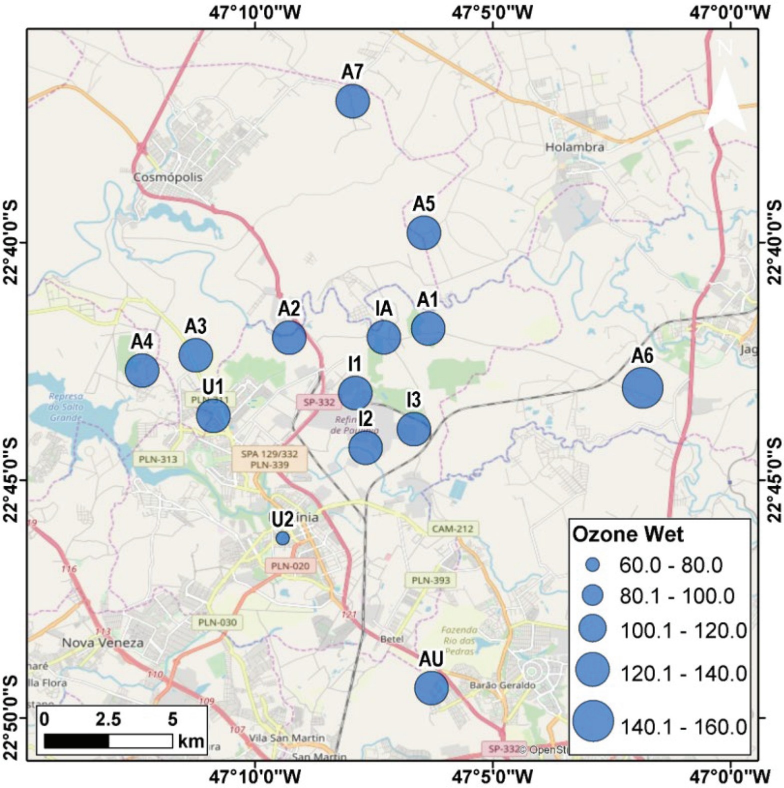

Fig. 5.9. Estimated O_3 concentrations (μg m^{-3}) at the biomonitoring sites during dry and wet periods, expressed as 24h average values during 14-day periods of tobacco Bel-W3 exposure

The adjusted biomonitoring model proved to be more adequate for estimating seasonal and spatial variations in the tropospheric O_3 in the studied region than the standardized biomonitoring protocol defined by VDI (2003). The estimated O_3 concentrations varied between 40 and 100 μg/m^3during dry exposure periods and between 60 and 160 μg/m^3during wet periods (Fig. 5.9), revealing the same seasonal tendency detached by data from the monitoring station located at the U2 site (Fig. 5.3). During the dry periods, the average O_3 concentrations estimated were lower at the U2 (40–50 μg/m^3), AU (50–60 μg/m^3), and A2 (70–80 μg/m^3) sites than at the other sites (80–100 μg/m^3). During the wet period, the average O_3 values estimated were also lower at the U2 site (60–80 μg/m^3), higher at the A6 site (140–160μg/m^3), and intermediate at the other sites (100–140 μg/m^3). Based on these estimates, we may assume that agricultural crops (A sites) and forest remnants are at higher risk imposed by O_3 than human populations in urban areas (U sites).

The results presented show that the biomonitoring approach using the tobacco Bel-W3 in regions where there is pronounced climatic seasonality, such as the MRC, can be improved by measuring leaf area and solar radiation. The new biomonitoring model allowed an estimation of O_3 levels, which was not possible using LII.

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CHAPTER SIX

ALTERNATIVE ROUTES FOR OZONE UPTAKE AND DAMAGE IN THE LEAF STRUCTURE OF TROPICAL PLANTS

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Abstract

Ozone (O_3) dissociation into reactive oxygen species (ROS) occurs in the aqueous medium, precisely in the leaf apoplast. Secretory glands located at the leaf surface produce and can reabsorb aqueous-based exudates (guttation droplets and nectar), which are also solutions placed for O_3 dissociation and ROS uptake. The relevance of morphological traits in conferring tolerance to O_3 effects has been widely demonstrated. However, the role of O_3 over the functioning of secretory glands and the consequences to the bearing plants are still unknown. In the discussion herein, we present evidence that glands producing aqueous-based exudates (hydathodes and extra floral nectaries) are direct routes of the entry of O_3-dissociated ROS into leaf tissues, and that this uptake is related to the rising of microscopic symptoms. To achieve this insight, plants were submitted to acute O_3 fumigation inside closed chambers, and routes of nectar and guttation droplet reabsorption were tracked, applying a fluorescent tracer over the exuded droplets. This methodology was also performed in a FACE system to verify whether the results obtained in chambers are reproducible in a real environment. In both closed chambers and FACE situations, the reabsorption of guttation droplets or nectar occurred through glands prior to the rising of microscopic O_3 symptoms. In addition, ROS were identified *in situ* following the same route of the exudate's uptake. The patterns of uptake described therein did not occur in plants from a filtered air chamber or control environment. Thus, there seems to exist alterations in the chemical composition of the exudates released that facilitate the uptake by glands, possibly due to the high concentration of dissociated ROS after O_3 exposure. This direct uptake of O_3-dissociated ROS causes their accumulation in the apoplast and adjacent cell structures and further increases microscopic alterations in these same sites. It characterizes an alternative route for O_3 uptake that may impact plant productivity due to the wide distribution of secretory glands in angiosperms.

Keywords: *Astronium graveolens* (Anacardiaceae); Extra floral nectaries; FACE; Fumigation chambers; Hydathodes; Guttation droplets; Nectar; *Passiflora edulis* (Passifloraceae); *Piptadenia gonoacantha* (Fabaceae); Sugarcane (Poaceae)

6.1 An overview of ozone leaf uptake

In pure water, ozone (O_3) decomposes faster than it does when in its gaseous phase. As pointed out by Roshchina and Roshchina (2003), "the reactions of O_3 in water and water solutions are of special interest, as the reactions with O_3 in living cells occur in a water environment". In aqueous solutions, O_3 can take two routes (summarized in Fig. 6.1), resulting in its dissociation in reactive oxygen species (ROS) that directly oxidize the compounds dissolved in the medium.

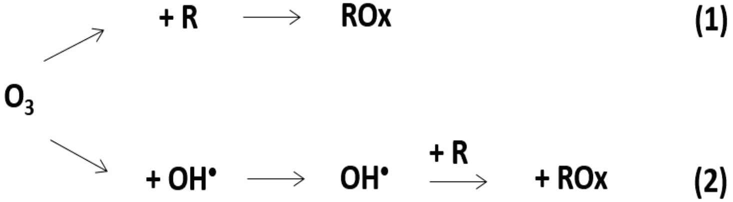

Fig. 6.1. Chemical routes of ozone (O_3) to act over stable radicals (R) in an aqueous solution: (1) direct reaction; and (2) radical-type reaction (ROS formation). The radical formation is represented here by the hydroxyl radical (OH•). The figure is adopted from Eriksson (2005)

Regarding the O_3-plant interface, it is well known that O_3 mostly enters the leaf through stomata and diffuses within the apoplast, rapidly decaying into ROS (Grantz et al. 2013). These initial reactions cause the first oxidative burst at the spongy parenchyma apoplast, precisely in the water film covering the mesophyll cells (Pellinen et al. 1999; Roshchina and Roshchina 2003; Eltayeb et al. 2007). Plants might mobilize antioxidants as protection against excessive or inappropriate ROS production, thus maintaining the oxidative/antioxidative equilibrium (Brandão et al. 2017). After exceeding these constitutive plant defenses, the resulting oxidants interact with target biomolecules causing injuries (Pell et al. 1997; Grantz et al. 2013). ROS are also responsible for the induction of secondary oxidative bursts that occur remotely from the stomatal entrance (Pellinen et al. 1999).

Variations in meteorological parameters, such as vapor pressure deficit and photosynthetically active radiation, directly affect the stomatal opening and the effective O_3 flux into plants via stomata and the severity of induced effects. This recognized environmental influence on stomatal closure has long motivated a series of studies in the northern hemisphere, aiming at proposing an ideal model for estimating the O_3 uptake by plants. The Deposition of O_3 for Stomatal Exchange (DO_3SE) model is a good example of such initiatives (Emberson et al. 2000). The interference of environmental conditions on the O_3 flux via stomata may also be expected in the tropical region. However, the effective O_3 flux into tropical plants has rarely been modeled. Assis et al. (2015) and Cassimiro et al. (2016) are among the few studies that evaluated the applicability of the DO$_3$SE model in tropical trees (*Psidium guajava* L. "Paluma" and *Astronium graveolens* Jacq., respectively).

Considerations about the formation of O_3-derived ROS in aqueous solutions on leaf surfaces, as well as their uptake by leaves, are underestimated. To date, we know that O_3 increases leaf permeability and wettability, although reports about these events are poorly documented (Dunning and Heck 1973; Grammatik Poulos and Manetas 1994; Schereuder et al. 2001). Ozone increases leaf permeability by changing the permeation properties of compounds within membranes (Kerstiens and Lendzian 1989) and wettability through cuticular changes (Dunning and Heck 1973; Schreuder et al. 2001). Theoretically, the altered membranes and cuticles are alternative non-stomatal routes of O_3 uptake and may contribute to the rise and spread of deleterious effects on plants.

The effects of O_3 over some other physiological phenomena related to aqueous solutions produced by plants and usually (or under certain conditions) reabsorbed remain entirely neglected in studies of O_3 uptake. Several plant species constitutively bear external secretory glands that produce/reabsorb aqueous solutions, including hydathodes, nectaries, and hydrophilic (mucilage-secretory) glands. Anatomically, these glands can be simple pores (openings in the epidermis), secretory trichomes (only of protodermal origin), or emergences (originated from protoderm, ground meristem and even procambium).

Knowledge about the involvement of secretory glands in restricting O_3 damage is not exactly new, but it has not been completely addressed. The descriptions are usually limited to their exudate, the chemical compounds that effectively interact with O_3 or its dissociated byproducts. The possible reactions of O_3 with non-dissociated organic compounds are summarized in Fig. 6.2. Gland exudates are involved in all three possible reactions. For example, terpenoids released by glandular trichomes (Jud et al. 2016; Li et al. 2018) or other inner glands (e.g., idioblasts and laticifers – see Cardoso-Gustavson et al. 2014) act as O_3 scavengers in the boundary layer (reaction #1). Flavonoids occurring inside secretory trichomes (Valkama et al. 2003) might oxidize due to their antioxidant properties (Agati et al. 2012) (reaction #2), and high weight polysaccharides produced by collectors (Cardoso-Gustavson et al. 2014) might directly stabilize O_3 molecules (Eriksson 2005) (reaction #3).

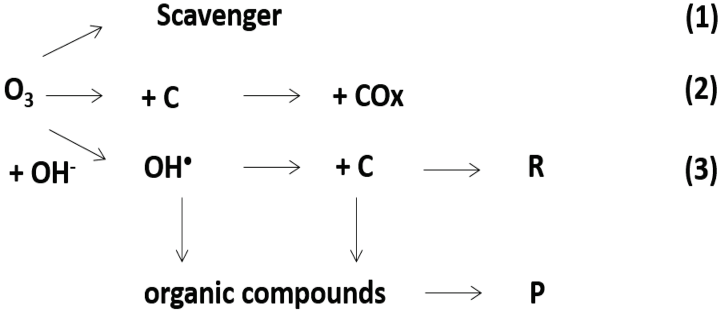

Fig. 6.2. Three possible reactions of O_3 with non-dissociated organic compounds in water, according to Roshchina and Roshchina (2003): (1) scavenging of O_3 by a scavenger; (2) a direct reaction with a dissolved organic non-dissociated compound (C) to form an oxidized compound (COx); and (3) a radical type of reaction, so that O_3 reacts with **OH^-** ions to form the $\dot{O}H$ radical. This radical reacts with molecules of organic compounds (C) to produce reactive radicals (R). This chain can be broken into new stable products (P)

However, the involvement of secretory glands in the uptake of O_3 by products is unknown. This pathway may be a significant alternative route in tropical plant species, as these leaf structures are commonly found in many taxa. We performed a series of case studies aiming to deepen this discussion, as will be described in the next section.

6.2 Secretory glands and ozone

To start this discussion, it seems reasonable to hypothesize that glands releasing and reabsorbing aqueous-based exudates (e.g., guttation droplets, nectar) would be suitable places for O_3 dissociation and direct ROS uptake. If so, the localization of these glands may be directly involved with the appearance and spread of O_3 symptoms and would also be an alternative to explain cases when the stomatal conductance is low, and the antioxidant system is efficient but leaves still show morphological symptoms (i.e., visible injury). Here, we focused on hydathodes and extra floral nectaries (EFN) that release their exudate outside the plant surface. Both structures are widely distributed in angiosperms.

Hydathodes mediate the guttation process enabled by the combined action of root pressure and transpiration. Guttation droplets are aqueous solutions from the xylem sap containing ions, metabolites, and proteins (Fahn 1979). The guttation phenomenon is observed in a wide range of angiosperms and other vascular plants. Guttation can be affected by several abiotic factors that decrease the transpiration rate (darkness, high CO_2, drought). In such conditions, the xylem sap transport mostly relies on root pressure and results in guttation from the leaf margin through the hydathodes (Eller et al. 2013; Cerutti et al. 2017).

Unlike floral nectaries, which are primarily involved in pollination, EFN are commonly implicated in indirect plant defense, as they can reduce herbivory by attracting invertebrate predators (Bentley 1977; Rocha et al. 2019). Nectar is an aqueous solution containing sugars, amino acids, proteins, and secondary metabolites as other minor constituents (Nicolson and Thornburg 2007). Nectar can be released from plants to the environment through stomata, secretory trichomes, or other non-specialized epidermal cells (Fahn 2002; Nepi 2007). Nectar reabsorption occurs in the opposite direction of its release (Nepi and Stpiczynska 2008; Cardoso-Gustavson and Davis 2015).

Several techniques have been applied to track water routes from the environment to the apoplast (see discussions in Eller et al. 2013; Cardoso-Gustavson et al. 2013a). Lucifer Yellow CH (LYCH)is a fluorescent apoplasticatoxictracer to plant tissues (Oparka and Prior 1988) that has been successfully applied in the visualization of mist uptake (Eller et al. 2013; Pina et al. 2016), cell permeability caused by herbicides (Cardoso-Gustavson et al. 2018), and nectar dynamics (release/uptake) due to its chemical property to link to glucose and penetrate the cell protoplast (Cardoso-Gustavson et al. 2013a; Cardoso-Gustavson and Davis 2015).

In the following sections, we describe three case studies that evidenced the direct uptake of ROS through secretory glands. Two of them were conducted in closed chambers with plant species bearing hydathodes (sugarcane) or EFN (*A. graveolens* and *Piptadenia gonoacantha*). LYCH was directly applied to the secretory glands to isolate the O_3 effects and build the concept. Then, we tested the model in the third case study developed in a FACE system using *Passiflora edulis* as a plant model and also LYCH as a tracer to identify the uptake routes of aqueous-based exudates in a natural O_3-polluted location. The rise of microscopic O_3-specific symptoms was evaluated following the detection of ROS by this specific reagent. Note that this alternative route does not replace the current model of O_3 uptake by plants; instead, they are complementary and should be considered in studies regarding plant species bearing these secretory glands.

6.2.1 Case study #1. Water uptake traced by LYCH in hydathodes of sugarcane

6.2.1.1 Plant material

Sugarcane (*Saccharum* spp.) is an important C4 crop used to produce sugar in many tropical and subtropical countries in diverse climates and growing conditions worldwide (Marques et al. 2016). Brazil is notable fore placing fossil fuels with renewable ethanol from sugarcane and being, together with the USA, a leader in the global production of bioethanol (Pereira et al. 2015). Elevated concentrations of O_3 have been registered in Brazilian regions where sugarcane is cultivated. Peak O_3 concentrations up to 147 ppb have been registered, constantly exceeding the one-hour São Paulo state critical limits (80 ppb) and the Accumulated O_3 Exposure Over a Threshold of 40 ppb (AOT40), exceeding the critical levels recommended for agricultural crop protection (CLRTAP 2015) since 2010 (Moura et al. 2014; Moura et al. 2018b). The sensitivity of this species to O_3 was first demonstrated (Moura et al. 2018b) in an experiment carried out in a free-air controlled exposure (FACE) facility. Two sugarcane genotypes (IACSP95-5000 and IACSP94-2094) were exposed for 90 days to three levels of O_3 concentrations: ambient (24-h mean of 39.4 ± 7.9 ppb and AOT40 of 12.6); ambient x1.2 (24-h mean of 48.1 ± 10.1 ppb and AOT40 of 20.0); and ambient x1.4(24-h mean of 54.0 ± 10.1 ppb and AOT40 of 25.7). Therefore, its productivity may be compromised, considering that O_3 reduces photosynthesis, growth and other plant functions (Felzer et al. 2007).

6.2.1.2 Fumigation experiment

Culm fragments of sugarcane (variety RB867515 provided by Embrapa-Araras, São Paulo, Brazil) were planted in 10-L pots filled with commercial substrate (Plantimax-Eucatex^{TM}) and constantly watered by capillarity. The plants were cultivated over four months inside a greenhouse with filtered air until the beginning of the experiments. The plants were then transferred from the greenhouse to the fumigation chambers three days before the fumigation exposure (acclimation period). The chambers used for fumigating sugarcane were composed of a stainless-steel structure covered by a film of Teflon, in the dimensions 85 × 94 × 85 cm (W × D × H) (Souza and Pagliuso 2009). Two O_3 treatments were used [filtered air (FA) and FA+O_3]. The FA treatment was obtained by removing gross and fine particulate matter and gaseous pollutants. The

FA+O_3 treatment was obtained by enriching the filtered air with 100 ppb of O_3. Ozone was generated under electrical discharge by dissociating oxygen in filtered air, using an O_3 generator (Ozontechenic). The O_3 levels were monitored using an Ecotech 9810B photometric monitor, previously calibrated. Plants were fumigated for 4 h for 4 days (sugarcane).

The LYCH application and visualization of secretory glands followed the procedures developed and detailed in Cardoso-Gustavson et al. (2013b) and Cardoso-Gustavson and Davis (2014). Droplets of LYCH solution were applied over standing guttation from the hydathodes. The control of this experiment was performed by applying distilled water to the same structure. LYCH applications were performed prior to the fumigation exposure in closed chambers.

After the treatment, the portions of plants submitted to LYCH were excised and intensely washed with distilled water, immediately fixed in Karnovsky's glutaraldehyde solution, and stored at 4°C. Samples were hand-cut, and slides mounted in glycerin: PBS buffer, pH 7.2 (1:1), and immediately observed under either blue and green filter sets or the DIC filter in a Leica DM 5500B widefield microscope. Images were captured and processed with Leica LAS-AF software.

Fixed fragments of fumigated sugarcane leaves containing hydathodes were also dehydrated and embedded in histories in Technovit 7100 (Heraeus Kulzer) and sectioned at 1.5 µm. Slides were stained with toluidine blue O, pH 6.9 (Feder and O'Brien 1968). Observations were performed in a Nikon Eclipse Ti-E microscope, and images were captured with Nikon NIS Elements software.

6.2.1.3 Main results

Summarizing the pivotal results, an enrolling and reddish coloration of the leaf margin started exactly in the regions containing hydathode pores where guttation phenomena occurred (Fig. 6.3A–D; microscopic details of hydathode pores in Fig. 6.4A–B). The fluorescence emission from LYCH applied over the guttation droplets prior to the fumigation period showed no entrance of the dye to inner tissues in filtered air samples (Fig. 6.4C). On the other hand, LYCH uptake was visualized from hydathode pores towards the inner tissues of plants 12h after O_3 exposure (Fig. 6.4D–E), reaching vascular tissues (Fig. 6.4F–G). ROS were detected with 3,30-diaminobenzidine (DAB) reagent following the LYCH route in the apoplast and protoplast of sclerenchyma cells close to the hydathode pores and bulliform cells, and in the cell wall of bundle sheath cells (Fig. 6.4H–J) 24 h after the end of the fumigation period. Oxidative bursts led to the programmed cell death process observed after 48h of exposure (Fig. 6.4K).

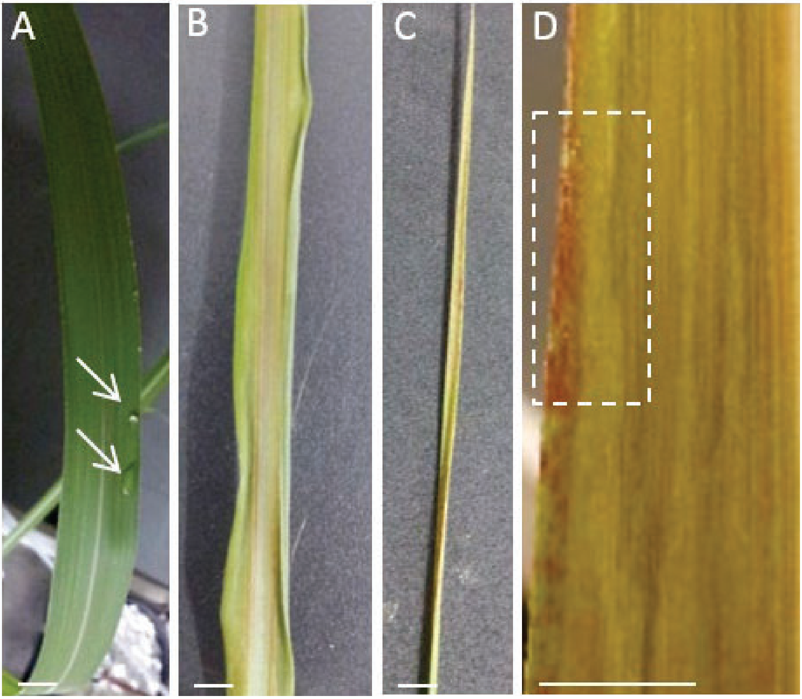

Fig. 6.3. Morphological alterations of the sugarcane leaf blade after O_3 fumigation. (A) Guttation droplets over the adaxial surface in the morning before the beginning of fumigation exposure. (B–C) Enrolling of the leaf margin from the apical (B) and median (C) regions of the leaf blade starting in the morning of the second day of exposure. (D) The manual unrolling of the leaf blade allows the visualization of a reddish O_3-specific injury, more intense at the margin of the leaf blade close to the hydathodes (dashed square). The colored symptom is observed in the morning of the third day of exposure. Note that the enrollment occurs from the margin towards the adaxial surface of the leaf blade. Scale bars: 1 cm

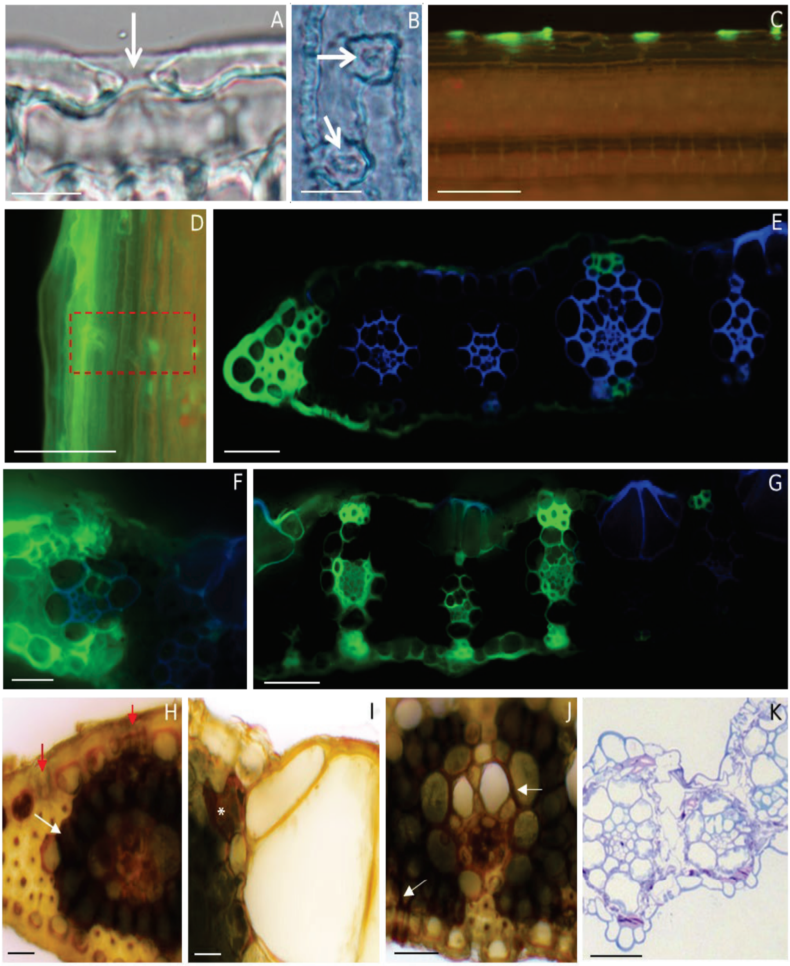

Fig. 6.4 Application of LYCH 1% aqueous solution prior to fumigation exposure and uptake into the inner leaf tissues of sugarcane 12 h later. (A–B) Hydathode pores: transversal (A) and frontal (B) views under the DIC filter. (C–D) LYCH application over guttation droplets in leaves from plants kept inside filtered air (C) and O_3 fumigation (D) chambers. Note the intense uptake highlighted by the red dashed square in (D). (E–G) LYCH uptake through the apoplast of leaf cells, starting from the hydathode pores (E) at the margin towards the central region of the leaf (G) Note the LYCH fluorescence from the apoplast of bundle sheath cells (F–G). (H–J) The region where LYCH was observed was the same as where H_2O_2 was further detected using DAB reagent 24 h after the exposure. Note the positive result below the hydathode pores (red arrows in H, asterisk in I), and the apoplast

of the mesophyll and bundle sheath cells (white arrows in H and J). (K) Leaf section stained with toluidine blue: the programmed cell death process in mesophyll cells 48 h after the exposure from regions following the same pattern of guttation droplet uptake and H_2O_2 accumulation. Scale bars: 75 μm (C–D), 50 μm (E–K), and 30 μm (A–B)

Young sugarcane plants were submitted to acute O_3 fumigation at a fragile phase of their development, i.e., their establishment and growth after vegetative propagation. Both early developed and older leaves (the first ones to appear after vegetative propagation) exhibited similar morphological and anatomical responses to acute fumigation regardless of their age. The direction of the cellular differentiation and maturation of sugarcane leaves was basipetal (i.e., from the tip to the base of the leaf). The process of apical and marginal meristem growth was simultaneous during the early plastochrons (data not shown), as also observed in *Oryza* and *Musa* (Fahn 1979). The direction of cellular differentiation is deeply related to the risein O_3 symptoms at specific cells. Since one of the most harmful consequences of O_3 exposure is accelerated leaf senescence, mainly in chronic exposures (Vollenweider et al. 2003; Vollenweider and Günthardt-Goerg 2006; Gielen et al. 2007), it would be expected that "older cells" (i.e., the ones that firstly differentiated) would be the first to be a target for death. Indeed, this pattern was verified in sugarcane leaves: O_3 affects mostly and firstly the apical and marginal regions, and then the central region of the leaf blade.

The usual O_3 reddish visible foliar injury can be associated with microscopic alterations of oxidation and necrosis mainly of photosynthesizing cells. Necrosis was caused by the collapse of the mesophyll, bulliform, and colorless cells. This fast cell death pattern characterizes the hypersensitive-like response (also referred to as the HR-like response), defined as a specific and common response to acute O_3 doses (Vollenweider et al. 2003; Vollenweider and Günthardt-Goerg 2006). The occurrence of HR-like symptoms was not randomly spread as usually reported (Vollenweider et al. 2003; Vollenweider and Günthardt-Goerg 2006), but followed a specific pattern starting from the margin of the leaf towards the vascular bundles of the center of the leaf, exactly following the water entrance and LYCH pathway through leaf tissues. The HR-like reaction occurs when a large amount of ROS reaches the cell causing a quick death. This is completely in line with the occurrence of this event, starting on cells close to the hydathodes and other tissues where water (thus ROS) was constantly inflowing. These results showed that water uptake coincides with the sites where ROS accumulate, and O_3-specific symptoms appear.

6.2.2 Case study #2. Nectar uptake traced by LYCH in the EFN of *Astronium graveolens* (Anacardiaceae) and *Piptadenia gonoacantha* (Fabaceae)

6.2.2.1 Plant material

A. graveolens and *P. gonoacantha* are Brazilian native species that have been successfully applied as models to understand the effects of O_3 and other air pollutants in tropical forests (for a review, see Domingos et al. 2015; Nakazato et al. 2018).

Their high susceptibility to O_3 doses was verified in previous experiments (Moura et al. 2014), which revealed that both species developed visible symptoms in both scenarios (as illustrated in Figs. 6.5 and 6.6). In chronic O_3 doses, seedlings of both species were exposed to an O_3 regime consisting of a 70 ppb O_3 square wave for 6 h for around 50 days under the controlled conditions of indoor exposure chambers (Moura et al. 2018a; Souza and Pagliuso 2009). As a result of this controlled exposure, the plants developed O_3 visible injuries with specific structural marks.

In *A. graveolens*, the visible injury was defined as small brownish intercostal stipples homogeneously distributed within composed leaves and more pronounced on the adaxial side of leaflets (Fig.5B *vs* A). The striking structural change was the large wart-like cell wall thickenings protruding into the apoplast, mostly occurring in the spongy parenchyma but sometimes also occupying the intercellular space tissues of the palisade parenchyma (Fig. 6.5D *vs* C). They were composed of pectins (Fig. 6.5I) and increasingly oxidized abaxially (Fig. 6.5J). The chloroplasts in the palisade parenchyma showed the degenerative condensation of organelle content, thylakoid membrane injury, and a higher plastoglobule density (Fig. 6.5E, H*vs* F, G).

In *P. gonoacantha*, the visible injury was defined as adaxial stipples, infrequently developed in chlorotic areas (Fig. 6.6B–C *vs* A). Ozonealso leads to the fall of *P. gonoacantha* leaflets (Fig. 6.6B). It is possible to identify discrete groups of disrupted cells showing cytorrhysis features, with cell walls folding, severe membrane injury, and only partially degraded remnants of the cell (Fig. 6.6E–G *vs* D–F).

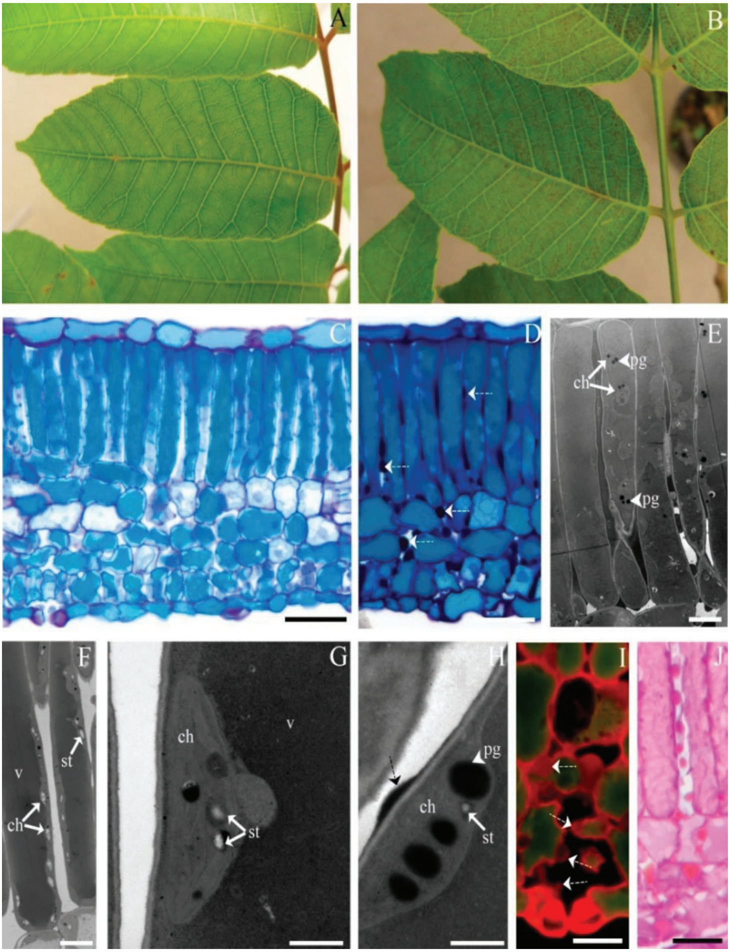

Fig. 6.5 Morphological and microscopic alterations caused by O_3 stress to the leaves of *Astronium graveolens*in response to experimental controlled conditions. (A, C, F–G) Asymptomatic samples from filtered air. (B *vs* A) Visible injury in O_3-exposed seedlings showing brownish stipples on the adaxial leaf surface. (D *vs* C) Massive wart-like protrusions on the cell wall protruding into the intercellular space (dotted arrows). (E, H*vs* F, G) Chloroplasts in the palisade parenchyma showing the condensation of the organelle content and a higher plastoglobule density (arrowheads). (H–I) Pectinaceous wart-like protrusions into the apoplast (dotted arrows). (J) Oxidation of the latter structures increased abaxially.Structures –ch: choroplasts;pg: plastoglobule; st: starch; v: vacuole. Stains – (C–D) Toluidine blue; (I) Coriphosphine, pectins; (J) PAS test, total polysaccharides. Scale bars: (C– D) 25 µm, (E–F) 5 µm, (G–H) 1 µm, and (I–J) 10 µm

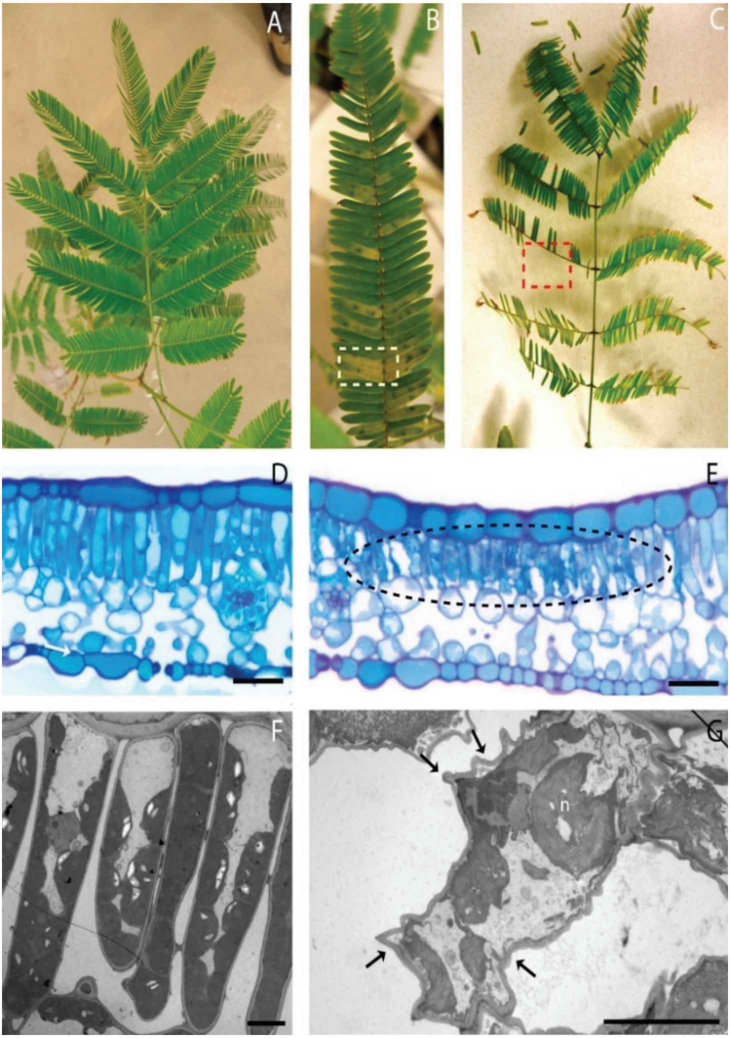

Fig. 6.6 Morphological and microscopic alterations caused by O_3 stress to the leaves of *Piptadenia gonoacantha* in response to experimental controlled conditions. (A, D and F) Asymptomatic samples from filtered air. (B–C *vs* A) Visible injury in O_3-exposed seedlings showing adaxial stippling in chlorotic areas (dashed square, B) and accelerated leaflet shedding (red dashed square, C). (F *vs* D) Discrete groups of disrupted cells (dashed ellipse). (G*vs* F) Palisade parenchyma cells with disrupted chloroplasts and folded cell walls (arrow). Structures–n: nucleus. Stain – (D-E) Toluidine blue. Scale bars: (D–E) 25 µm, and (F–G)5 µm

The high susceptibility to the acute and chronic O_3 doses described by Moura et al. (2014, 2018a) contrasted with the results of Aguiar-Silva et al. (2016), which concluded that *P. gonoacantha* is a tolerant species to oxidative stress, by comparing the activity of antioxidant enzymes (ascorbate peroxidase, glutathione reductase, catalase and superoxide dismutase).

These conflicting results between Aguiar-Silva et al. (2016) and Moura et al. (2018a) were elucidated by the understanding of the role of secretory glands in an O_3 scenario, as described below.

6.2.2.2 Fumigation experiment

We exposed saplings of both species to filtered air (FA) and FA+O_3 treatments within the closed chambers, as described in detail in section 2.1. The saplings were fumigated with 100 ppb during 4 h of a single day.

The LYCH application and visualization of secretory glands also followed the procedures developed and detailed in Cardoso-Gustavson et al. (2013b) and Cardoso-Gustavson and Davis (2014). Both species have EFN (nectary trichomes) over the leaflets. In addition, *P. gonoacantha* has EFN (nectary emergences) at the base of the leaf rachis. Droplets of LYCH solution were applied over nectar droplets from extra floral nectaries and over leaf regions containing glandular trichomes. The control of this experiment was performed by applying distilled water over the same structures. LYCH applications were performed prior to the fumigation exposure.

After the treatment, the portions of plants submitted to LYCH were excised and intensely washed with distilled water, immediately fixed in Karnovsky's glutaraldehyde solution and stored at 4°C. Samples were hand-cut, and slides mounted in glycerin: PBS buffer, pH 7.2 (1:1), and immediately observed under either blue and green filter sets, or the DIC filter in a Leica DM 5500B widefield microscope. Images were captured and processed.

We followed the simplified terminology of Nepi (2007) to describe the general anatomy of nectaries.

6.2.2.3 Main results

A. graveolens showed morphological symptoms 24 h after the fumigation period (Fig. 6.7A–B), with strong chlorosis and the beginning of brownish spots in the intercostal region of leaflets (Fig. 6.7B). The LYCH was applied to the EFN (nectary trichomes) located at the adaxial surface and leaf margin (Fig. 6.7A) prior to fumigation exposure. Plants kept inside the filtered air chambers did not show nectar uptake, at least during the analyzed period. On the other hand, fumigated plants showed a strong LYCH uptake from nectary trichomes towards the midrib (Fig. 6.7C–F).

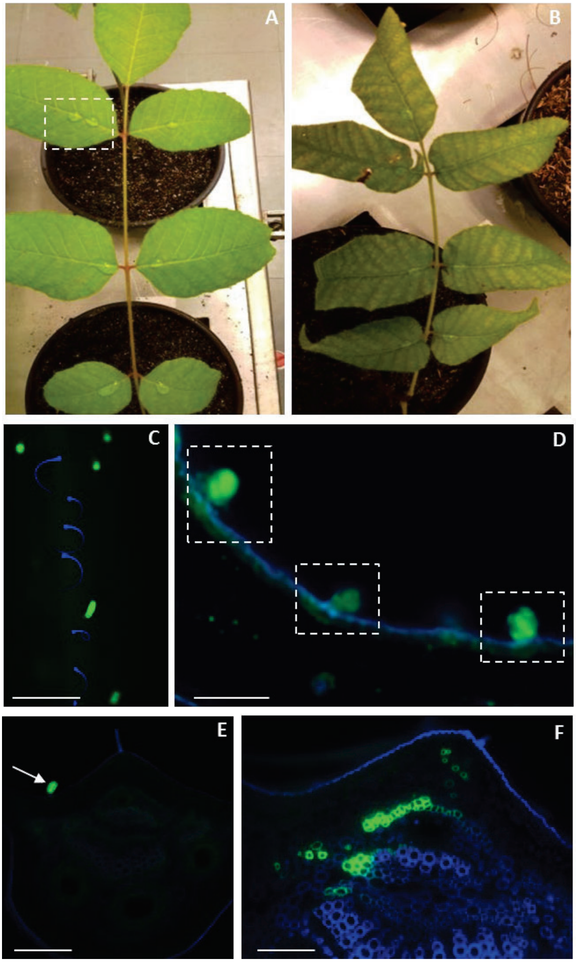

Fig. 6.7. The uptake of Lucifer Yellow CH (LYCH) 1% aqueous solution from extrafloral nectaries (EFN) into the inner leaf tissues of *Astroniumgraveolens*. (A–B) LYCH was applied prior to fumigation exposure at the adaxial surface of leaflets (dashed square in A), and leaves were collected 24 h after exposure (B). (C–D) LYCH uptake by EFN (nectary trichomes) located at the adaxial surface (C) and the leaf margin (D). (E–F) LYCH uptake through nectary trichomes (E) towards vascular tissues (F) of the midrib. Scale bars: 100 µm (C) and 50 µm (D–F)

P. gonoacantha has two morphologies of EFN: a trichome type located at the base of leaflets (Fig. 6.8A, D) and an emergence type at the base of the rachis (Fig. 6.8B–C). Both types had little or no nectar reabsorption, evidenced by the LYCH in plants kept in the filtered air chambers (Fig. 6.8F). In contrast, an intense fluorescence emission from this fluorophore was observed in the EFN of fumigated plants (Fig. 6.8E, G). In addition, the leaflets from fumigated plants showed complete chlorosis, and some of them fell 24 h after fumigation exposure (Fig. 6.8C). These symptoms occurred mainly in leaflets located in ramifications close to the EFN at the rachis.

Although nectar reabsorption is a phase of the nectary dynamics, the report of reabsorption by EFN is still incipient – see a discussion of possible reasons for that in Cardoso-Gustavson and Davis (2015). Here we showed that this event is accelerated in an O_3 scenario. Indeed, the passive uptake of LYCH via the nectary trichomes of *A. graveolens* and *P. gonoacantha* was observed only when these plants were under fumigation. In a filtered air situation, the reabsorption process in nectary trichomes was not observed (at least during the analyzed period). An incipient reabsorption was observed in the nectary emergences at the base of the *P. gonoacantha* rachis even in filtered air conditions. The reabsorption process was indicative that it occurs within a day in the absence of a collector. This event contrasts with the reabsorption processes already described in EFN, only occurring after several days without visitors (Cardoso-Gustavson et al. 2013b; Cardoso-Gustavson and Davis 2015). This phenomenon has ecological implications that are beyond the scope of this study. However, the uptake was even more intense in plants submitted to fumigation. Interestingly, the leaflet fall was observed only in ramifications bearing functional (i.e., at the secretory phase) nectary trichomes and close to the nectary emergence. This fall was possibly caused by the excess uptake of O_3-dissociated ROS in these regions, which causes cell death when in excess (Langebartels et al. 2002; Van Breusegem and Dat 2006).

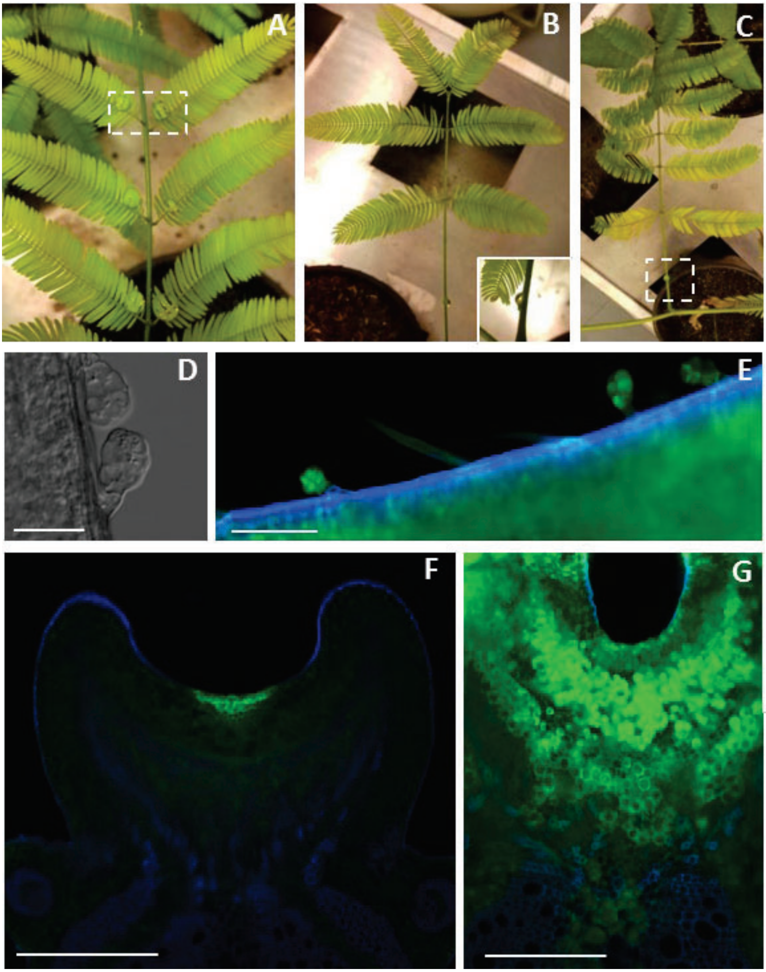

Fig. 6.8 The uptake of LYCH 1% aqueous solution from extra floralnectaries (EFN) into the inner leaf tissues of *Piptadenia gonoacantha*. (A–C) LYCH was applied over EFN (nectary trichomes) at the base of the adaxial surface of leaflets (dashed square in A) and over nectar droplets (inset in B) prior to fumigation exposure. EFN from leaflets and the one at the base of the rachis (nectary emergence; dashed square in C) were collected 24 h after acute fumigation. (D–E) LYCH uptake by EFN from leaflets (details of these glands under the DIC filter in (D) towards mesophyll cells (E). (F–G) An incipient and intense LYCH-nectar reabsorption by EFN at the rachis of plants kept inside filtered air (F) and O_3 fumigation (G) chambers, respectively. Scale bars: 100 µm (F), 75 µm (G), and 50 µm (D–E)

Indeed, the nectary trichomes were plasmolyzed in a fumigation scenario, and the entrance of LYCH occurred passively and similar to the uptake observed in floral nectary trichomes at the post-secretory phase (Cardoso-Gustavson and Davis 2015). Interestingly, the region of water uptake was the same as where the typical O_3 visible symptoms were observed in *A. graveolens* (Fernandes et al. 2016). Conversely, the leaflet fall observed in *P. gonoacantha* was also preceded by the nectar uptake, again following the nectar reabsorption route.

In summary, our results evidenced that the direct uptake of O_3-dissociated ROS may contribute to explaining the patterns of injury previously described in *A. graveolens* and *P. gonoacantha* (Moura et al. 2014; Moura et al. 2018a). In addition, the intense ROS absorption may explain the failure of the antioxidant system to combat the ROS excess in a localized site. However, the antioxidant system is comparatively highly efficient in maintaining the redox balance in the whole plant when compared to other native species (Aguiar-Silva et al. 2016).

6.2.3 Case study #3. Nectar uptake traced by LYCH in *Passiflora edulis* (Passifloraceae)

6.2.3.1 Plant material

Passiflora edulis is a Brazilian native liana with high economic and pharmacological importance (Souza et al. 2018). The apparent high susceptibility of *P. edulis* to chronic O_3 doses was verified by Fernandes et al. (2018) in the experiment conducted in a FACE system for 98 days. During this period, the plants were exposed to 22, 41 and 58 ppb h AOT40. *P. edulis* developed chlorosis in single and trilobate leaves (Fig. 6.9C–D *vs* A–B). The striking structural changes were chloroplast degeneration, reduced chloroplast size or irregular shape (Fig. 6.9F *vs* E), linked to accelerated cell senescence (Günthardt-Goerg and Vollenweider 2007). However, this species was revealed to be O_3-tolerant in the same experiment based on the ability of maintaining a high redox balance in the whole plant, although exhibiting specific O_3 symptoms (Fernandes et al. 2018).

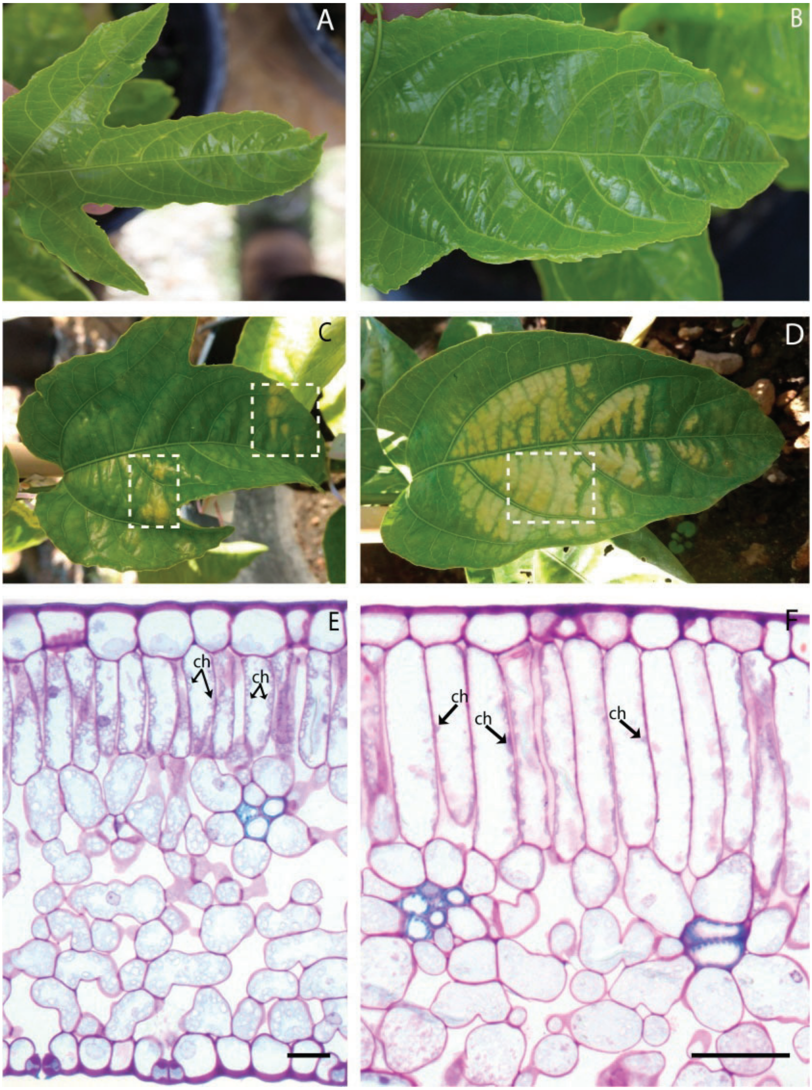

Fig. 6.9 Morphological and microscopic alterations caused by O_3 stress to the leaves of *Passiflora edulis* in response to experimental controlled conditions. (A–B and E) Asymptomatic samples from filtered air. (C, D *vs* A, B) Visible injury in O_3-exposed seedlings showing chlorosis on a three-lobed leaf (C *vs* A) and a simple leaf blade (D *vs* B). (F *vs* E) Reduction in the amount, size and density of chloroplasts along the leaf blade. Structure– ch: chloroplasts. Stain – (E-F) Toluidine blue. Scale bar: 50 µm (E–F)

It is possible that non-stomatal O_3 uptake through extra floral nectaries (EFN) helped to clarify this apparent contradiction (high redox balance vs. morphological injuries) in conducting a FACE experiment, comparing with the results performed with plants in a greenhouse with filtered air, as described below.

This species bears petiolar EFN (emergence type) that produce an intense amount of nectar rich in simple sugars and other secondary metabolites as minor constituents (Cardoso-Gustavson et al. 2013b). These authors described and discussed the anatomy, histochemistry, and evolutionary role of these glands extensively. Structurally, the EFN of *P. edulis* are complex, having several layers of specialized nectary cells (Fig. 6.10A).

6.2.3.2 FACE experiment

The FACE (Free Air Controlled Exposure) experiment was carried out in Italy (43°48’59” N, 11°12’01” E, 55 m a.s.l.). Details of the experimental facility are given in Paoletti et al. (2017, 1407-1414). The results shown here are part of the experiment described in Fernandes et al. (2018). The plants were submitted to three distinct O_3 levels: ambient air (AA; 22 ppb h AOT40); an intermediate O_3 level (AA + O_3 x 1.5; 41 ppb h AOT40), and an elevated O_3 level (AA + O_3 x 2; 58 ppb h AOT40) for 98 days of the summer season. The system consisted of three plots (5× 5× 2 m, L× W× H, respectively) for each O_3 treatment. The O_3 concentration and meteorological conditions were continuously monitored. The data arepresented in Fernandes et al. (2018).

Saplings of *P. edulis* (approximately 20 cm high) for the FACE experiment were obtained from an Italian nursery (43.935351 N, 10.928174 E) (Fernandes et al. 2018). The saplings, kept inside a green house with filtered air in Brazil, were obtained from living plants maintained at Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas (CPQBA, Paulínia, Brazil).

The LYCH application and visualization on secretory glands also followed the procedures developed and detailed in Cardoso-Gustavson et al. (2013b) and Cardoso-Gustavson and Davis (2014). Droplets of LYCH solution were applied over the EFN of *P. edulis*. The control of this experiment was also performed by applying distilled water over the same structures. LYCH was applied daily during 1month to *P. edulis* plants kept inside a greenhouse with filtered air and during the last 20 days of the 98-day exposure period in the FACE system.

After the treatment, the portions of plants submitted to LYCH were excised and intensely washed with distilled water, immediately fixed in Karnovsky's glutaraldehyde solution and stored at 4°C. Samples were hand-cut, and slides mounted in glycerin: PBS buffer, pH 7.2 (1:1), and immediately observed under either blue and green filter sets, or the DIC filter in a Leica DM 5500B widefield microscope. Images were captured and processed with Leica LAS-AF software.

Some details of leaf anatomy were obtained in the fixed fragments of petiolar extrafloral nectaries of *P. edulis* after dehydrating, and embedding in Paraplast Plus (Leica Biosystems) sectioning at a 5 µm thickness. Slides were stained with safranin and Astra blue (Gerlach 1969). Observations were performed using a Nikon Eclipse Ti-E microscope and images were captured with Nikon NIS Elements software.

We followed the simplified terminology of Nepi (2007) to describe the general anatomy of nectaries.

6.2.3.3 Main results

In plants maintained inside the greenhouse, the tracer persisted mainly in the EFN cuticle (Fig. 6.10B), eventually achieving the nectary epidermis (Fig. 6.10C), but it did not reach the vascular tissues. However, *P. edulis* plants exposed to different O_3 levels in the FACE system showed a very interesting pattern of LYCH uptake. Nectar-LYCH uptake was observed from the nectary epidermis towards the inner nectary tissues at ambient O_3 (Fig. 6.10D), and towards the vascular tissues at the intermediate and elevated O_3 levels [Ambient O_3 x 1.5 (Fig. 6.10E), and Ambient O_3 x 2 (Fig. 6.10F), respectively]. It is also important to notice the cuticle detachment and rupture in glands at higher O_3 levels, which was not observed in ambient O_3 or greenhouse situations.

The reabsorption process was observed in the EFN of *P. edulis* only after a long period of the FACE experiment. Remarkably, Cardoso-Gustavson et al. (2013a) did not notice the signals indicative of nectar reabsorption (applying the same methodology described here). Indeed, although the cuticle was detached when nectar was released from the nectary epidermis, these authors observed that cuticle disruption only occurs naturally with the intervention of ants (or nectar robbers). Once the nectar collector is absent, the reabsorption process does not occur due to the presence of a relatively thick cuticle and cuticular flanges (see considerations of this process in Cardoso-Gustavson et al. (2013a)). Under O_3 conditions, however, these glands seem to reach the post-secretory phase faster than expected, i.e., the EFN undergo senescence processes.

Thus, the EFN lose their ability to restrict the nectar entrance, and reabsorption occurs – see considerations of the passive uptake of nectar in *Hibiscus* flowers at the post-secretory phase in Cardoso-Gustavson and Davis (2015).

In summary, the nectar uptake traced by LYCH in *P. edulis* showed the O_3 effects over glands in a real environment simulated in the FACE system, corroborating our previous evidence obtained in closed chamber experiments.

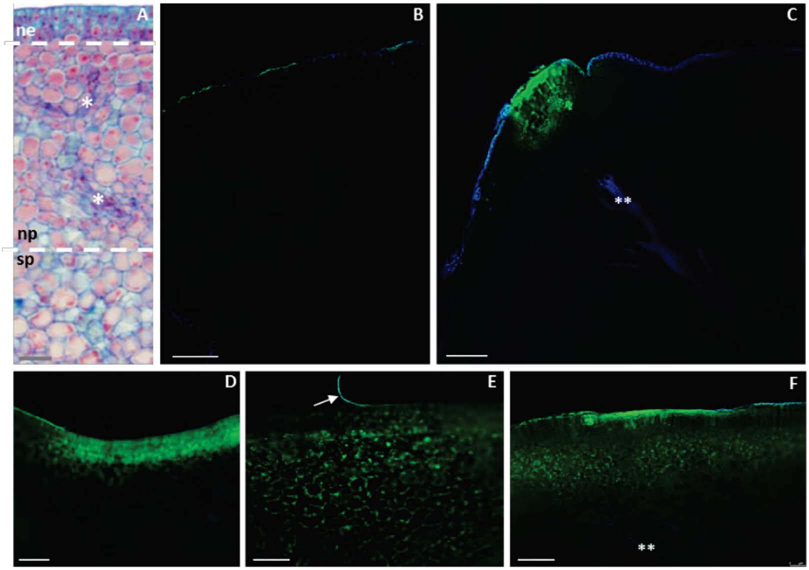

Figure 6.10 Nectar uptake by the extrafloral nectaries (EFN) of *Passiflora edulis* tracked by LYCH 1% aqueous solution. (A–C) The EFN of plants kept inside a greenhouse. (A) The structure of the EFN at the secretory phase (section stained with safranin and Astra blue): ne: nectary epidermis; np: nectary parenchyma; sp:subnectary parenchyma; ph: phloem terminations. Note the phloem terminations in the nectary parenchyma (*). (B–C) The LYCH-nectar uptake after one month. The LYCH remains mostly in the cuticle (B), reaching only the nectary epidermis (C). Note the xylem terminations in the subnectary parenchyma (**). (D–F) A daily application of LYCH in the last 20 days of a 94-day exposure in a FACE system. (D) Ambient O_3. (E) Ambient O_3 x 1.5; note the detached cuticle (arrow). (F) Ambient O_3 x 2. LYCH uptake reaching phloem cells occurs in all EFN regardless of the treatment. Xylem terminations are highlighted (**). Scale bars: 75 µm (B–F) and 30 µm (A)

6.3 The consequences of O_3 uptake by external glands to crop productivity and species ecology

Ozone stress can cause changes in the structure and function of ecosystems (Scebba et al. 2003). Regarding plant productivity, an extensive review of O_3 effects on net primary productivity was presented by Ainsworth et al. (2012), considering only the stomatal entrance of this gas. Experimental studies performed in the Mediterranean region indicated that O_3 induces losses on large (wheat, corn) and row crops (grapevine, watermelon, tomato) of high economic value (Fumagalli et al. 2001). Despite the high economic relevance of *Passiflora* and sugarcane in tropical environments (highlighting Brazil as the world's largest producer of both cultures), the impacts of O_3 over their productivity have only recently been assessed (Moura et al. 2018b; Fernandes et al. 2018). Since the impacts of O_3 on the productivity of gland-bearing species are potentially high, and the distribution of these species is wide in tropical environments, we believe that these glands should be considered in further models of O_3 flux.

In a high O_3 scenario, the ecology of species bearing EFN would be altered, mainly regarding the rupture of trophic interactions with nectar collectors, due to (i) alterations in nectar composition caused by the ROS-derived O_3 dissolution that affects the antioxidants and other chemicals occurring in nectar (Stabler 2016; Holopainen 2020), (ii) changes in the volatile emitted by the EFN that are disrupted by O_3 (Maffei 2010; Pinto et al. 2010), and (iii) alterations in the nectary physiology with ROS accumulation leading EFN to the post-secretory phase (i.e., an acceleration of gland senescence), and the consequent interruption of nectar production.

Considering that most of the tropical angiosperms bear EFN and/or hydathodes (Weber and Keeler 2013), this route should be considered in further studies to evaluate the impact of high levels of O_3 over crop production and forest ecology.

6.4 Conclusion and Perspectives

Overall, our case studies presented evidence of the direct uptake of O_3-dissociated ROS by glands, explaining the patterns of injury observed in sugarcane, *A. graveolens*, *P. gonoacantha* and *P. edulis*. Leaf glands are the pivotal sites of aqueous-solution uptake, and the high concentration of ROS in these structures may explain why leaflets fall so fast when *A. graveolens* and *P. gonoacantha* are submitted to O_3. The experiments with

P. edulis in the FACE system confirmed and supported the results from closed chamber experiments.

In summary, we showed that this O_3 uptake route through secretory glands may be complementary to the well-accepted stomatal pathway and cannot be neglected, as other non-stomatal routes (e.g., via the plant cuticle) can be (Kerstiens and Lendzian 1989). The model proposed here preconizes that water uptake by external glands helps to explain the pattern of injuries caused by O_3; it reacts with aqueous exudates (guttation droplets, nectar), producing ROS outside the leaf. This ROS-enriched water can be taken up through secretory glands (hydathodes, EFN). The intense inflow of ROS-enriched water overpasses the plant defense capacity, thus causing macro/microscopic injuries predictably. This model may explain the effects caused by O_3 when stomata are closed, the spatial patterns of morphological symptoms away from stomata pores, or the occurrence of severe localized morphological/microscopic symptoms in species with a high redox balance.

As future perspectives, the uptake model described here should be further explored, with the aim of elucidating all the processes triggered by O_3 once dissociated in the glands' exudates.

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CHAPTER SEVEN

IMPACTS OF TROPOSPHERIC OZONE ON THE PHYSIOLOGICAL ATTRIBUTES OF PLANTS

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Abstract

Tropospheric ozone (O_3) is the foremost gaseous pollutant in the environment, generating active oxygen species (AOS) upon entry into plants via the stomata. Considering the direct and indirect phytotoxicity of O_3 and its recent increments in the environment, considerable attention is paid to vegetation responses to increasing levels of O_3. The present chapter reviews the current literature related to the responses of plants' physiological attributes under ambient and elevated O_3 concentrations. Responses are recorded based on photosystem II functionality, the stomatal apparatus regulating stomatal conductance, the hormonal balance/signaling, and the water balance in woody species and crop plants under various exposure levels of O_3. Most of the results suggested that O_3 influences physiological processes through multiple concurrent targeting through AOS. Among the cellular components, the chloroplast is the prime target of O_3, which directly harms photosynthesis through Rubisco, the key regulator of energy transfer and carbon fixation. The majority of the studies have found significant adverse impacts of O_3 exposure on most physiological attributes that directly correlate with O_3 exposure (acute or chronic). The responses varied among the plant types as well as between cultivars and clones. This review clarifies the consequences of O_3 stress on physiological attributes. It also illustrates how different physiological processes are linked through interconnected regulatory pathways, which modify plants' responses.

Keywords: Physiology; Ozone; Photosynthesis; Stomatal conductance; AOS

Abbreviations: Ozone, O_3; Charcoal filtered air, FC; Non filtered air, NF; Photosynthetic rate, *Pn*; Stomatal conductance, g_s; Maximum activity of Rubisco, V_{cmax}; Maximum electron transport rate, J_{max}; Maximum quantum efficiency of photosystem II, Fv/Fm; Actual quantum yield of the photosystem, Fv'/Fm'; Coefficient for photochemical quenching, qp; Non-photochemical quenching, NPQ; Quantum yield of electron transfer, ΦPSII; Transpiration, Tr; Water use efficiency, WUE; Photosynthetic nitrogen use efficiency, PNUE

7.1 Introduction

Physiological processes such as photosynthesis, respiration, stomatal movement, transpiration, nutritional and hormonal balance are the most vital and diverse functions of plants. From the ecological perspective, these functions are well-coordinated and control the overall biogeochemistry of the Earth’s system. Any dysfunction of physiological processes has a drastic impact on a species' overall health or a community or ecosystem.

Plants are regularly influenced by their local environments and are mostly under different environmental pressures such as soil, light, water availability, pest attack, drought, herbivory, etc. Under abiotic and biotic stresses, plants' responses throughout evolution have evolved on a diverse scale. Apart from changes in plant growth, morphology, and biochemical diversification, physiological changes are the most interesting and plastic responses known to date.

Among the diverse abiotic stress factors, tropospheric or surface ozone (O_3) has emerged as one of the leading stress factors for land plants (Feng et al. 2019a; Tai et al. 2014). The adverse effects of O_3 on plants are realized based on the growth reductions, changes in community pattern, and yield losses of important crop plants, and the decline in forest productivity (Feng et al. 2019a; Pina et al. 2017; Singh et al. 2018; Schauberger et al. 2019). The negative influence of O_3 on vegetation is certainly realized on all continents of the world, and its current trend is unprecedented (Calatayud et al. 2011; Dumont et al. 2014; Ismail et al. 2014; Moura et al. 2018a; Feng et al. 2019a; Schauberger et al. 2019). As far as the current and future estimations are concerned, it is expected to rise in most bio-diversified countries in the world, such as India and China (Pommier et al. 2018; Tai et al. 2014).

The mechanism of O_3-induced stress responses in plants is well known (Dai et al. 2017; McAdam et al. 2017; Papazian et al. 2016; Singh et al. 2018.). As O_3 enters the apoplast through leaves' stomatal opening, it soon generates diverse, active oxygen species (AOS) in the sub stomatal surface. Afterward, it reacts with different cells' various components and initiates a local cascade of reactions, leading to stress response initiation through signaling by AOS themselves and a sophisticated signaling pathway (McAdam et al. 2017; Vainonen and Kangasjärvi 2015). One of the first targets of O_3 is the cell membrane of guard cells, along with epidermal and palisade cells. As O_3 and AOS directly enter the cytoplasm, the initiation of diverse antioxidative stress responses occurs in plants (Betzelberger et al. 2010; Dai et al. 2017; Grulke and Heath 2020; Papazian et al. 2016). One of the significant outcomes of such responses is the loss of control of stomata. This alters the carbon intake for photosynthesis, as the entry of CO_2 along with O_3 is reduced. The partial stomatal closure reduces O_3 entry until the stomata become fully closed under acute O_3 stress (Dumont et al. 2014).

Evidence suggests that O_3 directly or indirectly influences the stomatal response, photosynthesis by influencing both light and dark reactions, transpiration, hormonal balance, and nutrient uptake (Chen et al. 2009; Drapikowska et al. 2016; Dumont et al. 2014; Gao et al. 2017; McAdam et al. 2017; Moura et al. 2018a; Shang et al. 2019; Xu et al. 2019). Most of the reviews and meta-analyses performed on physiological responses under O_3 stress have been limited to either crop plants or forest trees or restricted to a particular geographical region or continent (Feng et al. 2019b; Morgan et al. 2003; Pleijel et al. 2019; Wittig et al. 2009). A wide range of physiological responses has not been comprehensively reviewed for different crop plants and woody species. Moreover, attempts of previous reviews have focused on responses under direct O_3 stress; however, in an environment, O_3 is not a single stress factor, and different stresses co-exist together, which may induce the synergistic or antagonistic effects (Bernacchi et al. 2006; Calzone et al. 2019; Gao et al. 2017; Papazian et al. 2016).

Considering these limitations, we made an attempt to review the O_3-induced responses on different physiological parameters such as photosynthetic rate (*Pn*), stomatal conductance (g_s), mesophyll conductance, chlorophyll fluorescence, water balance, and phytohormone status for important crop plants, woody species, and natural vegetation from different regions of the world. We also considered other stress factors such as drought, light, and nutrient availability in influencing physiological responses under O_3 stress. The primary objectives of the review are to identify (i) O_3-induced variations in the photosynthetic rate, (ii) stomatal response, (iii) light and dark reactions based on photosynthetic kinetics and chlorophyll fluorescence parameters, and (iv) variations in water balance and phytohormone under O_3 stress among different vegetation types.

7.2 Method

To understand the variations in the physiological responses of vegetation, the World Wide Web was searched for keywords such as photosynthetic rate, photosynthetic assimilation, net CO_2 assimilation, intercellular CO_2 concentrations, stomatal conductance, mesophyll conductance, the quantum efficiency of PSII, the quantum yield of electron transfer at PSII, maximum RuBP regeneration capacity, non-photochemical quenching, rate of triose phosphate utilization, respiration, transpiration rate; maximum rate of Rubisco carboxylation, ethylene, abscisic acid, jasmonic acid and ethylene in relation to ambient and elevated O_3 in different search engines such as Google Scholar, SciVerse, PubMed, Scopus and Web of Knowledge. Papers were selected based on studies that directly assess plants' response to either acute or chronic O_3 exposure or in combination with other stresses such as drought and light, having details of clear experimental design with replications and proper statistical assessment. Manuscripts where O_3 measurement data were mentioned without monitoring, and standard protocols were not followed in physiological measurements were not included in the review.

7.3 Photosynthesis

Twenty-one studies involving 15 crop species and 15 studies involving 24 woody species from different regions of the world were considered to estimate the effect of O_3 on the photosynthetic rate (*Pn*) (Tables7.1 and 7.2).

7.3.1 Crop plants

More than 80% of the studies on crop plants indicated a significant decline in Pn under O_3 exposure either in the growth chamber or under an ambient environment or exposure at an elevated O_3 concentration. The decrease in *Pn* is found to be directly correlated with the O_3 dose and the sensitivity of an individual cultivar (Table 7.1). The analyses showed significant reductions ranging between 2 - 83%, with an average decrease of 34% in *Pn* for different crop cultivars among the crops. The reductions in *Pn* for different wheat cultivars ranged from 8–71%, with an average reduction of 32% (Table 7.1). Compared to wheat, rice showed an average percentage decline of 33.5%, and for soybean a reduction of 30.4%, suggesting the significant effects of O_3 in influencing *Pn* in different crop species. Feng et al. (2018) recorded 20–71% reductions in *Pn* among five winter wheat cultivars. In contrast, Singh et al. (2018) recorded 8–32% reductions among 14 Indian wheat cultivars under ambient +30 ppb elevated O_3 exposure. Betzelberger et al. (2010) found only an average 10% reduction in ten soybean cultivars at 100 ppb O_3 fumigation for 23 days under FACE (Table 7.1).

The impact of O_3 on leaf photosynthesis is mostly realized during the later stages of growth as most of the studies identified a higher decline in the rate of photosynthesis during the post-vegetative stage or during the grain filling stage (Betzelberger et al. 2010; Chen et al. 2011; De Bock et al. 2012; Morgan et al. 2004). The decline in pigment concentration and the reduction in antioxidative enzymes' activities have been correlated with delayed photosynthesis. It may be argued that at the initial growth stages, O_3 stress induces plants to activate a faster metabolism, which results in a higher activity, which may benefit plants to cope with the initial stress. De Bock et al. (2012) showed a significant decline in the *Pn* at the later growth stage in oilseed rape and broccoli leaves (Table 7.1). Bernacchi et al. (2006) found no significant effect on *Pn* in the early leaves of *Glycine max* (L.) Merr. (soybean) under O_3 exposure; however, the combined exposure of O_3 and CO_2 positively influenced the *Pn*. In *G. max*, the loss of photosynthesis was evident during the flowering stage under elevated O_3 exposure, while no significant decline was recorded at the vegetative stage (Morgan et al. 2004). In another study on soybean, Chen et al. (2009) recorded 44 and 47% reductions in photosynthetic CO_2 uptake, respectively, under acute and chronic O_3 exposure, suggesting the sensitivity of soybean to O_3 stress (Table 7.1).

Table 7.1 Summary of studies indicating the O_3-induced percentage eduction in photosynthetic rate (*Pn*) and stomatal conductance to water vapor (g_s) in different crops (ns denotes non-significant variation and sig represents a significant reduction at $p \leq 0.05$)

Crop	**Type or plant material**	**O_3 exposure**	**Reduction in *Pn*(%)**	**Reduction in g_s(%)**	**Reference**
Five winter wheat cultivars	Cultivars	Exposure to ambient and elevated (E-O_3, 1.5 x ambient O_3)	20–71	3–41	Feng et al. (2018)
Two rice (O. *sativa* L.) genotypes, NB, and SL46	Genotype	O_3 fumigation of 100 ppb from 0900 to 1600 h for 23 days	37.3–45.6 24.8–26.7		Chen et al. (2011)
Ten Soybean cultivars	Cultivars	The 8 h growing season average O_3 was 46.3 and 37.9 ppb in ambient plots in 2007 and 2008 and 82.5 and 61.3 ppb in elevated plots in 2007 and 2008, respectively	11	15	Betzelberger et al. (2010)
O_3-sensitive (S156) and O_3-resistant (R123) genotypes of *P. vulgaris*	Genotype	Control and suburban	2–83	sig increase	Drapikowska et al. (2016)

Glycine max		Current and elevated (current O_3x 1.2)	ns early growth stage	-	Morgan et al. (2004)
			20–30		
Wild black mustard	*Brassica nigra*	O_3 exposure for 5 d, 70 ppb after herbivory O_3 exposure for 16 d, 70 ppb	26	51	Papazian et al. (2016)
			71.9	61	
G. max	Cultivar	Acute O_3 exposure of 400 ppb for 6 h	44	-	Chen et al. (2009)
	NE3399	90 ppb O_3 for 8 h d^{-1} for 28 d	47		
G. max		O_3 exposure of 1.23 × current concentration	ns	ns	Bernacchi et al. (2006)
White clover (*T. repens*)	Clones with contrasting sensitivity to ozone (NC-S and NC-R)	7 h^{-1} day, 5 days duration	6.1	ns	Crous et al. (2006)
		average concentration of 0, 40, 80, 110 ppb	65.9	60	
Two commercial sugarcane (*Saccharum* spp.) genotypes	Genotype	1.4 xambient	12.4	26	Moura et al. (2018b)
	IACSP94-2094		24.9	33	
	IACSP95-5000				

Cow pea (*Vigna unguiculata*)	Blackeye	Non-filtered with added O_3 of 50 ppb from 11.00 to 16.00 (NF + O_3) for 88 days	60	62	Tetteh et al. (2015)
	Asontem		55	56	
Snap bean (*Phaseolus vulgaris*)	Genotype	60 ppb O_3	ns	ns	Flowers et al. (2007)
	R123 (tolerant)		15 ns	ns	
	R331 (tolerant)		38	ns	
	S156 (sensitive)				
Lamotte adianae	Forb	Charcoal filtered air (CF) and non-filtered air plus 30 ppb ozone (NF+30).	26	ns	Calatayud et al. (2011)
Winter wheat (*T. aestivum*)	Cultivars	1.5 x ambient	61	68	Cao et al. (2009)
	Yannong 19		27.9	37.5	
	Yangmai 16				
Broccoli lower canopy leaves		O_3 non-filtered ambient air (NF) and non-filtered air with the addition of 20 and 40 ppb O_3	83	79	De Bock et al. (2012)
Oilseed rape			sig	sig	
Wheat	Cultivar	O_3 (NF) and ambient + 20 ppb O3	20.8–22.8	23.1	Yadav et al.

	Old (HUW234) and modern (HD3118)	(NF+)	14.8–19.5	19.2	(2020)
14 Indian wheat cultivars	Cultivar	30 ppb elevated O_3 above ambient level	8.0–32.8	10.3–31.5	Singh et al. (2018)
Radish (*Raphanus sativa*)		Filtered (FCs) and non-filtered (NFCs) treatments under ambient O_3	19	54	Tiwari and Agrawal (2011)
Brinjal (*Solanum melongena)*			32	26	
Mustard (*Brassica campestris*)	Kranti	Filtered (FCs) and non-filtered open top chambers (NFCs) under ambient O_3	20–39	-	Singh et al. (2009)
Wheat	Cultivar	Charcoal filtered air (FCs)	15–36	23–38	Sarkar et al. (2010)
	Sonalika	Non-filtered air (NFCs)	31–51	22–28	
	HUW 510	NF +10 ppb O_3			
		NF + 20 ppb O_3			
Mung bean	Six Indian cultivars of *Vigna radiata*	Ambient + 10 ppb O_3 for 6 h day^{-1}	9–36	1–33	Chaudhary et al. (2013)
Rice (*Oryza sativa*)	Four Bangladeshi cultivars	O_3 exposure at 60 and 100 ppb from 10.00-17.00 from July to November	18–66	22–72	Akhtar e al. (2010)

Cao et al. (2009) found that the sensitivity of wheat cultivars to O_3 is directly related to their photosynthetic ability as under O_3 exposure a sensitive cultivar (Yannong 19) had a higher reduction in *Pn* compared to an O_3-tolerant one (Yangmai 16). The cultivar-specific response to elevated O_3 was further highlighted by Singh et al. (2018), where 14 different cultivars showed a diverse range of reductions in *Pn*. The cultivar HD28 showed the highest decline and was found to be the most sensitive cultivar in terms of yield reduction under elevated O_3 (Table 7.1). Yadav et al. (2020) also found variations in *Pn* between differentially sensitive modern and old wheat cultivars. Sarkar et al. (2010) found lower percentage reductions in Sonalika (15–36% decline) compared to HUW 510 (31–51%) under elevated O_3 exposure (NF + 10 and NF +20 ppb). Moura et al. (2018b) found a differential sensitivity in the *Pn* of two sugarcane genotypes (IACSP94-2094 and IACSP95-5000) under free O_3 exposure (1.4 x ambient). An ozone-sensitive clone showed a greater negative influence of O_3 on *Pn* than the resistant clone of white clover (*Trifolium repens* L.cv. Regal) (Crous et al. 2006).

Flowers et al. (2007) studied differentially sensitive genotypes (S156, R123, and R331) of the snap bean (*Phaseolus vulgaris* L.) to O_3 and found that photosynthesis is largely governed by protecting Rubisco with the antioxidative defense system. The differential sensitivity is thus a result of the active protection or maintenance of the key enzymes of photosynthesis.

An ozone-induced reduction in *Pn* is largely governed by either stomatal or non-stomatal factors. In the first case, the entry of O_3 through stomatal opening causes AOS generation in the apoplast. To counterbalance, stomata either entirely or partly close to reduce the access of O_3; as a result, stomatal conductance is reduced. The lowering of stomatal conductance causes a reduction in the internal concentration of CO_2, and as a result, photosynthesis is inhibited or down-regulated (Akhtar e al. 2010). It is very evident that most of the plants employ this type of mechanism to reduce O_3 stress, and as a result, photosynthesis is down-regulated.

In contrast, many studies have also identified neither reductions in stomatal conductance nor internal CO_2 under O_3 exposure to down-regulate *Pn* (Cao et al. 2009; Papazian et al. 2016; Tetteh et al. 2015; Drapikowska et al. 2016). This decline is mostly attributed to non-stomatal factors such as direct inhibition of dark reaction enzymes or direct damage of photosynthetic machinery by O_3 (Flowers et al. 2007). In an African variety of cowpea (*Vigna unguiculata* L.), Black eye reduction in the *Pn* was attributed to thylakoid membrane dysfunction as a result of long-term exposure of O_3 (NF + 50 ppb) (Tetteh et al. 2015). Papazian et al. (2016) found a reduction in chlorophyll content (47.8%) in *Brassica nigra* (wild black mustard) under long-term O_3 exposure compared to the control as the main reason for the decline in *Pn*. Tetteh et al. (2015) found both stomatal and non-stomatal factors to beresponsible for reducing *Pn* in *V. unguiculata*.

It is generally observed that the negative effect of O_3 on photosynthesis is neutralized under elevated CO_2 exposure (Bernacchi et al. 2006). Drapikowska et al. (2016) found a negative association between net *Pn* with a percentage of necrotic area in O_3-sensitive (S156) and O_3-resistant (R123) genotypes of *P. vulgaris* L. (bean). Calatayud et al. (2011) found a decline in *Pn* in perennial forb *Lamotte adianae* under O_3 exposure. They attributed it to a significant reduction in chlorophyll content (10%), and an increase of 33% in daytime respiration.

7.3.2 Woody species

More than 75% of the studies on woody species showed a significant decline in *Pn* under O_3 exposure. The significant reductions in *Pn* ranged from 5–80%, with an average decrease of 29% for different tree species (Table 7.2). Based on the overall results, trees seem to maintain their photosynthetic activity better than crop plants under O_3 stress to some extent. Further, the seedlings have a higher sensitivity to O_3 compared to the mature trees (Paoletti et al. 2009).

Zhang et al. (2014) reported that non-stomatal factors such as CO_2 fixation and reduction in photosynthetic pigments are the significant causes of a decline in *Pn* in one-year-old *Metasequoia glyptostroboides* seedlings under NF + 60 ppb elevated O_3 exposure for two years using OTCs. Hoshika et al. (2013) found no significant effect of O_3 on photosynthesis in the early leaves of white birch, while the late leaves were negatively affected. The lower sensitivity of the first leaves was attributed to the avoidance mechanism by lowering stomatal conductance. Watanabe et al. (2018a) reported that reducing the biochemical assimilation capacity at the chloroplasts was the primary factor for the decrease in *Pn* under O_3 exposure in Siebold's beech (*Fagus crenata*) saplings. The study also identified the variable response of *Pn* between upper and lower canopy leaves to O_3 due to the differential uptake of O_3 at different canopy levels.

Pinus canariensis exposed under elevated O_3 (50 ppb for 88 days) in OTCs showed no significant difference in *Pn* and antioxidant levels, suggesting that the tolerance of *P. canariensis* to O_3 must be further evaluated to understand the mechanism (Then et al. 2009). Watanabe et al. (2013) recorded a higher reduction in *Pn*in Siebold’s beech (46%) compared to oak (*Quercus mongolica* var. crispula) (15%) under an elevated O_3 exposure of 60 ppb (Table 7.2). Such a higher percentage decline in both species was due to a significant increment in dark respiration as antioxidative activity might have elevated due to the elevated O_3. A reduction in *Pn* in an O_3-sensitive hybrid poplar clone (“546”) was attributed to the impairment of different photosynthesis processes such as Rubisco carboxylation and assimilation under O_3 exposure (Gao et al. 2017). Moura et al. (2018a) also found an increase in respiration with a reduction in assimilation under 70 ppb O_3 exposure for *Astronium graveolens*, while in *Croton floribundus,* an increase in respiration did not affect the *Pn*.

Eichelmann et al. (2004) recorded variations in average *Pn* between silver birch (*Betula pendula* Roth) clones under elevated O_3 exposure with a reduction in Rubisco content, which might be the main factor for the decline in photosynthesis. Oksanen (2003), however, recorded a significant decrease in net photosynthesis in both (*Fagus crenata*) sensitive and tolerant clones of *Betula pendula* (European white birch). Elevated O_3 did not decrease net photosynthesis undera 1-d exposure of acute O_3 (75,112.5 and 150 ppb) to two-hybrid aspen (*Populus tremula* L. x *P. tremuloides* Michx.) clones; however, a significant reduction was recorded under chronic O_3 exposure (70 ppb for 16 d) (Vahala et al. 2003). Calzone et al. (2019) observed a significant reduction in the CO_2 assimilation rate of *Punica granatum* L. under O_3 exposure due to a decrease in stomatal conductance. Xu et al. (2019) observed mesophyll conductance as a significant factor for reducing Poplar's photosynthesis under O_3 exposure.

Schaub et al. (2003) recorded a variable response in the *Pn* of *Prunus serotina*, *Fraxinus americana*, and *Acer rubrum* under non-filtered chambers having 98% ambient O_3, under filtered chambers with 50% ambient O_3, and in open plots with 100% ambient O_3. The results demonstrated the over- or under estimation of physiological responses due to the effects of artificial chambers and microclimatic conditions. Paoletti et al. (2009) also found a higher sensitivity to O_3 in the chamber than in the field trees of Manna ash (*Fraxinus ornus*) exposed to ambient O_3.

Table 7.2 Summary of studies indicating the O_3-induced percentage eduction in photosynthetic rate (*Pn*) and stomatal conductance to water vapor (g_s) in different trees (ns denotes non-significant variation and sig represents a significant reduction at $p \leq 0.05$)

Tree	Type of plant material	O_3 exposure	Reduction in *Pn*(%)	Reduction in g_s(%)	Reference
Ailanthus altissima	One-year-old seedlings	NF from 09.00 to 18.00 and NF supplied with 40 ppb	27	39–40	Gao et al. (2016)
Fraxinus chinensis			21	29	
Platanus orientalis			17	18	
Robinia pseudoacacia			31	25–33	
Psidium guajava	Cloned saplings	Six exposures, Autumn 2007 (Apr–Jun 2007); Spring 2007 (Oct–Dec 2007); Summer 2008 (Jan–Mar 2008); Autumn 2008 (Apr–Jun 2008); Winter 2008 (Jul–Sep 2008); and Spring 2008 (Oct–Dec 2008).	4.48–58	39–51	Pina et al. (2017)
Fraxinus ornus	2-year-old	O_3 exposure regime was a square wave of 150 ppb, from	59.8	32	Paoletti et al.

	seedlings 4 m height tree	10.00 to 18.00, for 7 weeks.	32.7	33	(2009)
Ailanthus altissima	One-year-old seedlings	NF, averaged O_3 concentration of 42 ppb from 09.00 to 18.00), NF supplied with 40 ppb of O_3 (NF + 40, averaged O_3 concentration of 69 ppb from 09.00 to 18.00)	17	ns	Hoshika et al. (2014)
F. chinensis			28	ns	
P.orientalis			1	ns	
		NF supplied with 80 ppb of O_3 (NF + 80, averaged O_3 concentration of 100 ppb from 09.00 to 18.00)	29	ns	
			43	ns	
			35	ns	
Croton floribundus	One-year-old seedlings	70 ppb O_3 square wave for 6 h for 36 days	<10 ns	increase up to 103	Moura et al. (2018a)
Astronium graveolens			20–80	20–75	
Punica granatum	One-year-old saplings	AOT40 = 21.51 ppm h AO	22	35	Calzone et al. (2019)

		AOT40 = 58.74 ppm h EO			
Two hybrid aspens	Clone	Single 8-hour O_3 pulses of 75, 112.5, or 150 ppb	ns to acute O_3 exposure	ns	Vahala et al. (2003)
Clone 51 and 200"				43	
		continuous 19-day chronic O_3 exposure (75ppb)	sig reduction in both clones with higher in clone 51		
Two silver birch (*Betula pendula* Roth) clones K1659	1-year-old-clonal saplings	Ambient concentration exceeded 20 ppb (target 2 x ambient)	sig	sig	Eichelmann et al. (2004)
(denoted as clone 80) and V5952 (denoted as clone 4)					
Prunus serotina	1-year-old-seedling	Ambient air, 100% O_3; NF, 98% ambient O_3; CF, 50% ambient O_3	2–10	ns	Schaub et al. (2003)
F. americana			increase	31	
Acer rubrum			increase	ns	
O_3-sensitive hybrid poplar clone (“546”)	Rooted cuttings	Under three O_3 levels (CF, NF, and NF + 40 ppb)	sig	-	Gao et al. (2017)
Siebold’s beech (*Fagus crenata)*	Ten-year-old saplings	O_3 exposure of 60 ppb during daytime for 98 days	46	ns	Watanabe et al. (2013)
			15	ns	

Oak (*Quercus mongolica*)					
Pinus canariensis	15-month-old plants	1 x O_3 AOT40 = 14	12 increase but ns	ns	Then et al. (2009)
		2 x O_3 AOT40 = 53			
White birch (*Betula platyphylla*)	3-year-old seedlings	2011 Ambient = 25.7 ppb	12 ns	35–46	Hoshika et al. (2013)
	Early leaf	Elevated = 56.7 ppb	32	16–25	
	Late leaf	2012 Ambient = 27.5 ppb			
		Elevated = 61.5 ppb			
Metasequoia glyptostroboides	One-year-old seedlings	NF and NF + 60 ppb O_3	14–31 (First year) 18–26 (Second year)	22–28	Zhang et al. (2014)
Castanopsis sieboldii	Two-year-old seedlings	CF and 1.0 time	ns, 13.7	ns	Watanabe et al. (2018b)
Quercus glauca		and 1.5 xambient O_3 concentrations	ns	ns	
Q. myrsinaefolia			ns	ns	

The overall response of *Pn* to O_3 exposure in woody species showed a significant decline in most cases; however, the magnitude of the negative effect depended upon the duration and concentration of O_3 exposure, the relative sensitivity of species, the O_3uptake capacity, and the growth stage of trees (Table 7.2). Most studies were based on seedling-stage undergrowth chambers or under semi-natural conditions. In contrast, few studies are performed on mature trees, limiting the actual quantification of O_3-induced carbon fixation in the forest and urban trees.

7.4 Stomatal conductance

As a primary response, plants reduce stomatal conductance (g_s) under elevated O_3 to avoid gas entry and, subsequently, oxidative stress. To estimate the effect of O_3 on g_s, we evaluated 17 studies involving 16 crop species from different regions of the world, while for trees, 16 studies involving 20 tree species were considered (Tables 7.1 and 7.2).

7.4.1 Crop plants

More than 83% of the studies on crop plants indicated a significant decline in g_s under O_3 exposure either in the growth chamber or under the ambient environment. A decrease in g_s is correlated with the O_3 dose and the sensitivity of the individual genotype or cultivar. Our analysis showed a significant reduction in g_s for different crop cultivars ranging from 3–79%, with an average decrease of 33% among other crops. The decline for different wheat cultivars ranged from 3–68%, with an average reduction of 33%, similar to that observed for *Pn*. For soybean, the average decrease in g_s values was around 15% (Table 7.1).

In oilseed rape (*Brassica napus*), a decline in g_s was only recorded after maximum leaf area attainment. In contrast, g_s remained unchanged in broccoli (*B. oleracea*) under O_3 exposure (De Bock et al. 2012). Cao et al. (2009) found higher reductions in g_s in an O_3-sensitive cultivar (Yannong 19) of winter wheat compared to a tolerant cultivar (Yangmai 16). The authors also found a non-significant change in *Ci*, suggesting that non-stomatal factors reduced photosynthesis. Tetteh et al. (2015) also recorded a significant decline in g_s under 50 ppb O_3 exposure in two African varieties of cowpea (*Vigna unguiculata*) without any change in *Ci*. In two commercial sugarcane genotypes, elevated O_3 exposure directly reduced g_s from the start of the exposure over the entire experimental period (Moura et al. 2018b). Crous et al. (2006) recorded a significant reduction in g_s due to O_3 exposure in the O_3-sensitive clone of white clover (*T. repens* L. cv.

Regal) while the resistant clone showed no significant difference. The difference in g_s showed a direct correlation with O_3 sensitivity in clover. Progressive reductions in g_s and hydraulic conductance were also recorded in cotton plants exposed to elevated O_3 (Grantz et al. 1999). Betzelberger et al. (2010) recorded an average 15% reduction in g_s among ten cultivars of soybean grown under FACE. In five winter wheat cultivars, Feng et al. (2018) recorded a decrease in g_s ranging from 3–41% under O_3 exposure of 3 nmol O_3 m^{-2} s^{-1} phytotoxic O_3 doses (POD3) (Table 7.1).

Snap bean (*Phaseolus vulgaris*) genotypes with differential sensitivity to O_3 showed no difference in g_s under 60 ppb exposure; however, mesophyll conductance was significantly reduced by 55% in the sensitive genotype (Flowers et al. 2007). In soybean (*G. max*), elevated O_3 exposure did not considerably affect g_s; even at midday g_s remained unaffected (Bernacchi et al. 2006). Calatayud et al. (2011) recorded no significant effect of O_3 exposure in g_s in *L.adianae* (Table 7.1).

In wild black mustard (*B. nigra*), short-term (5d) and long-term (16d) O_3 exposure reduced g_s significantly; however, long-term exposure caused a 10% higher reduction in comparison to short-term exposure (Papazian et al. 2016). Drapikowska et al. (2016) found a positive correlation between g_s with the percentage of necrotic areas in O_3-sensitive (S156) and -resistant (R123) genotypes of bean (*P. vulgaris*).

Monga et al. (2015) recorded a significant reduction in the midday and afternoon g_s of durum wheat (*Triticum durum*) under O_3 exposure suggesting that prior O_3 exposure in the early hours of the day might have caused stomatal impairment. Among the five durum wheat cultivars, Sculpture (36%) and Colombo (24%) cultivars had the highest mean values of g_s as well as the maximum reductions under O_3 exposure (Monga et al. 2015). The authors correlated the reduction in g_s of Colombo to damage to the guard cells of the stroma. In contrast, a decrease in Calvin cycle activity, which caused an increase in internal CO_2 as a feedback mechanism, was found to be the cause of g_s change in Sculpture.

In a study by Brosché et al. (2010), the O_3 sensitivity of 93 natural *Arabidopsis thaliana* accessions along with five O_3-sensitive mutants was analyzed under acute O_3 exposure (300–350 ppb, 7h). Authors found g_s to be the most important trait to assess the O_3 sensitivity of the accessions with the most sensitive accession showing the highest g_s value. Both abaxial and adaxial g_s showed a significant correlation with O_3-induced visible injury. Brosché et al. (2010) also recorded higher whole-rosette g_s, leading to a higher ion leakage under O_3 stress.

7.4.2 Woody species

More than 60% of the studies on trees showed a significant decline in g_s under O_3 exposure. Our analysis showed a significant reduction in g_s for different tree species ranging from 16–75%, with an average decrease of 34% among studied trees. Based on the overall results, trees seem to maintain their g_s compared to crop plants under elevated O_3 exposure (Table 7.2).

The lowering of g_s under elevated O_3 exposure is a well-known early response mechanism of plants to prevent the entry of O_3. Hoshika et al. (2013) recorded a lower g_s in the young leaves of white birch seedlings under O_3 exposure, which prevented photosynthesis damage. One-year-old seedlings of *Metasequoia glyptostroboides* showed a significant decline in g_s under elevated O_3 (E-O_3, NF + 60 ppb) exposure in the first year, though during the second year of exposure it remained unchanged (Zhang et al. 2014). Pina et al. (2017) found a significant reduction in the g_s of *Psidium guajava*, ranging between 39 and 51% in different seasons in the urban forest of São Paulo, SE Brazil. Gao et al. (2016) also found a significant effect of O_3 exposure on g_s and stomatal limitation in four common urban greening species in China (Table 7.2).

Dumont et al. (2014) found the differential response of adaxial and abaxial surface g_s to O_3 exposure in three Euramerican poplar genotypes; however, the total g_s was significantly reduced, suggesting a different effect of O_3 on the stomata of upper and lower surfaces. Dumont et al. (2014) reported changes in the stomatal response and g_s through the O_3-induced modification of ion fluxes in the membrane and the induction of signal transduction. Moura et al. (2018a) recorded a reduction in the g_s value with an increase in dark respiration in *A. graveolens* under O_3 stress. The authors attributed a decrease in g_s to increased intercellular CO_2 as carbon assimilation was reduced due to O_3. In *A. altissima* and *F. chinensis*, Hoshika et al. (2014) recorded a decrease in g_s with increasing stomatal O_3 flux and the ratio of stomatal O_3 flux to net photosynthesis, suggesting an impairment of stomatal control under high stomatal O_3 flux. Stomatal response and g_s in trees observed during these studies indicated that O_3-induced responses are highly dependent upon O_3 flux and the biochemical detoxification capacity. Dynamic stomatal response and g_s are both affected by O_3 exposure and the aging of the leaves (Paoletti et al. 2009). Street et al. (2011) found a difference in response to g_s in *Populus trichocarpa* and *P. deltoides* under acute and chronic exposure of O_3. Variations in g_s were also marked between young and semi-mature leaves.

Then et al. (2009), on the other hand, observed no significant effect of O_3 on the g_s of *P. canariensis* seedlings under free-air O_3 fumigation at

Kranzberg Forest, Germany. Non-significant variation in g_s was attributed to prevailing O_3 levels, which were not sufficient to induce any stress response. Watanabe et al. (2013) also found no significant variations in g_s or stomatal limitation under O_3 exposure in both Siebold's beech and Oak. Similarly, Schaub et al. (2003) also recorded minimal variations in g_s under O_3 exposure in *Prunus serotina, Fraxinus americana* and *Acer rubrum*. Oksanen (2003) found an interesting trend of g_s in O_3-sensitive and -tolerant clones of European white birch *(Betula pendula*) where g_s was first decreased significantly in the early season while a significant and consistent increase in g_s followed after that for the rest of the season. Stomatal conductance of hybrid aspen clones 51 and 200 also showed non-significant variations under chronic O_3 exposure (Vahala et al. 2003). In *Punica granatum*, g_s did not show any significant variation under O_3 and salt stress (Calzone et al. 2019) (Table 7.2).

7.5 Maximum activity of Rubisco (V_{cmax}) and the maximum electron transport rate (J_{max})

The maximum rate of Rubisco carboxylase activity (V_{cmax}) and the maximum rate of photosynthetic electron transport (J_{max}) are often used for understanding the accurate prediction of carbon flux rates. It is well known that under O_3 exposure, the synthesis of Rubisco declines and as a result, demand for both NADPH and ATP is reduced, which may alter or reduce the activity of electron transport.

Elevated O_3 exposure (60 ppb) caused a significant decline in V_{cmax} and J_{max} in the *M. glyptostroboides* tree (Table 7.3). The effect increased as the leaves aged; however, the ratio of $V_{\mathrm{cmax}}/J_{max}$ remained unchanged in different growing seasons (Zhang et al. 2014). Xu et al. (2019), on the other hand, found a significant decline of 39% in V_{cmax} while J_{max} remained unchanged under NF + 20 ppb O_3 exposure in the hybrid poplar clone. The study also identified a significant increase in the $V_{\mathrm{cmax}}/J_{max}$ ratio under O_3 exposure. Watanabe et al. (2018a), in Siebold's beech and Japanese oak, found no effect of leaf nitrogen content on the response of V_{cmax} and J_{max} to elevated O_3 as both reduced under O_3 exposure while leaf nitrogen remained unchanged. However, the study identified a distinct relationship between leaf nitrogen per unit area with V_{cmax} (Table 7.3).

Table 7.3 Summary of studies indicating the O_3-induced percentage eduction in the maximum activity of Rubisco (V_{cmax}) and the maximum electron transport rate (J_{max}) in different trees (ns denotes non-significant variation)

Tree	**Type of plant material**	**Ozone exposure**	V_{cmax}	***Jmax***	**Reference**
Ailanthus altissima	One-year-old seedlings	NF and NF + 40 ppb	42	-	Gao et al. (2016)
Fraxinus chinensis			-	27	
Platanus orientalis			32	36	
Robinia pseudoacacia			37	-	
F.ornus	2-year-old seedlings	150 ppb	57	50	Paoletti et al. (2009)
	4 m height tree		47	33	
O_3 sensitive hybrid poplar clone ("546")	Young Leaf	CF, NF and NF + 40 ppb	14	13	Gao et al. (2017)
	Old Leaf		33	18	
O_3-sensitive hybrid poplar clone "546"	Rooted cuttings	CF, NF + 40 ppb	39	ns	Xu et al. (2019)
Metasequoia glyptostroboides	One-year-old seedlings	NF and NF + 60 ppb	27–37	24–40	Zhang et al. (2014)

Castanopsis sieboldii	Two-year-old seedlings	CF, ambient x 1.0 and ambient x1.5	17	ns	Watanabe et al. (2018b)
Quercus glauca			ns	ns	
Q. myrsinaefolia			ns	ns	

Gao et al. (2017) recorded significant variations in V_{cmax} and J_{max} in the young and old leaves of the O_3-sensitive hybrid poplar clone (“546”) under O_3 exposure with old leaves showing a higher reduction in V_{cmax}; however, the reduction of J_{max} was similar in both types of leaves. Eichelmann et al. (2004) recorded differences in the J_{max} value under O_3 exposure in two silver birch (*Betula pendula*) clones with clone 4 identified as O_3-sensitive and a simultaneous decrease in Rubisco activity and Rubisco content.

As a response to O_3 exposure, injured leaflets of *Fraxinus ornus* showed a decline in V_{cmax} and J_{max} while the $J_{\text{max}}/V_{\text{cmax}}$ ratio increased (Paoletti et al. 2009). The increased ratio indicates that the supply of energy as ATP and NADPH was comparatively less affected than the carboxylation efficiency (Paoletti et al. 2009). One-year-old seedlings of four common urban greening species exposed to elevated O_3 showed a decline in V_{cmax} and J_{max}, identified as the significant reason for reducing photosynthetic CO_2 assimilation (Gao et al. 2016). The authors also found variations in V_{cmax} and J_{max} among trees, with *Ailanthus altissima* having a maximum reduction in V_{cmax} (42%), while *Platanus orientalis* showed the highest decline in J_{max} (36%) (Gao et al. 2016) (Table 7.3).

In the snap bean (*Phaseolus vulgaris* L.), Flowers et al. (2007) found a significant reduction of V_{cmax} by 43% in a sensitive genotype (S156) while no significant change was recorded for tolerant genotypes (R123 and R331) under 60 ppb O_3 exposure. O_3-sensitive and -tolerant clones of white clover (*T. repens* L. cv. Regal) under O_3 exposure showed a differential response for both V_{cmax} and J_{max}. An O_3-sensitive (NC-S) clone showed 28 and 26% reductions in V_{cmax} and J_{max}, respectively. At the same time, no significant change was detected in the tolerant clone (NC-R) (Crous et al. 2006) (Table 7.4). The authors also observed a significant negative correlation between cumulative O_3 fluxes with V_{cmax} in NC-S.

Table 7.4 Summary of studies indicating the O_3-induced percentage reduction in the maximum activity of Rubisco (V_{cmax}) and the maximum electron transport rate (J_{max}) in different crops

Crop	**Type or plant material**	**Ozone exposure**	V_{cmax}	***Jmax***	**Reference**
Snap bean (*Phaseolus vulgaris*)	R123 (tolerant)	60 ppb O_3	ns	-	Flowers et al. (2007)
	R331 (tolerant)		ns		
	S156 (sensitive)		43		
White clover (*T. repens*)	O_3 sensitive (NC-S)	0, 40, 80, 110 ppb	increase by 11	increase by 17	Crous et al. (2006)
	O_3 tolerant (NC-R),		28.6	26	
Glycine max	Vegetative leaf	Current and current O_3 x 1.2	Ns	ns	Morgan et al. (2004)
	Flowering leaf		sig	sig	
Two rice (*O. sativa*) genotypes	NB, a modern variety of the japonica subspecies	100 ppb	49	39	Chen et al. (2011)
			24	16	
	and SL46				
Lamottea dianae	Forb	25	26	26	Calatayud et al. (2011)

Wheat	Old cultivar	NF and NF + 20 ppb	21	11.6	Yadav et al. (2020)
	Modern cultivar		13	6.3	

In *G. max* L., Morgan et al. (2004) did not observe any significant effect of elevated O_3 exposure on both V_{cmax} and J_{max} for a recent fully expanded topmost leaf, however, leaves formed during the flowering stage showed a significant reduction of 20–30% in both the parameters suggesting that the O_3 sensitivity of photosynthetic physiology is related to leaf age as well as to the total period of O_3 exposure. The study also found a significant reduction in the V_{cmax}/J_{max} ratio, suggesting that O_3had more influence on Rubisco activity than on RuBP regeneration capacity. Chen et al. (2011) also recorded a significant difference in two genotypes of rice (*O. sativa*) with reductions of V_{cmax} being 24 and 49% respectively in clone SL46 and NB, while reductions of J_{max} were respectively 16 and 39% under elevated O_3exposure of 100 ppb for 23 days (Morgan et al. 2004) (Table 7.4). In winter wheat cultivars, V_{cmax} and J_{max} showed a progressive decline of 14–68% and 12–66% with time under O_3 exposure reflecting that the negative impact of O_3 increases with the plant age (Feng et al. 2018). Yadav et al. (2020) found a higher reduction of both V_{cmax} and J_{max}, respectively, by 21 and 11.6% in an old cultivar of wheat compared to a modern cultivar (13 and 6.3%) under elevated O_3 exposure. In the perennial forb *L. dianae*, Calatayud et al. (2011) found a significant reduction of 26% in both V_{cmax} and J_{max} under NF + 30 ppb O_3 exposure. V_{cmax} and J_{max} showed a strong linear correlation, suggesting a similar effect of O_3 on both the parameters and a strong interdependence of Rubisco carboxylation efficiency with a maximum RuBP regeneration capacity.

7.6 Chlorophyll fluorescence

Light energy absorption by plants is crucial for energy assimilation and heat dissipation in the plant. Chlorophyll fluorescence kinetics represent light absorption, transfer, dispersal, channeling, transmission, and dissipation of light energy by leaf chlorophyll, ultimately helping us understand plant photosynthetic performance (Table 7.5). Most studies have identified a reduction in Fv/Fm (a measure of the efficiency with which excitation is captured by the open reaction centers of Photosystem II) under abiotic stresses such as O_3 (Table 7.5). This reduction results from damage to Photosystem II reaction centers or is due to an increase in non-radiative energy dissipation (De Bock et al. 2012).

In oilseed rape (*B. napus*), NF +20 ppb O_3 did not influence Fv/Fm, Fv'/Fm' (energy harvesting by open PSII reaction centers in the light), and performance index (PI). In contrast, NF + 40 ppb O_3 caused a significant decline in all the parameters after mature leaf area formation (De Bock et al. 2012). In broccoli, the lower canopy leaves showed significant reductions inFv/Fm, Fv'/Fm' and PI under elevated O_3treatment (De Bock et al. 2012) (Table 7.5). The O_3 exposure for 75 d caused a significant decline in Fv/Fo and Fv/Fm by 29.8 and 6% in the winter wheat cultivar Yannong 19, while for cultivar Yangmai 16, reductions were only 8.5 and 1.5%, respectively, compared to control (Ji-Ling et al. 2009). The decline in values indicates O_3-induced damage to PSII and simultaneous photoinhibition (Cao et al. 2009). Under O_3 exposure, the Fo value of Yangmai 16 was higher than that of Yannong 19, indicating the lesser damage of PSII (Table 7.5).

In cultivar Yannong 19, photochemical quenching (qp) was also found to be reduced under O_3 stress. In contrast, in Yangmai 16, qp showed non-significant change with respect to control, indicating O_3-induced inhibition of electron transport from PSII to PS I. In Yannong 19, non-photochemical quenching (NPQ) showed an increment of 35.5% under O_3 exposure, while in Yangmai 16 non-significant changes were observed (Cao et al. 2009). As the transfer of an electron under stress is affected, plants transfer the excess light energy through heat dissipation, which leads to a decrease in qp. The decline in qp indicates the closure of the PSII reaction center, preventing the transfer of an electron between two photosystems while NPQ increases to remove excess energy as heat, ultimately reducing the utilization of light energy (Cao et al. 2009).

Among the three-snap bean (*P. vulgaris* L.) genotypes with known differences in sensitivity to O_3, S156 showed an increase in the F_0 value and a decrease in Fv/Fm under elevated O_3 compared to control. This suggests that O_3 induces a reduction in light capture and electron transport through alteration of the PSII reaction center while increasing energy dissipation through fluorescence as recorded by a significant decline in qp (Flowers et al. 2007). Compared to the sensitive genotype S156, the resistant genotype R123 showed a significant reduction in NQP under O_3 stress (Flowers et al. 2007) (Table 7.5).

Table 7.5 Summary of studies indicating the O_3-induced percentage eduction in chlorophyll fluorescence parameters (Fv/Fm, the maximum quantum efficiency of photosystem II; Fv'/Fm', the actual quantum yield of the photosystem; qp, the coefficient for photochemical quenching; NPQ, non-photochemical quenching; and ΦPSII, the quantum yield of electron transfer at PSII in different plant species)

Plant	**Type of plant material**	**Fv/Fm**	**Fv'/Fm'**	**qp**	**NPQ**	**ΦPSII**	**Reference**
Ailanthus altissima	One-year-old seedlings	-	16–17	8–16	-	ns	Gao et al. (2016)
F. chinensis			12	14	-	14–15	
Platanus orientalis			10	ns, 8		17	
Robinia pseudoacacia			ns	7–18		23	
F.ornus	2-year-old seedlings	6	-	-	-	-	Paoletti et al. (2009)
	4 m height tree	ns					
Punica granatum	One-year-old saplings	9–14	-	11	11–32	-	Calzone et al. (2019)
O_3-sensitive hybrid poplar clone (“546”)	Young Leaf	-	ns	ns		13	Gao et al. (2017)

	Old Leaf		25	12.5		25	
O$_3$-sensitive hybrid poplar clone “546”	Rooted cuttings	sig	sig	ns	ns	13	Xu et al. (2019)
Trifolium repens	Cultivar NC-S	sig	-	-	-	-	D’Haese et al. (2005)
	NC-R	ns					
Broccoli lower canopy leaves	Broccoli	4.5	31	-	-	-	De Bock et al. (2012)
Oilseed rape	Oilseed rape		sig				
Winter wheat (*Tritcium aestivum*) cultivars	Cultivars Yannong 19	6	-	sig	35.5 increase	-	Cao et al. (2009)
	Yangmai 16	1.5		ns	ns		
Snap bean (*Phaseolus vulgaris*)	R123 (tolerant)	ns	-	ns	5.6	-	Flowers et al. (2007)
	R331 (tolerant)	ns		ns	ns		
	S156 (sensitive)	9		11	ns		
Two rice (*O. sativa*) genotypes	NB, a modern variety of the japonica subspecies and SL46	-	6.1	17.6	-	29	Chen et al. (2011)
			8.7	6.9		ns	
Clover	*Trifolium repens*	ns	-	-	-	-	Scebba et al. (2003)

	T. pratense	4					
Wheat	Sonalika	10	-	-	-	-	Sarkar et al. (2010)
	HUW 510	17					
Radish	*Raphnus sativa*	6.9	-	-	-	-	Tiwari and Agrawal (2011)
Brinjal	*Solanum melongena*	2.6					
Wheat	Old (HUW234) and Modern (HD3118)	3.6–4.2	-	-	-	-	Yadav et al. (2020)
		ns					
Mustard	*Brassica campestris*. var. Kranti	ns	-	-	-	-	Singh et al. (2009)
Mung bean	Six Indian cultivars of *Vigna radiata*	3.1–18.2	-	-	-	-	Chaudhary et al. (2013)
Lamottea dianae	Forb	ns	-		17 increase		Calatayud et al. (2011)
				ns	ns	4	

In poplar, Xu et al. (2019) recorded a significant reduction in Fv/Fm, Fv'/Fm' and ΦPSII under elevated O_3 exposure; however, qp and NPQ remained unchanged between CF and NF + 40 ppb O_3. These results thus suggest that not all chlorophyll fluorescence parameters are simultaneously affected under O_3 stress. Similarly, Gao et al. (2017) found a difference in the behavior of chlorophyll fluorescence parameters of young and old leaves under elevated O_3 treatment as in the O_3-sensitive hybrid poplar clone ("546"), young and old leaves showed the contrasting behavior of chlorophyll fluorescence parameters under O_3 exposure. Fv'/Fm', qp, and NPQ were significantly reduced by 25, 12.5, and 25% in old leaves, while variations were non-significant in young leaves for Fv'/Fm' and qp under NF + 40 ppb O_3 exposure (Table 7.5). Young leaves under O_3 exposure also showed an increase in NPQ compared to plants under charcoal-filtered ambient air (CF) and non-filtered ambient air (NF) (Gao et al. 2017).

Crous et al. (2006) reported NPQ to bea key factor in determining O_3 sensitivity in O_3-sensitive (NC-S) and O_3-resistant (NC-R) bio-monitor clones of white clover, as under O_3 exposure qp showed an elevation in both clones; however, NPQ was always lower in the NC-S than in the NC-R clone. In *Betula pendula*, O_3 exposure initiated a differential response in the leaves of clones 4 and 80 where the quantum yield of electron transport was influenced only in clone 4 while both qp and NPQ showed no sensitivity to elevated O_3 treatment in both clones (Eichelmann et al. 2004). In soybean, Bernacchi et al. (2006) found no significant effect of elevated O_3 on photosynthetic electron transport on newly expanded leaves; however, a significant decrease was observed 1 d after exposure. Then et al. (2009) also recorded non-significant variations in chlorophyll fluorescence parameters under O_3 exposure in *P. canariensis* seedlings.

Chen et al. (2009) recorded a differential response of chlorophyll fluorescence parameters in soybean under acute and chronic O_3 exposure. Both exposures reduced PSII efficiency (Fq/Fm) by 20% with spatial heterogeneity in the leaves. The study also identified a decrease in Fq/Fm under acute exposure due to a decline in Fv/Fm and Fq/Fv; however, only Fq/Fv decreased under chronic exposure. The reduction (20%) in electron transport (Fq′/Fm′) was significant under both exposure conditions.

Chen et al. (2009) also recorded a difference in the increase of spatial heterogeneity of Fq′/Fm′ under chronic (10%) and acute (50%) treatments of O_3 in soybean. In Fv/Fm, Chen et al. (2009) found discrete patches of lower Fv/Fm than the control, whereas Fv/Fm was similar throughout the leaf surface. A significant reduction in Fv/Fm in acute exposure was more in the base than the leaf tip. Compared to control, acute O_3 exposure increased the spatial heterogeneity of Fv′/Fm′, Fq′/Fv′, and Fq′/Fm′ by 75, 37, and 46%, respectively. In contrast, the increase under chronic O_3 exposure was relatively lower, with percentage increases of 17, 35, and 10 in soybean, suggesting acute exposure significantly alters most of the chlorophyll fluorescence parameters (Chen et al. 2009).

In *Trifolium repens*, D'Haese et al. (2005) recorded a clear difference in the response of Fv/Fm ata 5-day 60 ppb O_3 exposure where the sensitive (NC-S) clone showed a significant decline in Fv/Fm while it remained unchanged in the tolerant clone (NC-R) (Table 7.5). A significant decrease in qp under O_3 exposure was also recorded by Morgan et al. (2004) in soybean, while the maximum apparent quantum efficiency remained unchanged until the last measurement day, when it showed a significant reduction suggesting damage to light-limited photosynthesis.

Scebba et al. (2006) assessed the Fv/Fm ratio, the actual quantum yield of PSII and the NPQ of 29 plant species, and recorded significant variations in 37% of plant species belonging to natural or semi-natural ecosystems. Overall, data showed a significant effect of O_3, species, and their interaction on Fv/Fm and NPQ while the actual quantum yield of PSII showed only significant variability with species, and the interaction of O_3 and species did not initiate a significant response (Table 7.5).

In ten cultivars of soybean, Betzelberger et al. (2010) recorded a significant decrease in the operating efficiency of photosystem II (Fq'/Fm') and the efficiency of CO_2 assimilation (FCO_2) under chronic elevated O_3 exposure. Chlorophyll fluorescence parameters did not respond to early O_3 exposure (5th day) in rice, while by day 23, the quantum efficiency of photosystem II had declined by 23%, Fv′/Fm′ by 6.1%, and Fq′/Fv′ by 17.6% (Chen et al. 2011). Sarkar et al. (2010) found a significant decrease of 10 and 17% in the Fv/Fm ratio of rice cultivars Sonalika and HUW510, respectively, under O_3 exposure, while Yadav et al. (2020) found a significant reduction of 4.2% only in an old cultivar of wheat while in the modern cultivar the ratio remained unchanged.

The acute O_3 exposure (150 ppb for 3 h) significantly reduced Fv/Fm by 4% in red clover (*Trifolium pratense*) while no significant effect was observed in white clover (*T. repens*). This reduction in Fv/Fm was attributed to an increase in Fo and a decrease in Fm (Scebba et al. 2003). Calatayud et al. (2011), in forb *L. dianae,* found that O_3 inducesa reduction in yield of PSII and qp; however, Fv/Fm remained unaffected, suggesting a reduction in light energy use at the expense of heat dissipation. Calzone et al. (2019) recorded a decrease in Fv/Fm, qp, and NPQ by 9–14, 11, and 11–32%, respectively, in pomegranate (*Punica granatum*) under O_3 and combined O_3 and salt stress (Table 7.5).

In 2-year-old seedlings of *Fraxinus ornus*, the Fv/Fm ratio and Performance Index were negatively affected under elevated O_3 exposure (Paoletti et al. 2009). Gao et al. (2016) recorded a significant reduction in Fv/Fm by 16, 12, and 10%, respectively, in *Ailanthus altissima, Platanus orientalis*, and *Fraxinus chinensis* under O_3 exposure, while a significant reduction in qp was recorded in all trees except *P. orientalis*. The actual quantum yield of PSII reduced more in *Robinia pseudoacacia* (23%) followed by *P. orientalis* (17%) and *F. chinensis* (14.5%), while NPQ remained unchanged in *A. altissima* (Table 7.5). These results suggest variable responses of different fluorescence parameters in test trees directly related to O_3 flux and the detoxification potential of species.

7.7 Water and nitrogen balance under O_3 stress

Nitrogen is the major component of pigments, enzymes of carbon assimilation, antioxidative enzymes, and metabolites. Nitrogen metabolism of plants is key to defense and physiological processes in plants. Nitrogen allocation to growth and defense is key to plant sensitivity under stress conditions.

Ozone stress increased mass-based leaf N concentration in hybrid poplar clones but significantly reduced area-based leaf N concentration (Shang et al. 2019). This reduction is due to the simultaneous decrease in leaf mass per unit area. The authors also recorded a positive correlation between area-based leaf N concentrations with the *Pn* (Shang et al. 2019). Shang et al. (2019) found a significant reduction in photosynthetic N-use efficiency (PNUE) and leaf N allocation to photosynthetic components in hybrid poplar clones; however, N allocation to cell walls and other components was enhanced, reflecting that N was allocated more towards structural components and defense than for photosynthesis. In 3-year-old white birch seedlings, O_3 exposure reduced PNUE in early and late leaves; however, a significant effect was only recorded for late leaves (Hoshika et al. 2013). In the same study, O_3 exposure induced a higher N content in young leaves suggesting that N accumulated more in young leaves to cope with O_3 stress (Table 7.6).

Nitrogen is an essential component of several enzymes and proteins involved in carbon assimilation, and it is expected that higher N in the leaf will help in stress tolerance. Watanabe et al. (2018a) recorded a decrease in V_{cmax} and J_{max} in leaves having a high N_{area} compared to leaves with a lower N_{area} under O_3 exposure in Siebold's beech; however, no such effect was detected in Japanese oak. Reduction in the N_{area} further leads to early senescence. The N_{mass} of lower canopy leaves also increased under O_3

exposure in Siebold's beech (Watanabe et al. 2018a). In ten-year-old saplings of Siebold’s beech (*Fagus crenata*) O_3 exposure declined the N allocation to the photosynthetic function, ultimately reducing PNUE (Watanabe et al. 2013). Elevated O_3 exposure in both the modern and old cultivar of wheat caused a significant reduction in PNUE by 6.7–12.4% (Table 7.6). The old cultivar, however, showed less decline compared to the sensitive modern cultivar (Yadav et al. 2020).

Both O_3-sensitive and O_3-tolerant cultivars of winter wheat (*T. aestivum*) showed a significant reduction of transpiration (Tr) under O_3 treatment. However, the decline was comparatively less in the tolerant cultivar, which helped it to maintain photosynthesis under O_3 stress (Cao et al. 2009) (Table 7.6). However, Yadav et al. (2020) recorded a non-significant reduction in Tr under O_3 stress in an old (HUW234) and a modern (HD3118) wheat cultivar. Tiwari and Agrawal (2011), on the other hand, found a significant increase in Tr in radish (*Raphnus sativa*) under O_3 exposure while brinjal (*Solanum melongena*) showed a significant reduction of 18.9% under ambient O_3 compared to the filtered chamber. Under 5-day 70 ppb O_3 exposure, Tr reduced by 15%, while under 16-day 70 ppb O_3 exposure, Tr reduced by 55% compared to control in *Brassica nigra* (Papazian et al. 2016). In different seasons, Pina et al. (2017) recorded a significant reduction in Tr ranging between 27 and 51% in *P. guajava* under a high O_3 environment in the urban forest of São Paulo, SE Brazil (Table 7.6).

Water use efficiency (WUE) in the old wheat cultivar showed no significant effect of NF + 20 ppb O_3. In contrast, the modern cultivar showed an 18% reduction in WUE, indicating that under O_3 stress, the sensitive cultivar failed to maintain water balance and photosynthesis (Yadav et al. 2020) (Table 7.6). Tiwari and Agrawal (2011) also recorded a reduction in WUE in radish and brinjal plants under NFC compared to FC. Radish showed a decline of 20.8% between the two species compared to brinjal (17.6%).

Table 7.6 Summary of studies indicating the O_3-induced reduction in transpiration, water use efficiency (WUE), and photosynthetic N use efficiency (PNUE) in different plant species (ns denotes non-significant variation)

Plant	Type of plant material	Ozone exposure	Reduction in transpiration (%)	Reduction in WUE (%)	Reduction in PNUE (%)	Reference
Ailanthus altissima	One-year-old seedlings	NF and NF + 40 ppb	-	3.7–25	-	Gao et al. (2016)
Fraxinus chinensis				6.7–7 increase		
Platanus orientalis				30		
Robiniap seudoacacia				21 increase		
O_3-sensitive hybrid poplar clone (“546”)	Young Leaf	CF, NF and NF + 40 ppb	-	ns	-	Gao et al. (2017)
	Old Leaf			31		
White birch (*Betula platyphylla* var. japonica)	Early leaf	Ambient (25.7 ppb)	-	-	ns	Hoshika et al. (2013)
	Late leaf	and elevated (56.7 ppb)			16–39	

Siebold’s beech	Ten-year-old saplings	60 ppb	-	-	31	Watanabe et al. (2013)
(*Fagus crenata*)					17.8	
Oak (*Quercus mongolica*)						
Psidium guajava	Cloned saplings	Ambient seasonal variations	27–51	-	-	Pina et al. (2017)
Winter wheat (*Tritcium aestivum* L.) cultivars	Cultivars Yannong 19	1.5 x ambient	57	-	-	Cao et al. (2009)
	Yangmai 16		27.9			
Radish	*Raphnus sativa*	FC and NFC	5.2 increase	20.8	-	Tiwari and Agrawal (2011)
Brinjal	*Solanum melongena*		18.9	17.6		
Wheat	Old (HUW234) and Modern (HD3118)	NF and NF + 20 ppb	ns ns	ns 18	6.7–8.7 9.4–12.4	Yadav et al. (2020)
Lamottea dianae	Forb	CF and NF+30	-	25	-	Calatayud et al. (2011)

Brassica nigra (wild black mustard)	5-day O_3 exposure 16-day O_3 exposure	Short 5 d, 70 ppb and 16 d, 70 ppb	12 55	-	-	Papazian et al. (2016)
Six Indian cultivars of *Vigna radiata*	Ambient + 10 ppb O_3 for 6 h day^{-1}	Ambient + 10 ppb	-	6.5–43.8	-	Chaudhary et al. (2013)

Gao et al. (2016) recorded significant variations in WUE among the four urban tree species exposed to NF + 40 ppb O_3. In response to elevated O_3 exposure, *Platanus orientalis* and *Ailanthus altissima* showed reductions in WUE by 30 and 25%, respectively, while for *Robinia pseudo acacia* and *Fraxinus chinensis*, WUE increased by 21 and 7%. These variations in different trees can be attributed to the individual susceptibility of species to elevated O_3 and differences in stomatal regulation. The young and old leaves of the O_3-sensitive hybrid poplar clone ("546") had distinct WUE and under O_3 stress, young leaves maintained their WUE under NF + 40 ppb O_3 while in old leaves a significant reduction of 31% in WUE was recorded compared to plants under NF (Gao et al. 2017). Calatayud et al. (2011) also recorded a significant reduction in WUE by 25% in forb species, *L. dianae*, under NF + 30 ppb O_3 exposure compared to control (Table 7.6).

7.8 Phytohormones

Salicylic acid (SA), abscisic acid (ABA), jasmonic acid (JA), and ethylene (ET) are well known for their role in oxidative stress caused by O_3 as well as for their role in plant signaling, but how these responses are elicited and how different hormones are coordinated are still largely unknown. In a perennial herb, *Leontodon hispidus*, elevated O_3 exposure reduced the sensitivity of stomatal closure to abscisic acid after 24 h (Wilkinson & Davies 2009). Under O_3 exposure, leaves produced higher ethylene concentrations after exposure for 9–16 days, while the leaf tissue ABA concentration remained unchanged after 11–14 days of O_3 exposure. The study also suggested that the O_3-induced reduction in stomatal sensitivity to ABA is mediated by ethylene (Wilkinson & Davies 2009).

Two-hybrid aspen (*Populus tremula* L. x *P. tremuloides* Michx.) clones were investigated for a hormonal response under chronic O_3 exposure. Both clones differed in their response to O_3 exposure as O_3-sensitive clone 51 showed a higher ethylene evolution than tolerant clone 200, while the salicylic acid concentration was higher in clone 200 (Vahala et al. 2003). Vahala et al. (2003) suggested the dual role of ethylene signaling under stress. It accelerates leaf senescence under low O_3 pressure, but under high or acute stress, ethylene is required for protection from necrotic cell death. At 75 ppb O_3 exposure, ethylene production was higher in clone 51 compared to clone 200, while at higher concentration, ET production was similar in both clones; however, O_3 exposure caused an increase in JA in clone 51 while in clone 200, no difference was observed (Vahala et al.). The overall study found ethylene dependent defense in the sensitive clone while the tolerant clone employed an SA dependent defense response to combat O_3 stress.

In both O_3-sensitive (NE-388) and -tolerant clones (NE-245) of hybrid poplar the JA level increased 3.2- and 2.7-fold under O_3 exposure compared to control plants (Koch et al. 2000). An increase of JA was recorded within 3 hours of O_3 exposure, while SA increased after 6 hours of O_3 exposure with a non-significant increment of 36 and 31% in NE-388 and NE-245, respectively. The overall response indicated that the tolerant clone enhanced the SA induced or activated program cell death pathway to counteract O_3 stress, while the sensitive clone failed to initiate a strong activation due to lower levels of either SA or JA (Koch et al. 2000).

In different natural accessions of *A. thaliana*, an acute 2 hours of O_3 exposure did not induce a significant change in ABA concentration; however, an 8-hour exposure increased the ABA level in all studied accessions, although most O_3-tolerant accessions showed a non-significant increment (Brosché et al. 2010). The study also did not get any significant correlation between the ABA concentration and O_3-induced leaf injury. Jasmonic acid concentrations in both O_3-tolerant (Kin-0, C24, and Col-0) and -sensitive (Est-1) accessions remained unchanged under O_3 exposure (Brosché et al. 2010).

Under acute O_3 exposure, Brosché et al. (2010) recorded a significant increase in SA concentrations in Ler-1 and Te-0 accessions of *A. thaliana* after 2 hours of O_3 exposure while all other accessions except Kas-1 and Est-1 showed significant increases after 8 hours of O_3 exposure. Among the accessions, Col-0 and Cvi-0 showed a five- to six-fold increment while Te-0 showed a twelve-fold increment in SA content after O_3 exposure (Brosché et al. 2010). In different natural accessions of *A. thaliana,* Brosché et al. (2010) recorded a positive association between O_3 injuries and JA concentration after 8 hours of O_3 exposure as JA is required for reducing O_3-induced oxidative stress.

McAdam et al. (2017) found a rapid increase in the ABA level under O_3 exposure, while ABA precursors' direct oxidation led to stomatal closure as an immediate response to O_3. The study also recorded a significant stomatal closure under a 3-minute pulse of 400 ppb O_3 in *A. thaliana* wild-type and the aba2-11 ABA biosynthetic mutant. Under O_3 exposure, wild-type *A. thaliana* and *S. lycopersicon* showed 1.74- and 1.25-fold increases in foliar ABA after O_3 exposure. The authors also observed a significant increase in the guard cell ABA level in the isolated epidermis of *A. thaliana* after a 3-minute short 400 ppb O_3 pulse due to the non-enzymatic conversion of ABA precursors (McAdam et al. 2017). McAdam et al. (2017) also found that an O_3-induced increase in ROS production leads to the closure of stomata by enhancing ABA concentration as authors recorded a lack of stomatal response in *P. sativum* and *A. vulgaris* where the foliar ABA level failed to increase under O_3 exposure.

7.9 Future perspective

More studies are needed for all vegetation types concerning physiological attributes under different O_3 exposure regimes. Future studies need to incorporate measurements in different growth phases, leaf types, canopy levels, and diurnal variations. Simultaneous measurements of both photosynthetic and chlorophyll fluorescence parameters need to be done. Flux-based measurements and the consequent physiological responses should be attempted. Techniques such as fluorescence imaging and the fluxomics approach must be tried along with conventional measures. New physiological markers for O_3 are required for large-scale biomonitoring programs. Nitrogen metabolism, leaf hydraulics, and hormonal response to O_3 exposure should be worked out in future studies. Species-specific responses should be carefully examined as several physiological factors are dependent upon anatomical and morphological characteristics and micro-meteorological conditions. In semi-natural conditions, chamber experiments must be validated under actual field conditions and ambient air pollution for policymaking.

7.10 Conclusion

Physiological responses of vegetation are found to be negatively influenced by O_3 exposure in the form of a reduction in photosynthetic carbon assimilation through a direct effect on stomatal conductance or through AOS-induced damage to carbon fixation. Most studies indicated a direct relationship between physiological processes with overall O_3 sensitivity in both trees and crop plants, although responses were species-specific. The current increasing trend of O_3 suggests a significant adverse effect on most of the physiological process, mainly through influence on the photosynthetic process and stomatal regulation, which ultimately reduces productivity in trees and yield in crop plants. Chlorophyll fluorescence parameters, the maximum rate of Rubisco carboxylase activity (V_{cmax}), and the maximum rate of photosynthetic electron transport (J_{max}) are identified as key parameters in understanding O_3-induced damage to the photosynthetic process. Other processes, such as transpiration, water use efficiency, nitrogen balance, and photosynthetic nitrogen use efficiency, are also modified by O_3, suggesting that this stress systematically influences several interlinked physiological processes. Phytohormones such as abscisic acid, jasmonic acid, salicylic acid, and ethylene are identified as key regulators for O_3-induced signaling responses in plants. More studies are required to understand the physiological sensitivity of different vegetation types to O_3 under field conditions considering other stress factors.

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CHAPTER EIGHT

IMPACTS OF OZONE ON THE ECOPHYSIOLOGY OF FOREST TREE SPECIES

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Abstract

Tropospheric ozone (O_3) is a phytotoxic air pollutant, and its effects on forest ecosystems are a major concern. According to O_3 exposure studies, current levels of O_3 would negatively affect growth, carbon allocation, phenology, and physiological functions of forest tree species, although there is a significant variation of O_3 susceptibility amongst species. Ozone is absorbed into the leaves through open stomata, there by reacting with components of cell walls and plasma membranes, and leading to leaf damage. Recent studies focus on modelling stomatal O_3 uptake and the mechanism of O_3 effects on internal processes of photosynthesis in the leaf. Ozone may also change the interaction with insect herbivores and symbiosis with ectomycorrhizal fungi. Other environmental factors, such as edaphoclimatic conditions, nitrogen deposition, and CO_2 concentration, might modulate the response of forests to O_3.

Keywords: Ozone; Forest Tree; Phenology; Ecophysiology

8.1 Introduction

Forests represent invaluable ecosystems offering a wide range of ecosystem services (Brockerhoff et al. 2017). Firstly, we identify the significance of atmospheric ozone (O_3) pollution on forest functions. According to Montréal Processing 1998, a leading international agreement on sustainable forest management, the following seven criteria are indicated: 1) conservation of biological diversity, 2) maintenance of the productive capacity of forest ecosystems, 3) maintenance of forest ecosystem health and vitality, 4) conservation and maintenance of soil and water resources, 5) maintenance of the forest contribution to global carbon cycles, 6) maintenance and enhancement of long-term multiple socio-economic benefits to meet the needs of societies, and 7) the legal, institutional, and economic framework for forest conservation and sustainable management. O_3 pollution can be identified as a threat for the "maintenance of forest ecosystem health and vitality" and consequently for other forest functions.

The effects of O_3 on trees have mainly been evaluated by experimental studies. Many types of O_3 exposure systems have been developed (Fig. 8.1). Ozone fumigation experiments using growth chambers are the most commonly used and can offer an advantage in mechanism - oriented studies owing to their controllability for O_3 concentration and uniform environmental conditions. On the other hand, micro-meteorological conditions and biotic stresses (e.g., herbivores and diseases) in chamber experiments are different from those of natural vegetation. In contrast, free-air O_3 enrichment of field-grown trees is a novel technique assessing the effects of O_3 under field conditions (e.g., Karnosky et al. 2007; Matyssek et al. 2007; Oksanen et al. 2007; Watanabe et al. 2013; Paoletti et al. 2017).

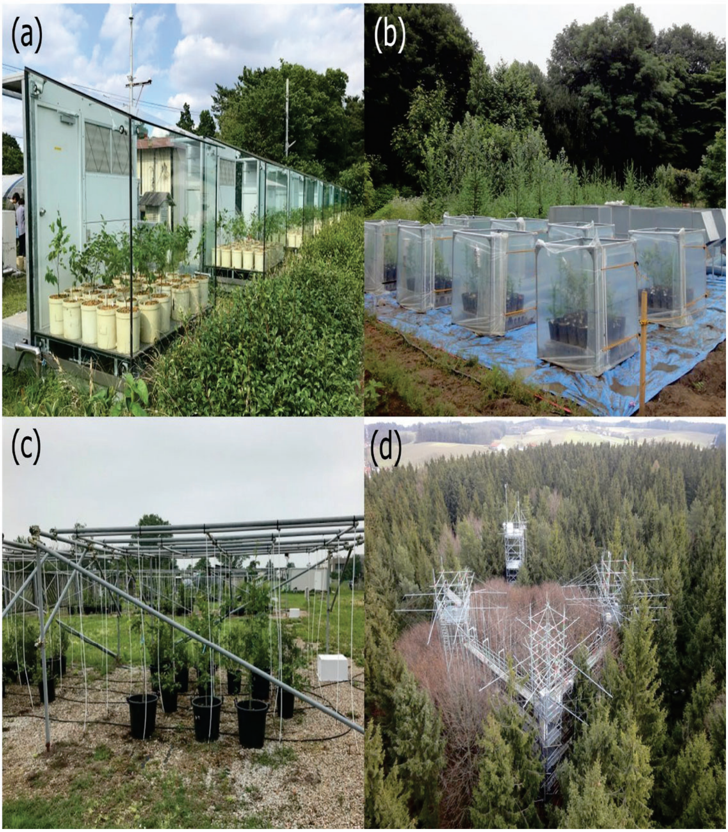

Fig. 8.1 (a) Naturally-lit environmental control chambers (TUAT, Japan), (b) open-top chambers (Hokkaido Univ., Japan), (c) free-air ozone fumigation systems for young plants (IRET-CNR, Italy) and (d) for mature forest ((Technische Universität München, Germany) (source: M. Watanabe).

In this chapter, we introduce ecophysiological traits of forest tree species such as growth, leaf gas exchange, photosynthetic nitrogen use, and interactions, with herbivores and symbiotic fungi under elevated O_3, and modifications of O_3 effects by other environmental factors, such as elevated CO_2, atmospheric nitrogen deposition, and soil water stress. The overview of the content is illustrated in Fig. 8.2.

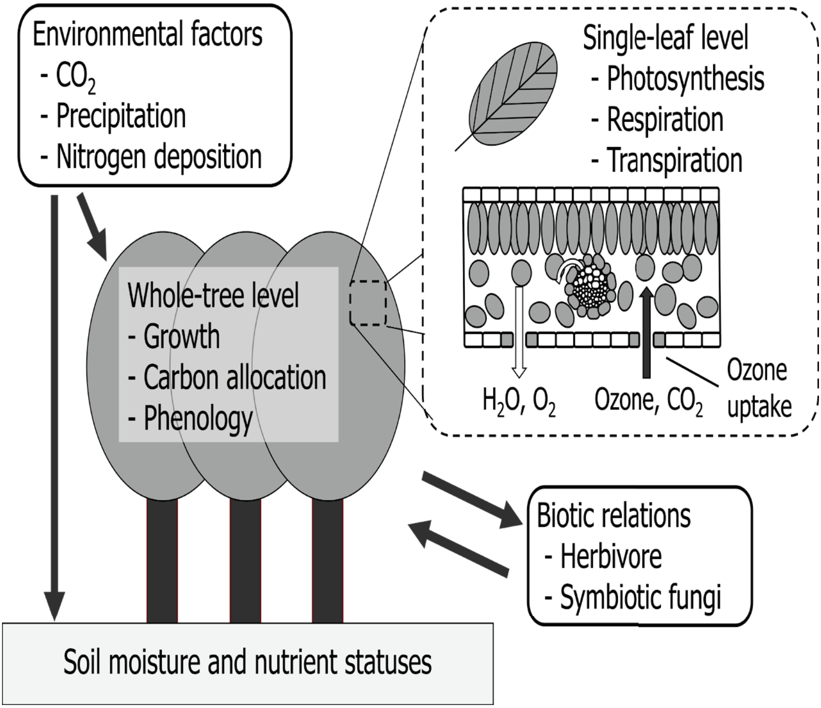

Fig. 8.2 Overview of ecophysiological traits of forest tree species under elevated ozone (O_3). Ozone enters leaves through stomata and causes a cascade of physiological processes not only in leaves but also in other organs. The effects of O_3 on ecophysiological traits may change biotic relations with herbivore and symbiotic fungi. Other environmental factors may modify the O_3 susceptibility of trees.

8.2 Effects of Ozone on the Ecophysiological Characteristics of Trees

In pioneer studies on the O_3 effects on woody species, acute effects of high O_3 concentration (>200 ppb) were investigated. Experimental studies on the effects of chronic O_3 exposure on the growth and physiology of forest trees mainly started in the 1990s. These studies indicated that ambient levels of O_3 below 100 ppb for several months can induce visible leaf injury and inhibit the growth and physiological functions of susceptible forest tree species, such as beech, birch, and poplar (Yamaguchi et al. 2011; Büker et al. 2015; Hoshika et al. 2020a).

Elevated O_3 entering the leaf through stomata causes damage to plants; e.g., decreases in photosynthesis and growth (Fig. 8.2). Therefore, a simple model of O_3 effects can be described as follows,

O_3 effect = stomatal O_3 uptake × damage caused by a unit of O_3 uptake

The stomatal O_3 uptake is called 'phytotoxic O_3 dose' (POD). The POD is the time integral of stomatal O_3 flux. Since O_3 concentration inside the leaf can be assumed as zero (Omasa et al. 1979; Laisk et al. 1989), stomatal O_3 flux is determined as the product of stomatal conductance for O_3 and ambient O_3 concentration based on gas-diffusion theory (micro-meteorological factors are also considered). Details of stomatal O_3 flux estimation are described by ICP Vegetation (2020). The latter factor in the formula relates to the detoxification of O_3 and reactive oxygen species derived from O_3, and the processes of repair and compensation against O_3 damage (Fuhrer and Booker 2003). However, the current knowledge is not sufficient to fully explain these mechanisms. It should be noted that complex physiological mechanisms regulate both factors explaining the O_3 effect (i.e. POD and damage).

8.2.1 Growth

Fumigation experiments indicate that when susceptible tree species are exposed to elevated O_3, the growth of dry matter generally decreases (Wittig et al. 2009; Yamaguchi et al. 2011; Li et al. 2017). These studies revealed significant differences in susceptibility to O_3 among plant functional types, i.e., deciduous/evergreen and broadleaved/coniferous. In general, O_3 susceptibility of deciduous tree species is higher than that of evergreen tree species (Reich 1987; Yamaguchi et al. 2011; Zhang et al. 2012; Büker et al. 2015; Li et al. 2017). The foliage of evergreen tree species is exposed to various biotic and abiotic stresses, such as strong wind, low temperature, herbivores, and diseases, for a longer time than that of deciduous tree species. Therefore, the foliage of evergreen trees should have a higher physical and chemical tolerance against stress factors. However, there is also a significant variation in O_3 susceptibility within a functional type. For example, *Castanopsis sieboldii*, a representative evergreen tree species in warm temperate forests of Japan, has a high susceptibility to O_3, comparable to the deciduous Siebold's beech (*Fagus crenata*) (Watanabe et al. 2008). In the meta-analysis by Wittig et al. (2009), the genus *Liriodendron*, a deciduous and broadleaved species, showed no growth reduction due to O_3 exposure. In Europe, the relationships between the POD and the growth reduction of several representative tree species have been investigated (Karlsson et al. 2007; Büker et al. 2015). An uptake-based analysis of the O_3 effect on Asian forest tree species is ongoing (Hu et al. 2015; Agathokleous et al. 2018).

Besides, O_3 affects the leaf turnover of trees with an indeterminate growth type, which produces new leaves during a growing season. Pell et al. (1994) demonstrated O_3-induced acceleration of old leaves' abscission with the production of fresh leaves in *Populus tremuloides* seedlings. These phenomena are considered as a kind of compensation response to elevated O_3 stress. A similar tendency was observed in *C. sieboldii* seedlings (Watanabe et al. 2008). The latter is an evergreen and broad-leaved species, and a decrease of previous-year leaves and an increase of 2nd flush current-year leaves were observed. However, the compensation response efficiencies against O_3 stress are not clear because old leaf fall means a loss of carbon, and new leaf production requires an additional cost of carbon. On the other hand, the O_3-induced stimulation of new leaf production without the abscission of old leaves was also observed in Siebold's beech, Konara oak (*Quercus serrata*), and Mizunara oak (*Q. mongolica* var. *crispula*) (Watanabe et al. 2010; Kitao et al. 2015).

8.2.2 Carbon allocation

There is Elevated O_3 changes in carbon allocation between above- and below-ground organs. Many O_3 fumigation studies indicated that O_3 induces more inhibition of root growth than above-ground growth, resulting in an increase in the ratio of shoot biomass to root biomass (S/R) under elevated O_3 (Wittig et al. 2009). However, it should be noted that nearly 70% of the studies showed no significant change of S/R due to exposure to O_3 (Agathokleous et al. 2016).

The reduction in carbon allocation to the root under elevated O_3 might result from a compensation response to maintain growth under elevated O_3 (Pell et al. 1995; Kitao et al. 2015) or an artifact due to the small pot size (Arp 1991). Increase of turnover and production of fine root and enhancement of soil respiration were observed in mature European beech (*Fagus sylvatica*) under free-air O_3 exposure (Matyssek et al. 2010). On the other hand, no difference between the above- and below-ground decline of biomass was found in adult poplar (Oxford clone: *Populus* *maximoviczii* Henry *berolinensis* Dippel.) subjected to ambient O_3 and relative to trees protected by ethylene diurea (EDU) for 8 years (Carriero et al. 2015). At present, the mechanism of the change in carbon allocation of tree species under elevated O_3 conditions is not fully understood. If the carbon allocation into the root decreases, carbon input from the root system to the soil by root turnover and exudate may be decreased.

On the other hand, relative carbon input from above ground by leaf and branch abscissions, will be high. The decomposition processes, including animal and microbial decomposers, differ between carbon from above-ground and below-ground. Therefore, changes in carbon allocation may have critical effects on the carbon cycle in forests.

8.2.3 Phenology

Acceleration of foliar senescence (earlier inhibition of physiological activity and nutrient concentration in leaves, and defoliation, etc.) is one of the typical phenomena found in trees exposed to elevated O_3 (Pell et al. 1999; Uddling et al. 2005; Vollenweider et al. 2019), while it somewhat differs from natural senescence (Matyssek and Sandermann 2003). Although the acceleration of foliar senescence and the above-mentioned old leaf abscission for the compensative new leaf production response under elevated O_3 might be partly the same mechanism, the difference has not been clarified. Yonekura et al. (2004) examined the effects of O_3 on phenological characteristics of Siebold's beech seedlings. Exposure to O_3 for one growing season (60 nmol mol^{-1} O_3 for seven hours per day) induced early leaf fall. Furthermore, seedlings exposed to O_3 in the previous growing season showed delayed bud break in the next spring. This phenomenon is called a "carry-over effect." A similar phenomenon is observed in mature European beech in Kranzberg Forest in Germany (Nunn et al. 2005). Several carry-over effects in phenology and growth, morphology, and physiology are reported in European birch (*Betula pendula*) seedlings (Oksanen and Saleen 1999).

8.2.4 Stomatal functions

Stomata are small pores on leaves, which have an aperture actively regulated by plants in response to multiple abiotic and biotic factors; Stomatal conductance, which represents the inverse of resistance in the gas diffusion process between ambient air and the air space inside a leaf and is a proxy of the stomatal aperture, is thus a major determinant of CO_2 uptake (photosynthesis) and water loss (transpiration) by plants. Ozone, by entering leaves through stomata and causing a cascade of physiological processes, can negatively affect photosynthesis (Reich 1987; Paoletti 2007; Wittig et al. 2007). As mentioned above, the POD is considered to be closely related to O_3 impacts on forest trees (Büker et al. 2015; Fares et al.2018). As also mentioned above, stomatal conductance is one of the most important factors for the POD estimation. A multiplicative numerical approach has been commonly used to estimate stomatal conductance (Jarvis-type model, Jarvis, 1976). Many experiments and observations have been made on forest tree species to improve estimations (e.g., Emberson et al. 2000, Alonso et al. 2008; Hoshika et al. 2012b; Kinose et al. 2014; Hu et al. 2015). In addition to the Jarvis-type model, a model based on an empirical relationship between the photosynthetic rate and stomatal conductance (i.e., the Ball-Woodrow-Berry model, Ball et al. 1987) was also applied to estimate stomatal O_3 flux (Büker et al. 2007; Fares et al. 2013; Hoshika et al. 2015a; Kinose et al. 2017).

According to the meta-analyses by Wittig et al. (2007) and Li et al. (2017), O_3 reduces stomatal conductance. This raises the question of whether O_3-induced stomatal closure could act as a response to avoid O_3 stress. Based on the optimal stomatal model, Hoshika et al. (2013, 2020) suggested that O_3 flux was efficiently limited by stomatal closure in early summer, indicating an avoidance of O_3 stress to allow maximum photosynthetic capacity. However, the decrease of stomatal conductance by O_3 exposure became small in late summer and autumn. Ozone also causes a less efficient stomatal control, the so-called “stomatal sluggishness” (Paoletti and Grulke 2005, 2010). The highest O_3 levels (>120 ppb as an average treatment) in the meta-analyses by Wittig et al. (2007) did not decrease stomatal conductance, whereas the same level of O_3 decreased the net photosynthetic rate. Watanabe et al. (2014b) pointed to an inconstant stomatal response of Monarch birch (*Betula maximowicziana*) seedlings to increase the POD due to both stomatal closure and sluggishness. Hoshika et al. (2015b) reported that O_3-induced stomatal closure was diminished with increasing leaf age in Siebold’s beech. Also, O_3 may increase night-time stomatal conductance in late summer or autumn, as reported in Siebold’s beech (Hoshika et al. 2013) and Oxford poplar clone (Hoshika et al. 2019). Because of this evidence, the modeling of stomatal conductance is of utmost interest.

Stomatal sluggishness also includes a slower stomatal response to environmental stimuli. Hoshika et al. (2012a) found a significantly slower stomatal response to changing light conditions (alternative light exposure between 100 and 1500 $\mu mol\ m^{-2}\ s^{-1}$ of photosynthetic photon flux density) in the leaves of Siebold’s beech. Although stomatal sluggishness mechanisms are still unknown, this response was attributed to the emission of ethylene due to O_3 exposure (Wilkinson and Davies 2010; Hoshika et al. 2019). It has been reported that the balance in turgor between the guard and subsidiary cells (Omasa 1990), lower abscisic acid sensitivity (Mills et al. 2009), and inhibition of CO_2 signaling (Dumont et al. 2014) are considered as possible mechanisms of slower stomatal responses. In the present section, two kinds of "stomatal sluggishness" were explained; one is that stomata do not close to the proper level (steady-state evidence), and the other is the slower stomatal response to environmental stimuli (kinetic evidence).

8.2.5 Photosynthesis

Photosynthesis is a critical process for growth and survival in nearly all plants (Lambers et al. 2008). It is well known that O_3 decreases the photosynthetic rate (Wittig et al. 2007). A decrease in the photosynthetic rate is one of the most critical factors that lead to tree growth reduction. Photosynthesis is comprised of the supply of CO_2 to the chloroplast from ambient air and the demand for CO_2 at the chloroplast. Stomatal conductance is one of the main factors controlling CO_2 supply into the chloroplast. The demand for CO_2 is determined by the capacity of biochemical reactions at the chloroplast, such as carboxylation in stroma and electron transport and ATP synthesis in thylakoid membranes.

There is a high correlation between the net photosynthetic rate and stomatal conductance (Ball et al. 1987) under normal ambient conditions (i.e., no O_3 effect). Co-reductions of the net photosynthetic rate and stomatal conductance may not indicate the stomatal limitation of photosynthesis. The analysis of the response curve of the net photosynthetic rate to the intercellular CO_2 concentration (A/C_i curve) is one of the most suitable methods to quantify the supply and demand of CO_2 in photosynthesis (Farquhar et al. 1980). The maximum rate of carboxylation (V_cmax) and the maximum rate of electron transport (J_max) are representative parameters of the demand function, and stomatal limitation is that of the supply function in the photosynthesis model. Many studies reported reductions of V_cmax and J_max by exposure to O_3, indicating that the chloroplast's biochemical capacity explains the O_3-induced reduction in the photosynthetic rate of trees (Watanabe et al. 2013; Zhang et al. 2014; Gao et al. 2016; Kinose et al. 2017). Reductions of Rubisco and chlorophyll contents under elevated O_3 support the importance of chloroplast activity in the negative impacts of O_3 on leaf photosynthesis (Yamaguchi et al. 2007; Watanabe et al. 2007; 2013).

In contrast, the responses of the stomatal limitation of photosynthesis to O_3 varied among studies. For example, Watanabe et al. (2018c) reported an O_3-induced increase of stomatal limitation in *C. sieboldii* seedlings. However, the same parameter decreased under elevated O_3 in Siebold's beech and Japanese oak saplings (Watanabe et al. 2018a). As mentioned above, the stomatal response to O_3 is complex, involving several physiological and molecular mechanisms. If the trees avoid O_3 uptake with some small loss of carbon assimilation the stomatal limitation would be increased. If O_3-induced stomatal sluggishness occurs, the stomatal limitation would be decreased. The two mechanisms will possibly compensate each other in the long term (Paoletti et al. 2020).

Mesophyll conductance, which is the conductance from the intercellular air space to the carboxylation site of the chloroplast, is another important factor for CO_2 supply in photosynthesis. Watanabe et al. (2018b) and Xu et al. (2019) indicated that mesophyll conductance is a sensitive process to O_3 in the photosynthesis of Siebold's beech and poplar seedlings, respectively. However, information on the effect of O_3 on mesophyll conductance is still limited.

Nitrogen is a nutrient that is strongly related to biochemical assimilation capacity (e.g., Lambers et al. 2008). A large fraction of the nitrogen in leaves is incorporated into proteins associated with the photosynthetic process (Evans 1989; Evans and Seemann 1989). There is a positive correlation between photosynthetic rates and leaf nitrogen contents (e.g., Watanabe et al. 2018a). Net photosynthetic rate (A) is described as follow,

$$A\ (\mu\text{mol m}^{-2}\ \text{s}^{-1}) = \text{PNUE}\ (\mu\text{mol mol}^{-1}\ \text{s}^{-1}) \times \text{leaf nitrogen content (mol m}^{-2}),$$

where PNUE is photosynthetic nitrogen use efficiency (net photosynthetic rate per unit leaf nitrogen content). Generally, exposure to O_3 has less effect on leaf nitrogen content except for the leaf senescence period (see section 3.1.3). On the other hand, O_3 decreased PNUE of several woody species (Maurer et al. 1997; Häikiö et al. 2007; 2009; Yamaguchi et al. 2007; Watanabe et al. 2013; Zhang et al. 2018), thus indicating that O_3 reduced the leaf nitrogen allocation into the photosynthetic apparatus (Watanabe et al. 2013).

When we consider the effects of O_3 on the whole-canopy carbon uptake of trees, it is important to understand the difference in O_3 sensitivity within the canopy. There are considerable variations of leaf traits within a canopy due to the difference in light intensity (Niinemets et al. 2015). Photosynthetic parameters, such as V_{cmax} and J_{max}, are higher in leaves exposed to higher irradiance (Rodríguez-Calcerrada et al. 2008; Niinemets et al. 2015). The difference in O_3 sensitivities between leaf positions within a canopy was also reported for several tree species. In sugar maple (*Acer saccharum*) and European beech (*Fagus sylvatica*), lower canopy leaves were more sensitive to O_3 than upper canopy leaves (Tjoelker et al.1995; Kitao et al. 2009), whereas poplar (*Populustristis*×*P. balsamifera*) and Siebold's beech showed higher O_3 sensitivity in upper canopy leaves (Tjoelker et al.1993; Watanabe et al. 2014a; 2018a; Kinose et al. 2017). There was no significant difference in O_3 sensitivities between the upper and lower canopy leaves in Mizunara oak (*Quercus mongolica* var. *crispula*) (Watanabe et al.2015; 2018a). Kinose et al. (2017) revealed that a more significant decrease of O_3-induced V_{cmax} in the upper canopy leaves of Siebold's beech seedlings was induced by a higher O_3 uptake in the upper canopy leaves. In their study, however, the reduction rate of V_{cmax} per unit O_3 uptake in the upper canopy leaves was small compared with that in the lower canopy leaves. They indicateda higher detoxification capacity for O_3 in the upper canopy leaves associated with greater photosynthates due to higher irradiance. These differences of O_3 sensitivity should be involved in canopy carbon photosynthetic models to estimate the forest scale carbon budget under elevated O_3 (Kitao et al. 2012; Watanabe et al. 2014a).

8.2.6 Respiration

The dark respiration rate in leaves is generally decreased under elevated O_3 (Wittig et al. 2009), although increases of respiration were also reported (Grulke 1999; Noormets et al. 2001; Kitao et al. 2009; Watanabe et al. 2013; Zhang et al. 2018). The conclusive mechanism of these differences has not been clarified yet. The extent of damage caused by O_3 may be one of the responsible factors to explain this discordance. If the O_3 damage is severe, all metabolic processes, including respiration, would be decreased. On the other hand, relatively little damage by O_3 may increase respiration to enhance the detoxification capacity of O_3-related reactive oxygen species and/or the repair of damaged tissues, which consumes carbohydrates (Landolt et al. 1997; Matyssek and Sandermann 2003; Agathokleous et al. 2019).

8.3 Biotic relations

8.3.1 Defense capacity against biotic stresses

Insects and diseases are dynamic components of forest ecosystems. However, trees can be more susceptible to outbreaks due to a particular environmental condition (e.g., Herms and Mattson 1992; Percy et al. 2002; Oßwald et al. 2012) and the presence of multiple stressors, such as drought and pollutants (Grulke et al. 1999). Ozone changes the concentration of defense chemicals in leaves under elevated O_3 (Percy et al. 2002; Peltonen et al. 2005). Sakikawa et al. (2016) reported that damage to the leaves of white birch (*B. platyphylla*) saplings by insect herbivores (mainly by leaf beetle: *Agelastica coerulea*) was decreased under elevated O_3. There are several reports on the effects of O_3 on the emission of biogenic volatile organic compounds (BVOCs) (Carriero et al. 2016; Yuan et al. 2016; Tani et al. 2017; Miyama et al. 2018). Some of the BVOCs also relate to defense against insect herbivores (Lambers et al. 2008), and O_3 may change the plant-insect interaction. However, information on the effects of O_3 on plant-insect/disease interaction in forest tree species is limited. The complex nature of the impact of O_3 on trophic interactions needs additional research.

8.3.2 Symbiosis with ectomycorrhizal fungi

The symbiosis of woody plants with ectomycorrhizal fungi in below-ground root systems is necessary for dominant tree species in both temperate and boreal forests (Marschner 2012). Large fractions, up to 30%, of photosynthates are used for ectomycorrhizae (ECM) colonization (Hampp and Nehls 2001). ECM help the absorption of water and essential nutrients such as phosphorous and sometimes nitrogen (e.g., Quoreshi et al. 2003; Cairney 2011). Several studies indicate that O_3 affects ECM colonization (Grebenc and Kraigher 2007; Wang et al. 2015; Mrak et al. 2019) (Fig.8.3). The reduction of the carbon supply to below-ground due to lower photosynthetic capacity and change in the fraction of carbon allocation under elevated O_3 are possible reasons for the negative impact of O_3 on ECM colonization. Furthermore, Wang et al. (2015) revealed changes in the composition of the ECM community in hybrid larch F_1 (*Larixg melinii* var. *japonica* × *L. kaempferi*) seedlings under elevated O_3, as O_3 decreased the fraction of *Tomentella* sp., and increased the fraction *Cadophora finlandia*.

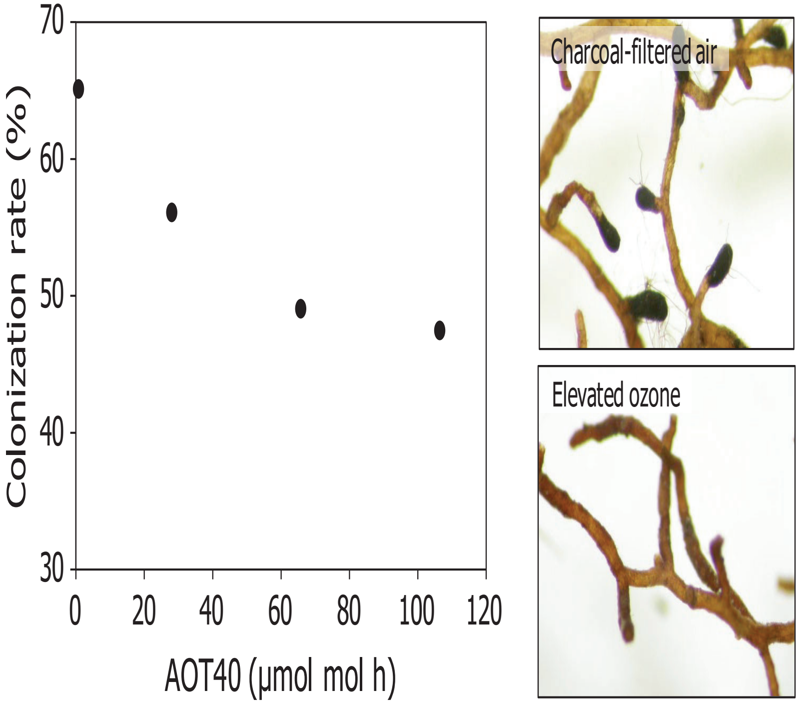

Fig. 8.3 The relationship between accumulated exposure over a threshold of 40 nmol mol^{-1} (AOT40, Agathokleous et al., 2018) of ozone and rate of ectomycorrhizal colonization in fine root tips of Konara oak (*Quercus serrata*) seedlings. Fine root tips of Konara oak seedling grown in charcoal-filtered air (upper photo) and elevated ozone (lower photo). Watanabe et al. (unpublished)

8.4 Combined Effects of Ozone and Other Environmental Factors

Forest ecosystems suffer from not only O_3 stress but also other environmental factors. Those factors might modify the effects of O_3 on the eco-physiological traits of trees described earlier in the chapter. Elevated atmospheric CO_2 concentration, increasing nitrogen deposition, and change in water availability, as a result of climate change, are critical factors that affect forest health condition (Paoletti et al. 2010).

8.4.1 Elevated Atmospheric CO_2

Atmospheric CO_2 levels have increased dramatically since the industrial revolution. They are over 400 μmol mol^{-1} (NOAA/GML, https://gml.noaa.gov/ccgg/trends/), and future rapid increases are expected, with values likely to reach 550 ppm by the middle of this century and 1000 ppm by the end of this century (Stocker et al. 2013). Elevated CO_2 would enhance tree growth if other factors such as nutrient and water availabilities do not constrain growth (e.g., Norby et al. 2010; Kitao et al. 2005). Higher atmospheric CO_2 concentration induces stomatal closure and therefore decreases stomatal O_3 uptake (Ainsworth et al. 2007). A greater amount of carbohydrates due to higher photosynthetic rates under elevated CO_2 may confer better detoxification and repair capacities against O_3 stress (Riikonen et al. 2004).

In fact, several experimental studies demonstrated the amelioration of an O_3-induced reduction in growth and/or photosynthesis under elevated CO_2 (e.g., Matsumura et al. 2005; Koike et al. 2012), whereas some of the studies did not show such an amelioration (Matsumura et al. 2005; Wang et al. 2015). Ozone-induced growth enhancements under elevated CO_2 were also reported in Siebold's beech, Konara oak, and Mizunara oak seedlings (Watanabe et al. 2010; Kitao et al. 2015). The experimental studies' did not totally support the hypothesis that "elevated CO_2 ameliorates negative impacts of O_3". Interestingly, when the amelioration or compensation of the O_3 impact under elevated CO_2 was observed, an elevated CO_2-induced decrease in stomatal conductance was also found (Matsumura et al. 2005; Watanabe et al. 2010, Kitao et al. 2015) except in hybrid larch F_1 (Koike et al. 2012), and vice versa. Therefore, the decrease of stomatal O_3 uptake may be one of the critical factors in the amelioration effect by elevated CO_2.

8.4.2 Nitrogen Deposition

The availability of nitrogen in the soil is a decisive factor in forest productivity. On the other hand, nitrogen deposition from the atmosphere to forest ecosystems is increasing due to the enhancement of human activities (Smil 1990; Vitousek et al. 1997; Galloway et al. 2004). Change in nitrogen availability as a result of increasing nitrogen deposition may affect the susceptibility of trees to O_3 because nitrogen is a component of the enzymes that catalyze many metabolic processes, including defense from O_3 (Dizengremel et al. 2013).

Although the meta-analyses by Feng et al. (2019) and Li et al. (2020) showed no modification of O_3 adverse effects by nitrogen addition, various interactions between O_3 and the soil nitrogen supply were demonstrated in case studies: enhanced susceptibility (e.g., Yamaguchi et al. 2007; Zhang et al.2018), decreased susceptibility (e.g., Watanabe et al. 2006; Sugai et al. 2019) and no change (Feng et al. 2011; Harmens et al.2017). Yamaguchi et al. (2007) reported an increase in the sensitivity of growth of Siebold's beech to O_3 under relatively high nitrogen loading. In this experiment, O_3 significantly reduced A_{sat} in the presence of relatively high nitrogen loading, but not in the absence of nitrogen loading. A similar tendency was observed in soluble protein and Rubisco content in leaves, whereas there was no significant interaction for nitrogen content. They concluded that the enhancement of O_3 susceptibility in Siebold's beech seedlings by soil nitrogen loading was due to more reduction of nitrogen allocation to soluble proteins, including Rubisco, and a consequent reduction of biochemical assimilation capacity in the chloroplast (Yamaguchi et al. 2010).

In contrast to Siebold's beech, the O_3-induced reduction in the growth of Japanese larch (*L. kaempferi*) seedlings was ameliorated by nitrogen loading (Watanabe et al. 2006). Pell et al. (1995) reported that the most considerable growth reduction of trembling aspen (*Populus tremuloides*) seedlings by O_3 was observed under the nitrogen supply that induced the highest tree growth rate. However, no growth enhancement by nitrogen loading was observed in Japanese larch seedlings in the experiment in Watanabe et al. (2006). Besides, the comparison experiment of Japanese larch and hybrid larch F_1 seedlings conducted by Sugai et al. (2019) supported these results. The growth of hybrid larch F_1 was enhanced by nitrogen supply, but nitrogen supply-induced mitigation of growth reduction by O_3 was not found. On the other hand, in Japanese larch, nitrogen did not enhance the growth but mitigated the negative effect of O_3 on growth. It would be essential to clarify whether or not nitrogen supply increases the growth when we consider a change in O_3 susceptibility by nitrogen supply.

8.4.3 Drought

The availability of water in the soil strongly affects plant production. Recent climate change at global, regional, and landscape levels modifies the water status in forest ecosystems. In several regions of the world, the amount of precipitation is projected to decrease due to climate change. Therefore, there is a possibility that many tree species are simultaneously affected by O_3 and drought. Plants close leaf stomata under drought conditions. Thus, the hypothesis "O_3 damage on trees decreases under drought condition because O_3 uptake through stomata decreases due to stomatal closure" is raised. Some studies support this hypothesis (Watanabe et al. 2005; Gao et al.2017; Hoshika et al. 2018a). However, others did not show amelioration effects (Karlsson et al. 1997; Yonekura et al. 2001, Alonso et al. 2014) or even showed enhanced O_3 effects (Pääkkönen et al. 1998). Stomata respond to O_3 and drought in an elaborate manner. Hoshika et al. (2020b) found that plant water-use strategies (i.e., isohydric or anisohydric) affect O_3-induced stomatal closure in Mediterranean trees. In well-watered plants, isohydric species limited O_3 fluxes by stomatal closure, whereas anisohydric species activated a tolerance response and did not actively close stomata. However, in combined O_3 and drought conditions, anisohydric species enhanced the capacity of avoidance by closing stomata to cope with the severe oxidative stress. The combination of O_3 and drought often alters the antioxidant system (e.g., Pellegrini et al. 2019). In late summer and autumn, regardless of the water use strategy, O_3 induced stomatal sluggishness, resulting in a lower capacity of stomatal control for regulating transpiration. Therefore, O_3 may enhance the susceptibility of trees to drought stress. As a result, no amelioration effect may be found in the experiment of O_3 and drought. Further research on O_3-induced stomatal sluggishness in the leaves of trees exposed to drought is needed. The role of aquaporins in O_3-drought interactive effects is also revealed (Harayama et al. 2019).

8.5 Future Perspectives

Many data are available regarding the effects of O_3 on forest tree species. However, most of these data were obtained from juvenile seedlings under controlled chamber conditions with short-term exposure (1-3 years). A long-term (over ten years) free-air O_3 exposure study with mature trees in a vegetation scale is necessary for the near future, because the free-air exposure method requires expensive experimental facilities for a realistic O_3 simulation, and a few individual trees can usually be investigated. Therefore, epidemiological approaches would also be useful to understand the impacts of O_3 on large trees in forests (Schaub and Calatayud 2013; Sicard et al. 2016; Braun et al. 2017; Paoletti et al. 2019). However, studies for understanding the physiological mechanisms of O_3 effects under the environmental control chamber are still important. Most of the environmental factors have the potential to modify the O_3 effect on forest tree species. To predict the effects of O_3 on forest ecosystems in changing environments, a deep understanding of the modification of the O_3 results by the other environmental factors is crucial. Most of the data on the effects of O_3 on woody species are obtained in Europe, North America, and East Asia. To consider the O_3 effects on forest ecosystems on a global scale, it is highly important to expand our knowledge to other regions/climate zones with various tree species.

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CHAPTER NINE

CROP PLANT RESPONSES TO OZONE-INDUCED OXIDATIVE STRESS: SIGNALING CASCADES AND ANTIOXIDATIVE MACHINERY

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Abstract

Amongst the abiotic stresses, tropospheric ozone (O_3), in particular, is one of the most harmful air pollutants with predicted ground-level increments under all climate change scenarios. High O_3 concentrations negatively affect various crop plants and their productivity by directly interfering with surface tissues or producing reactive oxygen species (ROS) in plant cells. Plants' acclimation response to O_3 involves changes in protein activities, gene expression, and antioxidative defense metabolism. It upsurges the production of plant hormones, such as ethylene, jasmonic acid and salicylic acid, changes in polyamine metabolism, and the activities of several phenylpropanoid pathway enzymes. ROS-mediated O_3 stress signals activate a network of finely balanced signaling cascades in the cell leading to cellular reprogramming of which protein post-translational control and calcium and hormone signaling pathways play crucial roles. In this review, the focus is on the uptake and perception of molecular O_3 at the cellular level, and how an intriguing response of ROS-mediated signaling pathways is linked to variable levels of antioxidants in different crop models, explicitly revealing the underlying molecular mechanisms of O_3 tolerance.

Keywords: Tropospheric ozone; Oxidative stress; Antioxidative machinery; Tolerance

9.1 Introduction

Tropospheric ozone (O_3) has been the most severely damaging air pollutant to vegetation (Karnosky et al. 2007). Over the past 150 years, increasing anthropogenic emissions of O_3 precursors and increased stratospheric mixing of O_3 into the lower atmospheric layers have led to an increase in the global average O_3 concentration up to 30−50 ppb, with significant variations spatially and temporally (IPCC 2013).

Under ozone stress, the sensitive plant species alter leaf physiology by increasing respiration rate, and reducing photosynthetic rate, stomatal functioning, and growth. Both above- and below-ground parts show alteration in C allocation and plant phenology (Ainsworth et al. 2012). Once inside the leaf, O_3 induces oxidative stress through the production of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide ($O_2^{\cdot -}$), and hydroxyl radicals ($OH^{\cdot}$) (Fiscus et al. 2005). Plants have a limited ability to detoxify ROS by scavenging them via both non-enzymatic antioxidants such as ascorbic acid, flavonoids, and phenolics or enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), or peroxidases (POD) (Blokhina et al. 2003). When left unscavenged, ROS induce accelerated senescence, leaf abscission, and cell death, all partially mediated by the plant hormones, ethylene (ET), jasmonic acid (JA), and salicylic acid (SA) (Fiscus et al. 2005; Kangasjärvi et al. 2005). Accelerated senescence leads to a down-regulation of photosynthetic genes and an up-regulation of genes involved in programmed cell death, consequently reducing whole-plant photosynthesis with an increase in leaf senescence and abscission (Wittig et al. 2009).

9.2 ROS signaling and antioxidants

Depending upon O_3 concentrations, O_3 stress can be differentiated into two distinct types: chronic and acute. Increased O_3 concentrations, either in chronic or acute O_3 exposure, induce several plant responses both at the biochemical and molecular levels (Cho et al. 2011). Hence, for plants to acclimate to O_3 stress, cellular reprogramming must be established for which high O_3 concentrations have to be perceived at the cellular level and then relayed to the nucleus. An example of an O_3-induced response is stomatal closure (Ainsworth et al. 2012), leading to O_3 acclimation (Hoshika et al. 2013). Without an acclimation program, oxidative stress will ultimately lead to cell death induced by specific signal amplification and transduction events, specifically the O_3-signaling events.

O_3 toxicity has long been believed to be mostly due to oxidative products resulting from O_3 degradation (Heath1994). It directly interferes with surface tissues of plant leaves or reaches mesophyll cells through stomata. The O_3 molecule is immediately degraded into H_2O_2, $^1O_2$, $OH^{\cdot}$ or $O_2^{\cdot-}$in the apoplastic space of plant cells, and further translocate inside the cells. But O_3 also induces active ROS production from several other sources. For example, O_3 induces H_2O_2 accumulation firstat the plasma membrane via NADPH-oxidases and at the cell wallvia POD. Then production continues in the cytoplasm, mitochondria, and peroxisomes (Vahisalu et al. 2010). ROS production is aprevalent response to oxidative stress, and the dual role of these molecules has been well established (Mittler 2002). These molecules have toxic effects on proteins, lipids, carbohydrates, and DNA and play a significant role in initiating signaling responses to stress (Jaspers and Kangasjärvi 2010). Therefore, cells have to unavoidably fine-tune the balance between these antagonistic roles of ROS throughout their life-time.

As a gaseous signaling molecule, ozone elicits cellular responses, resulting in drastic changes in gene expression (Cho et al. 2011). Since O_3 causes oxidation of unsaturated lipids in the plasma membrane, specific membrane lipids may serve as receptor molecules and promote O_3-induced lipid signaling chains (Baier et al. 2005). Studies have reported that plasma membrane lipid-based signaling molecules like JA and lipid hydroperoxides play essential roles in controlling various downstream processes (Kangasjärvi et al. 2005).

Generation of ROS in the apoplastic region causes the activation of Ca^{2+} channels, which in turn triggers the Ca^{2+} influx in the cytosolic region, thereby resulting in the activation of mitogen-activated protein kinase (MAPK) cascades in the cytosol, which plays a significant role in the signal transduction pathway (Cho et al. 2011). In turn, this activation of MAPK appears to be involved in the up-regulation of ET, SA, and J Asignaling pathways, which bring about gene expression changes. Castagna and Ranieri (2009) observed that ET and SA enhance lesion development in plants and ultimately cause cell death. Cellular death causes the production of certain products from lipid peroxidation, serving as substrates for JA synthesis, which further decreases the production of ET-dependent ROS and, ultimately, the spread of cell death (Kangasjärvi et al. 2005).

When O_3 enters into the leaf through the stomata, it serves as the first line of a control restricting O_3 flux via control mechanisms of the stomatal aperture. Thus, O_3 flux in the apoplastic region is largely determined via uptake by the stomatal aperture present on the leaves' surface; although, the mechanism through which O_3 influences stomatal conductance is still not clear. However, several studies have postulated activation of the O_3-induced abscisic acid signaling pathway and the outburst of ROS directly in guard cells (Kangasjärvi et al. 2005). ROS lead to cellular membrane damage and cause detrimental effects on the normal functioning of the cells. Plants have developed several mechanisms to use non-enzymatic and enzymatic antioxidants present in different cellular compartments to cope with the oxidative injury caused by O_3 stress (Singh et al. 2014). Studies by Moldau et al. (2011) suggest that ROS directly control stomatal movement as seen within minutes of O_3 flux into the leaves limiting its further uptake. The ROS-activated Ca^{2+}channel with a concomitant increase in the cytosolic Ca^{2+}, in turn, activates Ca^{2+} dependent protein kinases (CPKs). It activates protein kinase OPEN STOMATA 1 (OST1), which in turn activates several other ion channels in guard cells of which SLOW ANION CHANNEL 1 (SLAC 1) plays a significant role in stomatal closure (Vainonen and Kangasjärvi 2015). The MAPK cascade modifies stomatal function in response to H_2O_2. In *Nicotiana tabacum*, NtMPK4-silenced plants with impaired stomatal closure regulation showed enhanced sensitivity to O_3 (Gomi et al. 2005). While in *N. attenuate*, silencing of the NaMPK4 gene impaired stomatal closure induced by the exogenous application of H_2O_2 (Hettenhausen et al. 2012). Also, loss of function of MEKK1 resulted in H_2O_2 accumulation in stomata (Ichimura et al. 2006), suggesting a role of MEKK1 in regulating ROS homeostasis in stomata. Thus, there lies a close connection between O_3-mediated ROS and the fine-tuned signaling cascades regulating stomatal closure (Fig. 9.1).

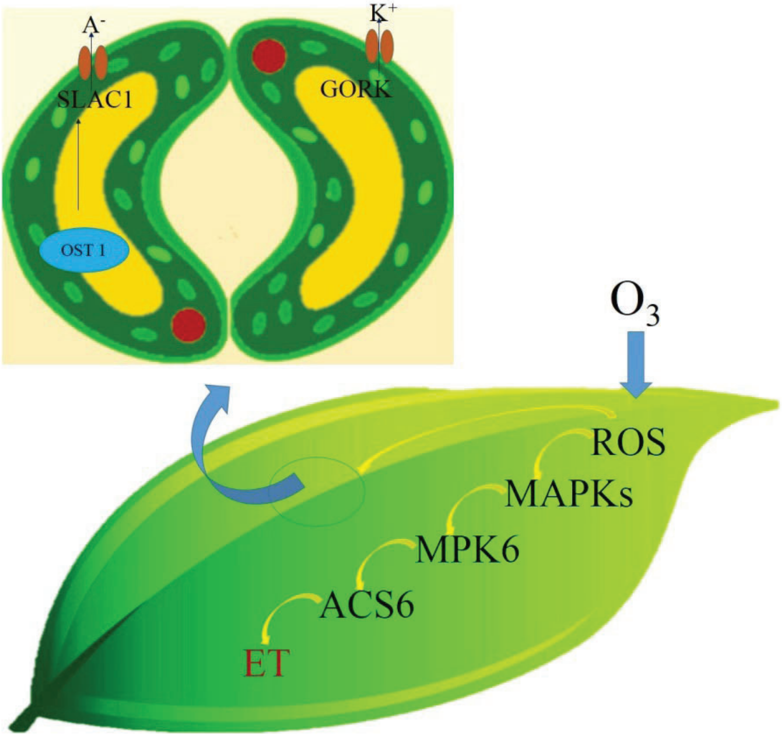

Fig. 9.1. The mechanism of the reactive oxygen species (ROS)-mediated closure of stomata and the simultaneous emission of plant hormone ethylene (ET) in response to O_3 stress. ROS activates protein kinase OPEN STOMATA 1(OST1), which controls the activity of the outward rectifying anion channelSLOW ANION CHANNEL 1 (SLAC1) along with another outward rectifying **K**$^+$ channel in guard cells (GORK) leading to stomatal closure. Also, ROS accumulation in the cytosol alters the redox balance in the cell, leading to activation of MAPKs, MPK6 resulting in the phosphorylation and stabilization of ACS6 in the cytoplasm and an increase in ET biosynthesis

When O_3 enters into the apoplast, it is immediately degraded into secondary ROS or reacts with cellular biomolecules such as protein, lipid, DNA and apoplastic fluid (Mishra et al. 2013). Detoxification of ROS at this site is considered to be the second line of defense (Castagna and Ranieri 2009). A diverse range of antioxidants, such as ascorbate (AsA), glutathione (GSH), and phenolic compounds, noticeably participates in the detoxification process of ROS (Foyer and Noctor 2011). The availability of AsA offers up to 30–50% of detoxification in different species sensitive to O_3 (Turcsanyi et al. 2000) while it has also been correlated with O_3 tolerance in many plant species such as wheat (Fatima et al. 2018), soybean (Roth et al. 2017) and rice (Frei et al. 2012). Besides, sucrose, RFOs (raffinose family oligosaccharides), fructans, and some lipophilic antioxidants, such as α-tocopherol or carotenoids, also contribute to antioxidant defense by directly quenching ROS in different organelles or organelle membranes, respectively (Das and Roychoudhury 2014). Other antioxidants, GSH, and enzymes of the Asada-Halliwell pathway, also play a major role in redox signaling (Foyer and Noctor 2011). Considering GSH, its apoplastic concentration has been found to be very low (Zechmann 2014), limiting its ability to scavenge ROS or contribute to AsA regeneration. Therefore, apoplastic GSH is expected to be involved in signaling rather than in the detoxification process (Zechmann 2014). The different cell compartments have unequal antioxidant concentrations and redox states (Foyer and Noctor 2016). Moreover, AsA concentration in the apoplast is around 0.2–1.5 mM, approximately 10–30 times lower than the cytosol (Van Hove et al. 2001). The AsA efflux mechanism runs via an anion channel, while the regeneration of AsA in the cytoplasm determines the efficiency of apoplastic detoxification (Smirnoff 2018). Other low molecular weight compounds present in the apoplast, such as phenolic glycosides formed due to the induction of phenylpropanoid pathways, also play an essential role in O_3 defense (Booker et al. 2012). Phenolic compounds in the apoplastic fluid of plants were shown to increase in response to O_3 (Grace 2007). In a study by Cabane et al. (2012), foliar and stem cells' cell wall showed an increase in lignin biosynthesis in a dose-dependent manner under O_3 exposure, suggesting its role in the detoxification process.

Enzymatic antioxidants such as SOD, CAT, APX, and GPX provide efficient detoxifying machinery against ROS production (Mittler 2006), and O_3 tolerance has been markedly associated with the expression of antioxidative enzymes such as SOD and APX (Lee et al. 2007). In the Ascorbate-glutathione cycle, APX is the key enzyme scavenging H_2O_2, while glutathione reductase (GR) is another potential enzyme involved in the antioxidative process in plants (Ashraf 2009). Under stress conditions, a large amount of H_2O_2 is transported and accumulated in the vacuole, acting as a sink for ROS (Koffler et al. 2014). The vacuoles are well replenished with polyphenols, especially anthocyanins, flavan-3-ol monomers, proanthocyanidins, and glycosylated flavonols (Dusart et al. 2019). AsA also contributes to vacuolar ROS scavenging, either directly or by coupling with phenolics and phenol oxidases. In contrast, vacuolar flavonols have been suggested to contribute to H_2O_2 detoxification by giving electronsto phenol peroxidases (Dusart et al. 2019). Furthermore, it has been shown that vacuolar AsA can reduce phenoxy radicals for the regeneration of phenolic compounds (Takahama and Oniki 1997). Therefore, an alternative AsA/phenolics/POD mechanism also efficiently contributes to cellular defense against ROS.

9.3 Sensing of ROS in the apoplast

ROS accumulated in the apoplast stimulate both intra- and extra-cellular signal transduction pathways. They change the cellular redox homeostasis, such as perception by apoplastic proteins. They also cause oxidative damage to proteins and disturb the transportation of H_2O_2 via aquaporins into the cytosol (Foyer and Noctor 2005; Kangasjärvi et al. 2005; Dynowski et al. 2008) and create a network of O_3 triggered signaling responses. ROS may even change the activity/conformation of some apoplastic proteins via redox-dependent post-translational modifications, and a number of cysteine-rich receptor-like-kinases (RLK) present at the plasma membrane are activated, which serve as apoplastic ROS sensors in response to elevated O_3 (Wrzaczek et al. 2010). ROS burst also involves extracellular POD and membrane-bound NADPH oxidases, also known as RESPIRATORY BURST OXIDASE HOMOLOGS (RBOH) that produce H_2O_2 (O'Brei et al. 2012) and $O_2^{\cdot -}$ (Foreman et al. 2003), respectively. The signal from apoplastic ROS is also relayed to the chloroplastvia heteromeric G protein within a short time after O_3 exposure (Joo et al. 2005). Thus, chloroplast derived ROS production plays various roles in plant immune responses, activation of defense-related genes, accumulation of SA (Maruta et al. 2012), down-regulation of photosynthesis-related genes (Brosche et al. 2012), and processes directly related to cell death (Liu et al. 2007).

The entry of H_2O_2 and oxidized dehydroascorbate (DHA) into the cytosol alters the cell's redox balance, which is sensed through redox signaling transcription factors (TFs). This altered redox balance and Ca^{2+} influx activate MAPK results in altered gene expression involved in hormone biosynthesis, antioxidant metabolism, increased respiration rates, and decreased photosynthesis (Ainsworth et al. 2017). The chloroplast signal is transmitted to the nucleus by several retrograde signaling pathways leading to changes in gene expression (Estavillo et al. 2013). Figure 9.2 depicts the molecular mechanism of O_3-generated ROS production and signaling pathways in a plant cell.

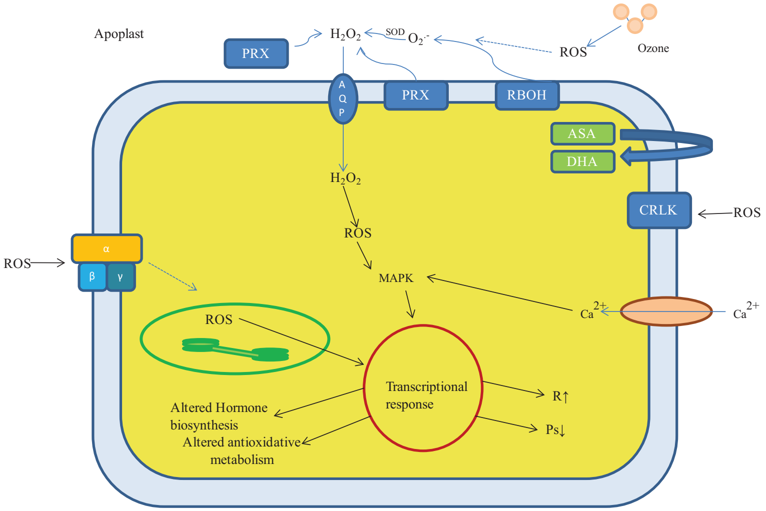

Fig. 9.2. A diagrammatic representation of O_3-induced reactive oxygen species' (ROS) production and signaling cascades in a plant cell. Here, AQP, PRX, RBOH, CRLK, ASA, DHA, SOD, and MAPK are plasma membrane aquaporins through which H_2O_2(hydrogen peroxide) enters inside the cell, extracellular and membrane-bound peroxidases, plasma membrane-bound NADPH oxidases, apoplastic protein, i.e., cysteine-rich receptor-like kinases, ascorbic acid, dehydroascorbate, superoxide dismutase, and mitogen-activated protein kinases, respectively. Heteromeric G-protein (α, β, and γ) also induces ROS burst in the chloroplast. Calcium influx mediated activation of MAPK ultimately activates a transcriptional response, leading to altered hormone biosynthesis, altered antioxidative metabolism, increased respiration (R), and decreased photosynthetic rates (Ps)

9.4 MAPK signaling and ethylene biosynthesis

MAPK signaling cascades are activated in response to a variety of abiotic and biotic stresses (Rodriguez et al. 2010). A MAPK signaling module comprises three protein kinases phosphorylating and activating each other; a MAPK kinase kinase (MEKK) activates MAPK kinase (MKK), which in turn phosphorylates and activates MAPK (MPK). MPKs phosphorylate a wide range of substrates from metabolic enzymes to transcription factors; therefore, MPKs' activity must be tightly controlled for a specific response such as phosphorylation and stabilization of ACS2/6 in the cytoplasm for ethylene biosynthesis. In a study, translocation of MPK3/6 to the nucleus leads to phosphorylation and activation of target TFs as the down-regulation of MKK5 and MPK3 in

Arabidopsis ecotype Ws-0 was associated with O_3 sensitivity (Mahalingam et al. 2006). In contrast, Ahlfors et al. (2004) found rapid activation in MAPKs, MPK3, and MPK6 in response to O_3 within 0.5 to 2 h of fumigation. Ozone exposure also leads to the induced expression of ethylene biosynthesis-related genes, including ASCs (1-aminocyclopropane 1-carboxylate synthase), ACOs (ACC oxidase), and SAM (S-ADENOSYLMETHIONINE SYNTHETASE) in different plant species (Kangasjärvi et al.2004). Even sensitivity to O_3 was possibly associated with genotype differences in the regulation of ET emission in soybean genotypes (Chutteang et al. 2015). In signaling pathways of the plant defense system, MAPK cascades play a pivotal role (Pedley and Martin 2005).

Activation of MAPKs by O_3 has been reported in tobacco (Samuel and Ellis 2002), rice (Kim et al. 2003), and Arabidopsis (Ahlfors et al. 2004). Some WRKY transcription factors (so named because of the WRKYGQK heptapeptide sequence at the N-terminal end, widely distributed in plants) are regulated by MAPK at both the transcriptional and post-transcriptional level defense-related signaling pathways and also positively or negatively regulate plant defense responses (Ishihama and Yoshioka 2012). WRKY TFs are a large family of regulatory proteins in plants, classified into three groups based on the number of WRKY domains and the features of the associated zinc finger-like motif (Eulgem et al. 2000). Most WRKY proteins play key roles in plant tolerance to both biotic and abiotic stresses while inducing genes involvedin stress perception, signal transduction, and also in redox regulation (Khan et al. 2018). However, very limited information is available about the role of WRKY proteins in plant responses to O_3 stress. These are involved in several developmental and physiological processes of plants and regulate several responses pertaining to signal perception/ transduction, redox regulation, and even secondary metabolism (Rushton et al. 2010). In a study by Li et al. (2015), isolated WRKY genes from *Salvia multiorrhiza* were induced differentially under O_3 exposure. WRKY53, a member of Group III b of TFs, is likely to be involved in plants' defense responses to stress as Besseau et al. (2012) used H_2O_2 and O_3 as elicitors and a positive modulation was observed. WRKY33 contains the SP cluster, the phosphorylation of which by MPK3/MPK6 is required to induce ACS2 (1-AMINO-CYCLOPROPANE-1-CARBOXYLIC-ACID SYNTHASE2) in ET biosynthesis (Li et al. 2012).

9.5 Ozone stress and secondary plant metabolism

The secondary metabolic pathway in any plant defense system plays an important role in the production of various phenolic compounds. A study by Guidi et al. (2005) has reported the up- or down-regulation of enzymes of secondary metabolism or their products under O_3 stress, while Paolacci et al. (2001) observed stimulation in the phenylpropanoid route and flavonoid branch by the hike in transcript accumulation of PAL (phenylalanine ammonia-lyase), CHS (chalcone synthase), and CHI (chalcone-flavanone isomerase) under O_3 exposure. Jasmonic and salicylic acids are also important components of plant signaling pathways and are often up-regulated following O_3 exposure leading to the instigation of defense responses (Zadra et al. 2006). Along with the antioxidative pathway, another metabolic pathway, such asthe phenylpropanoid pathway, also helps in the detoxification process in plants.

Ozone exposure results in the accumulation of phenolic compounds in plant cells, and many of these metabolites are formed via the phenylpropanoid pathway. The first enzyme of this metabolic pathway i.e., PAL has been shown to be up-regulated at the levels of transcription as well as an enzymatic activity under O_3 stress. In wheat cultivars, a rise in PAL activity under O_3 stress was correlated with phenolics' accumulation (Koch et al. 1998). Phenolic compounds such as flavonoids and anthocyanins are potential non-enzymatic antioxidants in plant defense systems again stoxidative stress (Booker et al. 2012). The rise in AsA could also act as a cofactor for the biosynthesis of polyphenols and flavonoids (Pellegrini et al. 2018). Some studies have reported the induction of phenylpropanoid and flavonoid pathway genes along with the stimulation of key enzymes of the phenolic pathway (PAL and CAD) under O_3 exposure (Booker et al. 2012; Guidi et al. 2005). Decreased CAD activity was also reported by Guidi et al. (2005) in resistant and sensitive genotypes of *Lycopersicum esculentum* under O_3 exposure.

9.6 Mechanism of O_3 tolerance in crop plants

In a study conducted by Moura et al. (2018), sugarcane genotypes had an effective antioxidative system to combat O_3 stress by elevating both APX and CAT activities against accumulated leaf concentrations of H_2O_2. Caregnato et al. (2013), using two different varieties of *Phaseolus vulgaris* L. revealed increased AsA and CAT protein concentrations after O_3 exposure, indicating their better cellular capacity against O_3 stress to reduce H_2O_2. Rai and Agrawal (2008) found a cultivar dependent response in rice whereas a more significant induction in the defense system was in NDR 97 (tolerant to O_3 stress) compared to Saurabh 950via increased SOD, POD, AsA, and phenol along with the lesser reduction in photosynthetic pigments and proteins. Increased enzymes (CAT, POD, APX and GR) and AsA and total phenolics were also observed by Mishra et al. (2013) in wheat cultivars. Tiwari and Agrawal (2011) found higher constitutive levels and higher increments of AsA and phenol in brinjal compared to radish, triggering better protective mechanisms in former species due to a greater increase in antioxidants under O_3 stress. Apart from these, Herbinger et al. (2002) showed that in two different cultivars of wheat, one drought resistant and the other sensitive to it, namely, *Triticumaestivum* L. cv. Nandu and *Triticum durum* Desf. cv. Extradur, respectively, O_3 stress did not show an additive effect and drought stress and, thus, did not alter antioxidants' concentration in these cultivars. The results of Wang et al. (2014) also showed a decrease in enzymatic antioxidants (SOD, POD, and APX) at different growth stages of wheat cultivars (Y16 and Y19) under continuous O_3 exposure.

Several studies have reported transcriptomic responses of plants to O_3. The gene expression pattern induced due to O_3 exposure has been an essential aspect of recent studies. Frei et al. (2010) evaluated the potential tolerance mechanism in rice crops by investigating the alteration in gene expressions induced due to O_3 exposure. The study documented strong expression of gene encoding antioxidant enzymes (CAT and POX) in O_3-sensitive genotype SL15. On the contrary, another gene encoding ascorbate oxidase was identified with lower expression in O_3-tolerant genotype SL41, suggesting its involvement in enhancing AsA status under O_3 exposure. Many genes involved in programmed cell death (ET or JA biosynthesis, MAPK signaling, and detoxification of ROS, CAT and POX) were also identified as O_3-responsive genes in a comparative microarray study using two chromosome segment substitution rice lines (Frei et al. 2010).

More recent studies employed high-throughput sequencing techniques (RNA-Seq) to monitor transcriptomic responses to O_3. This led to the identification of O_3-responsive genes and pathways associated with photosynthesis and respiration, oxidative stress, defense response, protein ubiquitination and organic acid biosynthesis in soybean (Waldeck et al. 2017), cellular integrity and metabolism (ascorbate-glutathione metabolism, hormone, wax and cutinbiosynthesis) in pak choi (Zhang et al. 2017) and antioxidant metabolism (ascorbate-glutathione cycle genes) in legume crops (Yendrek et al. 2015). Also, Marchicha et al. (2018) reported that O_3-induced oxidative stress triggered the activation of

WRKY TFs, which operated as positive or negative regulators of gene expression.

Cho et al. (2008) used 2-week-old rice (cv. Nipponbare) seedlings for exposure to O_3 (0.2 ppm for 24 h) to investigate the molecular responses of O_3 using several integrated approaches at the level of transcripts, proteins, and metabolites. Altered expression was shown by 1535 non-redundant genes, representing eight main functional categories, while genes involved in information storage and processing/transcription factors (10%) and cellular processing and signaling/signal transduction (24%) were highly present within 1h of O_3 treatment. Besides, the genes categorized into metabolism (18, 19%), information storage and processing (17, 23%) and cellular processing and signaling (20, 16%) were mainly regulated at 12 and 24 h; further, their main subcategories were secondary metabolites' biosynthesis, ribosomal protein, post-translational modification, and signal transduction, respectively.

2-D gel electrophoresis-based proteomics' analyses combined with a tandem mass spectrometer (MS) identified 23 differentially expressed protein spots with 21 non-redundant proteins in leaves exposed to O_3 for 24 h compared to respective control. Proteins identified were found to be majorly involved in cellular processing and signaling, followed by photosynthesis and defense mechanisms (14%). Capillary electrophoresis-MS-based metabolomic profiling revealed the accumulation of GSH, amino acids, andγ-aminobutyric acid in O_3 exposed leaves until 24 h compared to control. Thestudy showed O_3-triggered chain reactions of the altered gene, protein, and metabolite expressions involved in several rice cellular processes. Another survey by Tammam et al. (2018) revealed that 75 ppb of O_3 concentration up-regulated the genes of antioxidant activity of two rice cultivars (Sakha 101 and Giza 178), with concomitant increases in SOD and APX activities. In wheat, the O_3 tolerance mechanism was also exhibited by the up-regulation of genes of the antioxidative pathway and JA pathway (Pazarlar et al. 2017). Besides, non-enzymatic antioxidants such as AsA and thiols and enzymatic antioxidants such as APX and GR marked O_3 tolerance in different cultivars of wheat (Fatima et al. 2019; Yadav et al. 2019). Besides, Yadav et al. (2019) even showed that early sown wheat cultivars were more sensitive to O_3 stress marked by higher energy cost to produce non-enzymatic antioxidants.

In maize cultivars (HQPM 1 and DHM 117), Singh et al. (2014) reported increased activities of enzymes such as SOD, CAT, POX, APX, and GPX using NATIVE-PAGE analyses along with higher induction of secondary metabolites such as total phenol, flavonoids, and anthocyanin under O_3 stress. Also, Pandey et al. (2018) reported increased activities of antioxidative enzymes such as SOD, CAT, AOX, GR, and POD along with AsA in wheat cultivar HD 2976, marking its tolerance towards elevated O_3 stress. Contrary to this, Mikkelson et al. (2015) showed a higher accumulation of flavonoids and phenylpropanoids, such as feruloylquinic acid in barley under O_3 stress.

The physiological and biochemical mechanisms underlying plant responses to O_3 stress have been investigated by Zhang et al. (2017). The transcriptomic analysis of pakchoi (*Brassica campestris* ssp. chinensis) exposed to different acute doses of O_3 (ambient or non-filtered air (31 ppb) and elevated (252 ppb) for 2 days (8 h per day)) revealed a total of 675 differentially expressed genes under elevated O_3, including 219 DEGs with a decline in expressions and 456 DEGs with hiked expressions. Ozone stress stimulatedseveral cellular defense pathways, including phenylpropanoid biosynthesis, glutathione metabolism, sulphur metabolism, glucosinolate biosynthesis, cutin, suberin and wax biosynthesis and also transcription factors such as AP2-ERF, WRKY, JAZ, and MYB. This study provided a key insight into the molecular responses of pakchoi whereby the specific DEGs could be further used for the functional identification of essential allelic genes determining the O_3 sensitivity in pakchoi.

Roth et al. (2017) found that soybean genotypes had a general trend of Fiskeby III containing more ascorbic acid than Mandarin (Ottawa), regardless of treatment; therefore, O_3 seems to induce glutathione synthesis only in Mandarin. While Whaley et al. (2015), using RNA-seq analysis revealed genetic response and tolerance mechanisms under O_3 exposure in soybean cultivars (*Glycine max*) comparing an O_3-intolerant cultivar (Mandarin-Ottawa) and an O_3-resistant cultivar (Fiskeby III). Transcriptome analysis revealed cultivar-dependent expression of different genes as oxidative stress responses, where both Fiskeby III and Mandarin exhibited an increased expression of oxidative response genes as well asa rise in glutathiones, phenylpropanoids, and phenylalanine ammonia-lyases. Cultivar Mandarin showed more general stress response genes, whereas Fiskeby III had increased expression of metabolic process genes. However, Ahmad et al. (2015) observed that O_3 induced the up-regulation of the CC14 gene by three to fourfold and the overexpression of cysteine proteases in maize leaves after 20 d treatment, ultimately resulting in causing protein degradation and programmed cell death. Sarkar et al. (2010), with wheat cultivars, revealed a decrease in RUBISCO subunits using 1DGE analysis and induced accumulation of antioxidative enzymes such as SOD and APX and defense-related thaumatin-like protein using the Western blot technique while 2DGE revealed 38 differentially expressed protein spots common in both cultivars. This study further stressed the need for research work on the molecular network operating under O_3 stress, linking an increased antioxidant level and some stress-related proteins. Moreover, further study of Sarkar et al. (2015) showed increased SOD activities, CAT, POX, APX, and GR, along with induction in AsA, thiol, and total phenolics. Besides, 2DGE analyses also revealed 25 differential spots in both the cultivars with a reduction in RUBISCO subunits under O_3 stress. Studies made by Ghosh et al. (2020 a; 2020b) on *Triticum aestivum* cv. HD 2967showed that under wheat-weed interaction supplicated with O_3 stress, the weed (*Chenopodium album* L.) showed greater antioxidative potential with hikes in enzymes CAT, POX, GR, SOD and non-enzymatic antioxidants such as AsA, thiols and total phenolics. Another study conducted by the same group showed the additive effect of water stress and O_3, where, under the water deficit condition and elevated O_3, there was a more significant increase in antioxidative enzymes. A list of crop plants with the biochemical or molecular mechanisms of O_3 tolerance operating in them has been given in Table 9.1.

Table 9.1: Ozone tolerance mechanisms in different crop plants under acute or chronic O_3 stress. Here, ↑ and ↓ are increase or decrease, respectively, while APX, POD, CAT, SOD, GR, GSH, and AsA are ascorbate peroxidase, peroxidase, catalase, superoxide dismutase, glutathione reductase, glutathione, and ascorbic acid, respectively

Crop	O_3 dose	Response (biochemical/molecular)	Reference
Oryza sativa L. cv. Nipponbare	0.2 ppm for 24 h	↑ gene expression mainly of cellular processing, signaling, and metabolism	Cho et al. (2008)
Oryza sativa L. cv. Koshihikari	40, 60 and 120 ppb for 6 h d^{-1},9 days	↑antioxidants protein glutathione S-transferase, Mn-superoxide dismutase defense/stress-related proteins, pathogenesis-related (PR) class 5 protein (PR5), and two PR10 proteins OsPR10/PBZ1 and RSOsPR10	Feng et al.(2008)
Oryza sativa L. cvs. Saurabh 950 and NDR 97	Ambient mean varying between 23.4 and 45.4 ppb, 123 days	↑ SOD, POD, AsA, and phenol	Rai and Agrawal (2008)
Oryza sativa cvs. MalviyaDhan 36 and Shivani	10 and 20 ppb, 5 h d^{-1}, 120 days	Differential protein profile in NATIVE and SDS-PAGE	Sarkar and Agrawal (2010)
Oryza sativa L. cv. Nipponbare	120 nll^{-1}, 7h d^{-1}, 13 days	↑ gene expression of CAT, POD, and AsA oxidase ↓Total AsA and reduced AsA concentration	Frei et al.(2010)

Oryza sativa cv.MalviyaDhan 36 and Shivani	Ambient + 10 and 20 ppb for 12h d^{-1}, 120 days	↑activity of SOD, CAT, POX, APX, and GR along with induction in Asa, thiol and total phenolics. Besides, 2DGE analyses also revealed 25 differential spots	Sarkar et al. (2015)
Oryza sativa cvs. Sakha 101, Sakha 103, Sakha 106, Giza 178 and Giza 179	75 $ppbh^{-1}$, 28 days	↑ SOD and APX	Tammam et al.(2018)
Phaseolus vulgaris L. cvs. Fepagro 26 and Irai	122.6 ppb h, 6h d^{-1}, 7 days	↑AsA and CAT protein	Caregnatoet al.(2013)
Raphanus sativa L. var. PusaReshmi and brinjal (*Solanumme longena* L. var. Pusa hybrid-6)	40.8 ppb, 8h d^{-1}, 60 and135 days, respectively	↑AsA and phenol	Tiwari and Agrawal (2011)
Zea mays cvs., HQPM 1 and DHM 117	Ambient +15 and 30 ppb 5h d^{-1}, 150 days	↑SOD, CAT, POX, APX and GPX and ↑*in-gel* activity along with secondary metabolites such as total phenol, flavonoids and anthocyanin	Singh et al. (2014)

Hordeum vulgare cv., Pallas	100 ppb, 21 days	↑Flavonoids and phenylpropanoids	Mikkelson et al. (2015)
Melissa officinalis	200 ppb for 3h	↑shikimate dehydrogenase, phenylalanine ammonia-lyase, and cinnamyl alcohol dehydrogenase activities	Tonelli et al. (2015)
Pisums ativum	151.2 nll^{-1} ± 0.72 nll^{-1} for 8h d^{-1}	↑ GR transcript, apoplasticAsA, and GSH	Yendrek et al.(2015)
Capsicum baccatum	171.6 $\mu g/m^3$, 6h	↑Carotenoids and total phenolics	Bortolin et al. (2016)
Glycine max	27 ± 2 ppb and 74 ± 1 ppb,25 days	↑ expression of oxidative response genes as well as glutathione, phenylpropanoids, and phenylalanine ammonia lyases	Whaley et al.(2015)
Glycine max	110±10 $nmolmol^{-1}$ 9h, 48 days	↑Flavonoids, ↓PAL and PPO, LOX and POD	Zhao et al. (2016)
Glycine max genotypes	65.6 ppb7h d^{-1} for 5 days	↑AsA and GSH	Roth et al.(2017)
Glycine max (L.) Merr	75 ppbfor 5 days	↑genes related to defense, protein folding, steroid biosynthesis, and DNA recombination	Waldeck et al.(2017)

		↓endopeptidases	
Brassica campestris ssp. chinensis	252 ppb for 8h day^{-1}, 2 days	↑ gene expressionglutathione metablosimphenylpropanoid biosynthesis, sulphur metabolism, glucosinolate biosynthesis	Zhang et al.(2017)
Phoenix dactylifera	200 ppb for 1h, 2h, 4h, and 8h	↑ AsA and total phenol	Du et al. (2018)
Saccharums pp. genotypes IACSP94-2094 and IACSP95-5000	49 ± 12 ppb for 45 days	↑ APXand CAT	Moura et al. (2018)
Triticum aestivum L. cv. Nandu and *Triticum durum* Desf. cv. Extradur	Ambient + 50 ppb, 42 days	No additive effect along with drought stress; no change in antioxidant level	Herbinger et al. (2002)
T. aestivum L. cvs. Sonalika and HUW 510	Ambient + 10 and 20 ppb for 5h d^{-1}, 50 days	↑SOD and APX and defense-related thaumatin-like protein using the Western blot technique while 2DGE revealed 38 differentially expressed protein spots common in both cultivars	Sarkar et al. (2010)
T. aestivum L. HUW 37 and K 9107	Ambient+10 ppb 4h d^{-1}, 120 days	↑ activity of enzymes (CAT, POD, APX, and GR) along with AsA and total phenolics	Mishra et al. (2013)
*T. aestivum L.*cvs. Yangmai16 (Y16) and Yannong19 (Y19)	50% higher than ambient, 7 h d^{-1}, 110 days	↓SOD, POD, and APX	Wang et al. (2014)
Triticum aestivum L. cvs.	Ambient +30 ppb, 6 h d^{-1}, 120 days	↑AsA and GSH	

HD2987, PBW 502 and Kharchiya 65			Fatima et al. (2018)
Triticum aestivum L. cvs. HD 2967 and Sonalika	Ambient +20 ppb, 4 h d^{-1}, 150 days	↑SOD, CAT, APX, GR, POD and AsA	Pandey et al. (2018)
Triticum aestivum L. cvs.	Ambient +30 ppb, 6 h d^{-1}, 120 days	↑AsA and thiols	Fatima et al. (2019)
Triticum aestivum L. cvs. HUW 468, HUW 234, HD 3086 and HD 3118	Ambient +20 ppb, 5 h d^{-1}, 150 days	↑GR, APX, AsA, and phenolics	Yadav et al. (2019)
Triticum aestivum L. cv. HD2967 and *Chenopodium album* L. Fat hen	Ambient +20 ppb, 4 h d^{-1}, 150 days	↑SOD and APX	Ghosh et al. (2020a)
Triticum aestivum L. cv. HD2967	Ambient +20 ppb, 4 h d^{-1}, 150 days	↑APX, CAT, SOD, and GR	Ghosh et al. (2020b)

9.7 Conclusion

Ground-level O_3-induced oxidative stress via ROS upsets the whole cellular machinery, including all biochemical and physiological processes affecting plant growth, development, and productivity. Further, ROS act as a signal elicitor causing changes in cellular redox homeostasis, activating the MAPK pathway, alter gene expression, and antioxidant metabolism. This creates a network of O_3-triggered signaling responses. This review reveals the mechanism of O_3 tolerance in different crop plants linked to the variable levels of antioxidants and gene expression. But a complete mechanistic understanding of O_3-induced ROS production, and downstream processes is still lacking. Studies addressing such questions will result in a better understanding of ROS-regulated signaling mechanisms with gains in crop improvement where O_3 tolerance traits can be integrated with high yielding varieties for better crop productivity.

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CHAPTER TEN

ANALYSIS OF TRANSCRIPTOME, PROTEOME, AND METABOLOME APPROACHES TO OVERVIEW THE OZONE RESPONSES IN PLANTS: AN UPDATE

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Abstract

Human activities have led to an increase in tropospheric ozone (O_3) concentration, one of the most prominent air pollutants since the industrial revolution. It is a secondary air pollutant formed by the interaction of primary pollutants in the presence of sunlight. Ozone-generated reactive oxygen species (ROS) lead to oxidative stress in plants, resulting in the triggering of stress perception and signal transduction and the activation of molecular cascades, up- or down-regulation of genes, remodeling of the protein expression pattern, induction of defense-related mechanisms, and altered metabolic processes. Phenotypically these changes at the molecular level manifest in reduced growth and development, foliar injury, and senescence, compromising the plant's productivity. ROS are even known to cause oxidative damage to DNA. Expression analyses have found that the genes involved in signal transduction or processing, the defense pathway and redox homeostasis, hormonal regulation, photosynthesis, respiration, secondary metabolism, or senescence are affected. Alterations in transcription are usually mirrored at the proteome level. The proteins responding to O_3 are principally involved in signaling pathways, stress or defense response, chaperones, protein synthesis, electron transport chains, the photosynthetic carbon reduction cycle, and energy metabolism. The inter-networking between transcript and protein abundance, therefore, regulates the metabolite levels in O_3 stressed plants. Some metabolites such as ascorbic acid, thiols, and those derived from secondary metabolism act as powerful non-enzymatic antioxidants and help in maintaining cellular homeostasis. Considering O_3-induced phytotoxicity, an effort has been made in the present review to summarize the progress about transcriptomics, metabolomics, and protein dynamics of O_3-induced stress in plants. This up-to-date information harvested from integrative biology applications will improve the understanding of molecular factors influencing O_3 susceptibility or tolerance, which could guide breeders/ genetic engineers to develop O_3-tolerant/-resistant genotypes.

Keywords: Gene expression; Metabolites; Ozone; Protein; Signal transduction; Reactive oxygen species

10.1 Introduction

Climate change modules such as an increase in concentrations of tropospheric ozone (O_3), carbon dioxide (CO_2), and ultraviolet-B (UV-B) radiation, episodes of drought, fluctuating soil nutritional dynamics, and salinity are reducing the plant's productivity. Among all these, tropospheric O_3 is of great concern. Many studies from different parts of the world have shown that average concentrations of O_3 in many areas have increased, especially the tropical countries (Singh and Agrawal 2017; Ghosh et al. 2018); these changes may have come from changes in climatic pattern and human-emitted precursors of O_3 (Oltmans et al. 2006). Ozone in the troposphere is a secondary air pollutant produced by photochemical reactions involving various precursors like nitrogen dioxides, volatile organic compounds (VOCs), methane, and carbon monoxide in the presence of high sunlight (Ashmore 2005; Sampedro et al. 2020). Favorable climatological factors such as intense solar radiation, high temperature, stagnant wind, and low humidity favor the O_3 built up in the troposphere (Tiwari et al. 2008). Globally, the mean concentration of tropospheric O_3 has increased from 20–30 ppb to 30–50 ppb over the past one hundred and fifty years due to increased emissions of O_3 precursors (IPCC 2013). Ozone is an issue of concern since it can adversely affect human health and plants (Agathokleous and Saitanis 2020). Globally, O_3 has adverse effects and causes significant losses in crop productivity (Ainsworth 2017; Ghosh et al. 2020). Reports have shown that ambient O_3 concentrations with average 40 ppb concentrations have significantly reduced the yield of major food crops, including rice, wheat, soybean, and potato, by about 10% compared to O_3-filtered air (Feng and Kobayashi 2009). Moreover, being phytotoxic O_3 is known to induce cell death (Kangasjärvi et al. 2005), cause foliar injury (Cho et al. 2012), accelerate leaf senescence (Natali et al. 2018), and have detrimental effects on vegetative growth (Agathokleous et al. 2020) as well as the reproductive development of plants (Leisner and Ainsworth 2012). The underlying mechanism shows that O_3 causes oxidative stress by production of reactive oxygen species (ROS), which further leads to a chain of reactions (Foyer and Noctor 2005), causing significant negative effects on the cellular metabolism of the plants resulting in damage to membrane lipids, proteins, carbohydrates and nucleic acids (Blokhina et al. 2003). Subsequently, a stress response, as sensed by the plants, activates a network of signaling pathways (Komatsu 2008), which later results in the proliferation of molecular cascades involving stress perception, signal transduction, alterations in the gene and protein expression patterns (Hossain et al. 2013), accumulation of stress hormones and activation of defense mechanisms in plants (Overmyer et al. 2005, Nadgorska- Socha et al. 2013, Fatima et al. 2019) to maintain cellular redox homeostasis and therefore counteract the oxidative stress. The effects of O_3 on plants and the related responses have been shown in Figure 10.1. Even some low molecular weight metabolites play an important role as antioxidants to keep the ROS level tuned and prevent the cellular damage caused due to oxidative stress (Mittler 2002; Mittler 2017).

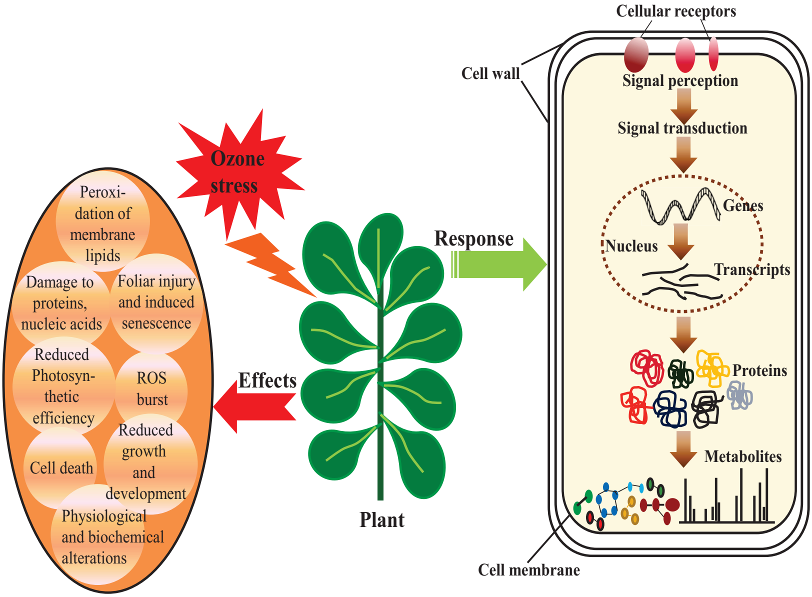

Fig. 10.1. An overview of the effects of O_3 stress on plants and the response at cellular levels. Ozone stress is perceived, and the related signal transduction leads to transcription of O_3 responsive genes. The translation leads to protein synthesis, and various pathways lead to the production of metabolites, which play a role in defense against O_3 stress

The use of high-throughput technology applications such as transcriptomics, proteomics, and metabolomics contributes to an improved understanding of plant biology; the development of various molecular markers to promote the crop improvement programmers through recent technologies and breeding events (Agrawal et al. 2013). The translated portion of the genome plays a vital role in the plant stress response and is the chief functional player. Therefore, proteomic studies deliver us a fine picture of the protein networks and metabolic pathways involved in stress tolerance (Hossain et al. 2013). Agrawal and co-workers (2013) have also indicated that most biological functions in a cell are performed by proteins and metabolites rather than by mRNA levels, and therefore gene expression results may not necessarily always provide information for the description of a biological system; since mRNA levels perhaps may not go in parallel with cognate protein abundance (Hatzimanikatis and Lee 1999; Kosová et al. 2011). Consequently, systems biology not only studies a particular phenomenon but investigates a broader view, which includes all of the elements of the biological system (Renaut et al. 2009). Several OMICS (transcriptomics, proteomics, and metabolomics) tools and techniques have been utilized to investigate the impact of O_3 stress on plants. Hence, the present compilation focuses on the survey and understands the O_3-induced signal transduction, changes at the transcriptome, proteome, and metabolome (particularly non-enzymatic metabolites) levels to analyze stress-responsive adaptations, pathways, and events at the molecular level in O_3 stressed plants. An integrated analysis of the transcriptome, proteome, and metabolome changes under O_3 stress can be a considerable contribution to unraveling the phenomenon underlying stress acclimation, screening stress-associated biomarkers, and depicting key pathways underlying plant tolerance to oxidative stress. This knowledge will help to guide agricultural breeders and biotechnologists to explore different mechanisms for plant resistance to oxidative stress and therefore fabricate O_3-tolerant/-resistant genotypes that could either adapt ina high O_3 environment or withstand resistance to O_3 stress.

10.2 Ozone-induced ROS production and the related signal transduction

Ozone enters the leaves through stomatal pores, governed mainly by stomatal conductance (Ainsworth et al. 2012). After their uptake in plants, breakdown of O_3 in aqueous conditions or by the reaction with cellular constituents leads to the generation of ROS such as superoxide (O_2^-) and hydroxyl ($^{\cdot}OH$) radical and hydrogen peroxide (H_2O_2) (Rao et al. 2000a). These ROS produced in plants can be cytotoxic at high concentrations while they act as key signaling molecules at lower concentrations (Gechev et al. 2006; Mahalingam and Fedoroff 2003; Marchica et al. 2019). ROS further lead to a chain of reactions, which causes substantial effects on plants' cellular metabolism. Different ROS receptors or sensors sense an induced production of ROS. H_2O_2 sensing in some receptors is also linked to the activation or induction of Ca^{2+}/calmodulin kinase since Ca^{2+} channel blockers can inhibit O_3 or H_2O_2 generated SIPK (salicylate-induced protein kinase) activation in tobacco (Cristina et al. 2010). For instance, OXI1 (OXIDATIVE SIGNAL-INDUCIBLE1) is a protein kinase that is essential for oxidative burst-mediated signaling in *Arabidopsis* (Rental et al. 2004), which is activated by Ca^{2+} signals (Pang and Wang 2010). While tobacco plants were treated with O_3 for a brief period, MAPK activation was observed through an in-gel kinase assay, and strong MAPK activation was seen within minutes of O_3 exposure (Samuel et al. 2000). Moreover, suppression of SIPK leads to a hypersensitive response in tobacco plants towards O_3 stress (Samuel et al. 2005). The receptors then activate a MAP kinase (MAPK) cascade, which plays vital roles in plant signal transduction pathways (Fig. 10.2). The MAPK cascades are major downstream components of sensors or the receptors that transduce extracellular stimuli in plant intracellular responses (Tena et al. 2001; Zhang and Klessig 2001). The general assembly of this signaling unit consists of a MAP kinase kinasekinase (MAPKKK) that phosphorylates a MAP kinase kinase (MAPKK) that sequentially phosphorylates a MAP kinase (MAPK) (Fig. 10.2; Kangasjärvi et al. 2005). This activation of MAP kinases generally leads to nuclear localization and activation of transcription factors, and effector proteins, kinases, transporters, enzymes, etc., bymodifying their activities through phosphorylation (Mittler 2002; Morris 2001; Neill et al. 2002; Cristina et al. 2010). The transcription factors then take part in the expression of genes concerned with defense-related mechanisms and redox control, including regulation of different antioxidative enzymes, primary or secondary metabolic pathways, heat shock proteins, and programmed cell death (PCD), which resulted in metabolic adjustments and biochemical alterations to maintain the redox homeostasis (Mittler 2002; Heath 2008; Pang and Wang 2010).

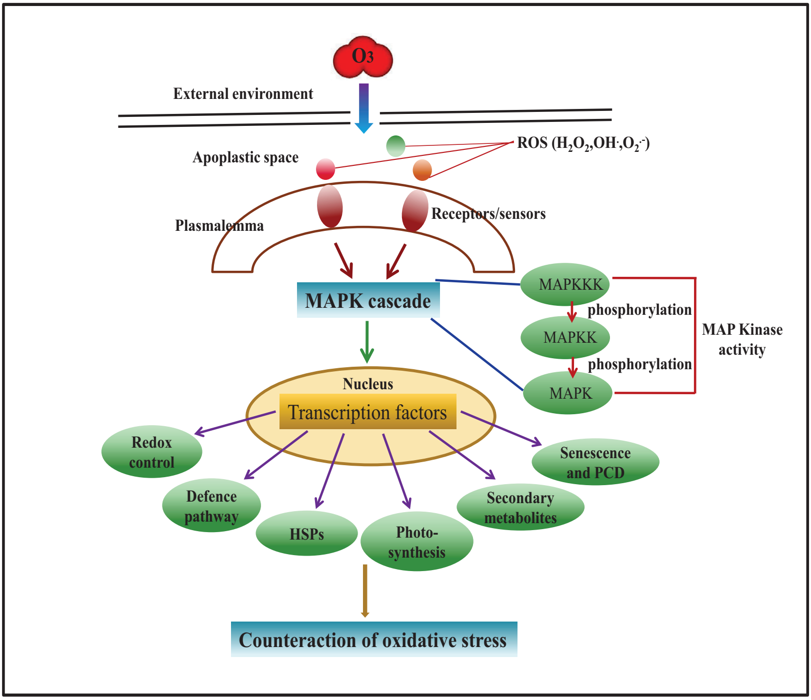

Fig. 10.2 A proposed representation for MAP kinase-mediated activation of ROS-induced signal transduction events during oxidative stress caused due to an O_3 encounter (Figure adapted from Mittler 2002)

Besides, activation of MAPK marks the up-regulation of plant hormones, such as ethylene (ET) synthesis in consort with salicylic acid (SA) (Castagna and Ranieri 2009); or the complex relations of ROS–plant hormone interactions, in which the ROS themselves may act as secondary messengers, point to the upstream reactions encompassing ROS that lead to the participation of phytohormones (Vranova et al. 2002). Ethylene biosynthesis by the induction of genes transcribing 1-aminocyclopropane-1-carboxylate synthase (ACS6) is one of the fastest responses to O_3 exposure (Kangasjärvi et al. 2005). Activation of ET synthesis by the induction of genes encoding for 1-aminocyclopropane-1-carboxylic acid synthase (ACS) found a 2.2-fold increase in ACS6 and a 3.9-fold increase in ACS2 expression. In contrast, isochorismate synthase (ICS), a precursor of SA production, recorded a 6.7-fold increase after 3 h of O_3 fumigation in *Arabidopsis* (Col-0) exposed to an O_3 concentration of 300 ppb (Tosti et al. 2006). A remarkable increase in the concentration of ET was observed in the O_3-sensitive cultivar of tobacco (Bel W3) upon exposure to O_3 (Ederli et al. 2006). When accumulated in high levels in O_3-exposed plants, phytohormones ethylene and salicylic acid are responsible for forming O_3-induced lesions on a leaf and even programmed cell death (PCD) (Overmyer et al. 2000; Overmyer et al. 2005; Yoshida et al. 2009). However, Tamaokiand associates (2003), in a transcriptome analysis of O_3-induced *Arabidopsis* mutants, found that ET produced in low levels could stimulate the expression of defense-related genes rather than cause PCD. Moreover, O_3-induced SA seems to have a dual function that depends on the level of its production (Tamaoki et al. 2003).

When there is an occurrence of cell death, specific lipid peroxidation products function as substrates for the synthesis of jasmonic acid (JA). Ozone elicits the JA synthesis via the modulation of expressions of genes involved in the process, such as lipoxygenases, allene oxide synthase, and 12-oxo-phytodienoate reductases (Rao et al. 2000b; Tosti et al. 2006). Furthermore, five genes linked to the JA pathway were also up-regulated when *Arabidopsis thaliana* of Col-0 ecotype was fumigated with an O_3 concentration of 300 ppb (Tosti et al. 2006). The action of JA is antagonistic, and therefore, it reduces ethylene-dependent ROS production and the spread of cell death (Kangasjärvi et al. 2005). For instance, O_3-sensitive ecotype, Cvi-0, which accumulates a large amount of SA, shows JA's low activity, signifying that the SA accumulation as a reaction to O_3 exposure is negatively regulated by JA signaling (Rao et al. 2000b). JA has also been shown to regulate the activation of genes encoding the enzymes involved in systemically induced defense responses (Farmer and Ryan 1992). A potential role of JA in defense was seen in O_3-sensitive tobacco Bel W3 showing that JA application before O_3 exposure reduced O_3-induced necrotic lesion formation and the level of H_2O_2 (Orvar et al. 1997). Besides, ROS also mediate abscisic acid (ABA)-induced stomatal closure responses. Apel and Hirt (2004) found that in guard cells of *Arabidopsis*, ROS stimulated ABA synthesis and induced stomatal closure via activation of plasma-membrane-locatedcalcium channels. An ET-dependent decline in stomatal sensitivity to ABA has also been documented by Wilkinson et al. (2010). Thus, there is a signaling network where plant hormones ET, SA, JA, and ABA play a central role. These phytohormones induce most of the O_3-responsive genes, and the equilibrium between them regulates the degree of O_3 sensitivity to the plants and related lesion formation or cell death (Kangasjärvi et al. 2005).

10.3 Impact of ozone on DNA and genome stability

Among the biomolecules which are negatively affected by O_3, is the genetic material DNA. There are various reports on O_3-induced DNA damage using different animals as models (Cheng et al. 2003; Labuschagne 2007), but very few studies have addressed the mutagenic effect of O_3 on plants (Aras et al. 2010; Tripathi et al. 2011; Vardar et al. 2014; Sarkar et al. 2015). Previously, ROS has been a major cause of oxidative damage to DNA (Bray and West 2005; Labuschagne 2007). Oxidative attack on DNA generates transformed bases and damaged sugar residues that undergo fragmentation and lead to DNA strand breakage. Piraino and associates (2006) have concluded in their investigation on *Trifolium repens* using AFLP markers that elevated O_3 concentrations present in mixed air pollutants in Novara Province of Italy during the study period might have contributed to DNA damage in tested plants. A RAPD analysis was performed by Sarkar et al. (2015) which reported that genome template stability varied from 100% in filtered chambers to 66.1% and 50.8% in rice cultivar Malviya dhan 36 and 70% and 55% in cultivar Shivani under two treatments of elevated O_3 (ambient+10 and 20 ppb), respectively. Different polymorphic bands were detected for each primer, perhaps due to the loss and gain of PCR fragments. In another study, Tripathi et al. (2011) analyzed the response of *Linum usitatissimum* (linseed) plants under elevated O_3 stress through the RAPD technique. Results showed that a 10 ppb increase over ambient O_3 concentration can cause approximately 50% of damage to linseed plants' genome stability. The unique band index (UBI) was calculated, which was 45.3% under elevated O_3 treatment. According to Liu et al. (2005), a plausible cause of bands' disappearance and appearance is genomic rearrangement, which resulted in a change of priming sites, strand breaks, and DNA photo products (pyrimidine dimers and 6, 4 photoproducts). These changes obstruct the normal polymerization of DNA in the PCR reaction. Another cause of DNA damage under O_3 exposure is the formation of DNA adducts, which are free radical damage products and this is a common event during O_3 exposure. Furthermore, alteration of DNA binding sites by O_3 adducts may influence mRNA transcription due to altered binding by DNA-binding proteins (Steinberg et al. 1990).

10.4 Ozone effects on expression of genes

For the past few decades, there has been a marked escalation in applying genome-level gene-expression profiling tools to explore systems biology. Variations in mRNA levels are generally due to the alterations in the transcriptional rate of genes (Komatsu 2008). A range of high-throughput molecular techniques such as microarray, expression of genes in real-time analyses, and the latest next-generation sequencing (NGS) is now progressively used to identify and characterize the genes regulating key processes and traits in the plant under normal and stressed conditions. In response to O_3, genes encoding the enzymes or proteins associated with cell wall metabolism, glycolysis, the citric acid cycle, sucrose biosynthesis, lipid, amino acid and protein turnover, defense reactions and signaling pathways, hormonal regulation, photosynthesis, photorespiration, the phenylpropanoid pathway, the shikimate pathway, senescence and PCD are reported to be significantly affected (Paolacci et al. 2001; Gupta et al. 2005; Janzik et al. 2005; D'Haese et al. 2006; Lee and Yun 2006; Li et al. 2006; Mahalingam et al. 2006; Tosti et al. 2006; Cho et al. 2008; Ludwikow and Sadowski 2008; Short et al. 2012; Tammam et al. 2019). Moreover, responses of specific genes to O_3 stress extensively overlap with those activated by other abiotic and biotic stresses, and they even share common transcriptional regulation (Lee and Yun 2006).

Several investigations have been performed with the model plant *Arabidopsis thaliana* concerning its O_3 responses. *Arabidopsis* treated with 150 ppb O_3 for six h d^{-1} for 14 days stimulated eight senescence-related genes, including *SAG18*, *SAG20*, and *SAG21* indicating that the acceleration of leaf senescence induced by O_3 is a regulated event comprising various genes associated with natural senescence (Miller et al. 1999). Different ecotypes of *Arabidopsis thaliana* Columbia-0 (Col-0), Wassilewskija (WS), Cape Verde Islands (Cvi-0), and a closely related, *Thellungiella halophile* (Th), were exposed to 20–25% above the ambient levels of O_3 in a free air concentration enrichment (FACE) experiment. In ambient air Col-0 comparatively showed higher expression levels of receptor-like kinases (RLKs), photosynthesis-related genes, and chaperones than Cvi-0 (Li et al. 2006). However, WS exhibited O_3-mediated reductions in the expression of genes associated with cell wall growth and alterations in mRNA levels of marker genes for programmed cell death. Furthermore, the authors talked about the diversity across four *Arabidopsis* ecotypes in relation to O_3 responses based on the differences in signaling and transcriptional response networks due to O_3 exposure (Li et al. 2006). The tolerant *Arabidopsis* genotype Col-0 was fumigated with an O_3 concentration of 300 ppb for six hours and modulation of gene expression during the treatment (post three hours of treatment, T3 h) and the recovery phase (6 hours from the end of the treatment, T12 h) was checked by microarray gene chip and real-time reverse transcriptase PCR.

It was observed that transcript levels of numerous WRKY, RLKs and MAPK, and five senescence-related genes were over-expressed in O_3-treated plants. Conversely, CAT2, GPX1, GPX7, and GST1 were found to be down-regulated only after 12 h from the commencement of O_3 fumigation (Tosti et al. 2006).

D'Haese and co-workers (2006) performed an integrated microarray analysis on *Arabidopsis* by exposing it for two days to filtered air or 150 ppb O_3 for 8 h day^{-1}. It was observed that forty-eight hours post O_3 fumigation, there was an induction of SA synthesis and genes involved in senescence and programmed cell death. Moreover, the induction of genes related to the synthesis of anthocyanin diglucosides and thioredoxin-based redox control was also observed. Expression of genes in phenylpropanoid and flavonoid pathways e.g., hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase, caffeic acid/5-hydroxyferulic acid *O*-methyltransferase, cinnamyl alcohol dehydrogenase, etc., was up-regulated after an O_3 treatment of 125 ppb for three hours and two days in theCol-0 accession of *Arabidopsis thaliana* (Booker et al. 2012). Employing a microarray analysis to investigate the effect of 500 ppb O_3 for 6h (acute O_3 stress) on gene expression in ten-day-old *Arabidopsis*; Col-0 seedlings showed that out of 1788 probes, approximately 1364 displayed increased expression while 424 showed reduced expression (Short et al. 2012). The most responsive genes related to protein turnover, vesicle transport, and calcium signaling with Ca^{2+} signaling being strongly signified as O_3-responsive genes (Short et al. 2012), perhaps indicating a crucial role of calcium-based cellular signaling as a response to acute O_3 exposure. It is well known that micro RNAs or miRNAs are involved in gene silencing. Iyer and co-workers (2012), using the plant miRNA array, identified twenty-two miRNA families that were differentially expressed within one hour of O_3 exposure in *Arabidopsis thaliana* ecotype Col-0. Besides, miRNA targeted genes showed a strong negative correlation to the miRNA expressed in response to O_3 fumigation, suggesting that miRNAs may assist in resource allocation by putting a check on developmental processes to cater to the demands of the oxidative stress in plants.

Rice transcriptomes have also been extensively analyzed for their response to O_3 stress. Rice leaves exposed to 200 ppb O_3 for one, twelve, and twenty-four hours were checked for the transcript levels of approximately 22,000 rice genes (cDNA clones) using a DNA microarray chip. It was observed that a total of 394, 983, and 615 genes were up-regulated in O_3-exposed leaves, whereas 38, 244, and 71 genes were down-regulated at one, twelve, and twenty-four hours, respectively. These genes were related to signal transduction and signaling processes, transcription factors, post-translational modification, and cellular metabolism that were altered in the leaves exposed to O_3 for twelve and twenty-four hours (Cho et al. 2008). Transcription factors that were altered were found to be ERE, MYB, NAC, WRKY, and MAPK cascades. Among the cellular metabolism, respiration and defense-related genes such as catalase, peroxidase, monodehydroascobate reductase, glutathione synthetase, and glutathione S-transferase were predominantly induced at twelve and twenty-four hours of O_3 exposures, whereas transcript levels of genes related to photosynthesis, were suppressed (Cho et al. 2008). In a study to evaluate O_3-induced foliar injury symptoms and the related gene expression, two-week-old rice cultivar Nipponbare leaves were fumigated with an O_3 concentration of 200 ppb for 1, 12, 24, 48, and 72 h, as a result of which expression of 146 genes was found to be increased with increased foliar injury up to 72 h, showing a strong positive correlation between the subset of expressed genes and observed O_3-induced visible injury. Authors emphasized that the genes associated with foliar injury symptoms may serve as a means of potential biomarkers to study the mechanisms of injury symptoms developed due to O_3 (Cho et al. 2012).

Panicles and grains of rice plants were analyzed through an oligo-DNA microarray technique for their respective transcriptomes. Among the O_3-responsive genes, 176 were up-regulated, and 444 genes were down-regulated in panicle compared to the 24 and 106 genes in grain, respectively. The differential genes were predominantly involved in signaling, defense activities, hormonal regulation, transcription, and proteolysis (Cho et al. 2013). Contrasting rice genotypes SL15 (O_3-sensitive) and SL41 (O_3-tolerant) were exposed to 120 ppb of O_3, and the gene expression profiling based on microarray hybridization yielded 470 probes expressing differentially in SL15 and 314 in SL41. Changes in gene expression were categorized as the ones involved in programmed cell death (ethylene or jasmonic acid metabolism), disease resistance, biosynthesis of antioxidants, and enzymatic detoxification of ROS (Frei et al. 2010). A study evaluated the impact of O_3 exposure (75 ppb) on rice cultivars Sakha 101 and Giza 178. The authors speculated that a delayed transcriptional response in the O_3-sensitive plants made them more susceptible to O_3. In contrast, in resistant plants, the rapid and sustained activation of several signaling pathways enabled the deployment of multiple mechanisms for minimizing O_3-induced ROS toxicity (Tammam et al. 2019). OZONE RESPONSIVE APOPLASTIC PROTEIN1 (OsORAP1) is induced due to O_3 stress. Four-week-old wild-type rice plants were treated either with the ambient concentration of O_3 or with elevated O_3 for twenty days. *OsORAP1* gene was highly induced by elevated O_3, reaching more than thirty-fold expression levels found in ambient O_3 on the tenth day of O_3 exposure (Ueda et al. 2015). Moreover, to overview an inter-varietal response, expression of *OsORAP1* gene has also been checked across different rice genotypes. It was observed that O_3 fumigation of a 100 ppb concentration led to a highly significant induction of *OsORAP1* expression across all the 18 selected genotypes (Ashrafuzzaman et al. 2020).

Glycine max (soybean) is an O_3-sensitive crop, and its genetic responses under oxidative stress could give clues about its underlying sensitivity and counteraction to minimize O_3 damage. mRNA expression levels of genes in soybean cultivars Jinpumkong and Cheongjakong treated with O_3 were explored using differential banding patterns. A total of 408 bands were expressed differently on agarose gel, and most of the differentially expressed genes were those linked to the cellular and metabolic processes (Moon et al. 2013). Mechanistic differences in legumes such as *Phaseolus vulgaris* L., *Glycine max* (L.) Merr. and *Pisum sativum* to elevated O_3 were analyzed through global transcriptomics, which showed an increased abundance of respiration-related transcripts. Differences specific to *P. sativum* included a pronounced increase in abundance of the *GLUTATHIONE REDUCTASE* transcript (Yendrek et al. 2015). Molecular mechanisms associated with the differential tolerance to O_3 between Mandarin (Ottawa) (sensitive) and Fiskeby III (tolerant) [*Glycine max* (L.) Merr.] genotypes were examined by Chutteang and associates (2016). An up-regulation in the expression of g*lutathione reductase 2* was detected in both genotypes under high O_3 treatment. However, *protein D1* expression was down-regulated in Mandarin (Ottawa) but remained unchanged in Fiskeby III (Chutteang et al. 2016). Besides, an RNA-seq analysis of these two soybean cultivars displaying variable sensitivity to O_3 was performed to compare the genotypic differences in their O_3 tolerance, which was partly attributed to a delayed response to O_3 in Fiskeby III (O_3-tolerant) and therefore showing the ability to maintain the expression of defense as well as stress-responsive genes. However, the intolerant Mandarin (Ottawa) revealed a higher gene expression level for an oxidative and general stress response in early time points of O_3 exposure, which were later reduced or perished (Whaley et al. 2015).

Several other species have also been tested for their responses to oxidative stress at the gene level. Relating to ozone impact on levels of the *Zea mays* L. cultivar Chambord, mRNAs encoding C_4 phosphoenolpyruvate carboxylase (PEPC), ribulose-l,5-bisphosphate carboxylase/oxygenase (RuBisCO) small and large subunits (SSU and

LSU, respectively) and RuBisCO activase were tested under non-filtered air (NF), and non-filtered air containing 40 and 80 ppb O_3 using open-top chambers. Except for *RuBisCO* mRNAs, all other measured transcripts from the fifth leaf were reduced by +80 ppb O_3 concentration. In contrast, in the tenth leaf, the +40 ppb O_3 atmosphere increased the gene expression levels of RuBisCO and RuBisCO activase (Leitao et al. 2007). In *P. vulgaris*, O_3 exposure of 120 ppb for 4 h caused an induction of the phenylpropanoid pathway and flavonoid synthesis, confirmed by the increased mRNA accumulation of *phenyl alanine ammonia lyase* (PAL), *chalcone synthase*, and *chalcone isomerase*, which supported the view that O_3 acts as an abiotic elicitor of secondary metabolic defense responses in plants (Paolacci et al. 2001). Two genotypes of *Medicago truncatula* were investigated for their differential responses to O_3 stress; the sensitive genotype Jemalong showed a down-regulation of defense response genes. The tolerant JE 154 genotype exhibited much more rapid and large-scale transcriptome changes twelve hours post O_3 exposure (Puckette et al. 2008).

A transcriptomic exploration of *Brassica campestris ssp. chinensis* exposed to an elevated O_3 concentration of 252 ppb for two days (8 h fumigation per day) yielded 456 DEGs manifesting increased expressions, and 219 DEGs with reduced expressions. Differentially expressed genes were predominantly linked with defense mechanisms, including glutathione and sulfur metabolism, glucosinolate and phenylpropanoid biosynthesis, and structural barriers like waxes and cutin (Zhang et al. 2017). Ina herbaceous perennial plant, *Centaurea jacea*, when exposed to an O_3 concentration of 200 ppb for 5 h, PAL activity was increased by 41 and 91% in resistant and sensitive lines respectively, compared to controls, in contrast to the transcripts of chalcone synthase, whose sensitive and resistant lines did not change after O_3 treatment (Francini et al. 2008). The transcriptome of O_3 stress-regulated genes (ORGs) in two pepper cultivars [*Capsicum annuum* cultivar Dabotop (O_3-sensitive) and *C. annuum* cultivar Buchon (O_3-tolerant)] were analyzed by cDNA microarray analysis (Lee and Yun, 2006). One hundred and eighty genes were up- or down-regulated more than three-fold under O_3 stress. Expressions of 84 of these ORGs increased and 88 were reduced, while the ORGs were specifically up-regulated in the O_3-sensitive cultivar. The authors concluded that these ORGs were involved in signaling pathways, defense, transport, metabolism, transcription, protein synthesis, protein degradation, and photosynthesis (Lee and Yun, 2006). In tobacco leaves, the activity of chitinase and β-1,3-glucanase and their respective mRNA levels was increased in response to O_3 exposure (Schraudner et al. 1992).

The enzyme 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase showed the highest induction after 4h of O_3 fumigation in *Nicotiana tabacum* L. cv. Bel W3, where it reached a three- to five-fold induction. Similarly, the transcript level of 3-Dehydroquinate dehydratase-shikimate 5-dehydrogenase and chorismate synthase showed an induction pattern after four hours, and this induction ranged from 1.8 to 2.6-fold and was highest for chorismate synthase while shikimate kinase remained unchanged by O_3 (Janzik et al. 2005).

10.5 Proteomic response to ozone stress

Cellular or a tissue proteome is immensely complex and variable at a specific time point due to its complex nature and wide dynamic range of proteins (Agrawal et al. 2013). The most popular approach used to analyze proteome or protein dynamics relies on separating proteins from complex mixtures on 2D gel expression, apart from the separation of gel-less protein, which is prevalent nowadays. Indeed, O_3 is a damaging air pollutant that affects plants adversely and can modify the intracellular protein dynamics (Agarwal et al. 2002a) since plant metabolism upon stress is reshuffled from active growth and development to acclimation towards stress (Kosová et al. 2011). Thus, proteomics studies can make a considerable contribution to probing the possible relationships between protein expression and stress acclimation in plants (Kosová et al. 2011). Maximum studies based on comparative proteomics of the O_3 treated and their respective control plants are predominantly done by employing 2-DE followed by MS analysis for protein identification (Table 10.1).

Table 10.1 A compilation of studies displaying the effects of ozonestress on the proteome of different crop plants

S. No.	Crop/cultivar/ genotype	Plant tissue/ organelle	Ozone Treatment	Proteomic Technique	Differential expressed proteinsspots	Major findings	Reference
1.	*Oryza sativa* L. cultivar Nipponbare	Leaf	200 ppb for 72 h	2-DGE/N-terminal and internal amino acid sequencing	52	Reduction in major photosynthetic proteins including ribulose-1, 5-bisphosphate carboxylase/oxygenase (RuBisCO); Induction of stress/defense related proteins	Agrawal et al. (2002a)
2.	*O. sativa* L. cultivar Nipponbare	Leaf	200 ppb for 24 h	2-DGE/N-terminal amino acid sequencing and 2-DE/*in vitro* phosphorylation	Large number	Changes in proteins related to defense and or stress like calcium homeostasis, cellular protection, and pathogenesis-related proteins; The appearance of new phosphoproteins of molecular mass ranging between 14 and 60 kDa as the primary O_3 response	Agrawal et al. (2002b)

3.	*O. sativa* L. cultivar Koshihikari	Leaf	elevated O_3 of 120 ppb for 9days	2-DGE/MALDI-TOF/MS, N-terminal sequencing	20	Drastic reduction in the abundance of photosynthetic proteins including large sub-units of RuBisCO and their fragments, RuBisCO activase, energy metabolism proteins, and thioredoxin peroxidase; Induced expression of antioxidants like Mn-SOD, glutathione S transferase, and defense/stress-related proteins like PR proteins	Feng et al. (2008)
4.	*O. sativa* L. cultivar Nipponbare	Leaf	200 ppb for 24 h	2-DGE/LC-MS/MS	23	Damages to photosynthetic assembly; Increased abundance of Chaperones like Clppro tease and other heat shock proteins HSP 90 and HSP 70, chloroplast localized ftsH homologue, and RuBisCO activase small isoform; Induction of defense-related proteins such as chloroplast L-APX, glutathione peroxidase, and PR proteins	Cho et al. (2008)

5.	*O. sativa* L. cultivars Kirara 397 Koshihikari Takanari	Leaf	73 ppb	2-DGE/nano-LC-MS/MS	31 18 11	Reduced expression of photosynthetic and glycolysis associated proteins in Kirara 397, but their increased abundance in Koshihikari; No significant decrease in the expression of proteins related to photosynthesis and energy metabolism; No effect on defense or disease-linked proteins in Takanari	Sawada et al. (2012)
6.	*Triticum aestivum* L. cultivars Sonalika and HUW 510	Leaf	ambient + 10 and 20 ppb	2-DGE/Q-TOF-MS/MS	38 differentially expressed spots common in Sonalika and HUW 510	Drastic reductions in RuBisCO and its activase, energy metabolism proteins like ATP synthase, aldolase, and phosphoglycerate kinase; Increased abundance of stress and defense-related proteins	Sarkar et al. (2010)
7.	*Zea mays* L. cultivar Guarare 8128	Leaf	200 ppb for 72 h	2-DGE/Q-TOF-MS/MS	12	Increased expression levels of antioxidants ascorbate peroxidase and catalase; Intense increase in low molecular weight (24 and 30 kDa) heat shock proteins and 41 kDa naringenin-7-O-	Torres et al. (2007)

						methyltransferase protein (NOMT) in younger leaves; Substantial loss in SOD abundance depending on the position of the leaf	
8.	*Glycine max* L. cultivar Enrei	Leaf and chloroplast	120 ppb for 3 days	2-DGE/MALDI-TOF MS	Leaf:20 Chloroplast: 32	Decline in proteins associated with photosynthesis and carbon assimilation; Increased expression of the antioxidant defense system and carbon metabolism proteins	Ahsan et al. (2010)
9.	*G. max* L. cultivar Pioneer 93B15	Leaf and root	116 ppb	2-DGE/LC-MS/MS and redox-sensitive thiol labeling	Leaf: 29 Root: 36	Alterations were found in the proteins associated with signaling, antioxidants, carbon metabolism and photosynthesis; amino acid, flavonoid, and isoprenoid biosynthesis	Galant et al. (2012)
10.	*Phaseolus vulgaris* L.cultivar IDIAP R3	Leaf	200 ppb for 72 h	2-DGE/Q-TOF-MS/MS	25	A severe decline in 19 and 20 kDa SOD proteins in younger leaves; Increased abundance of 25 kDa ascorbate peroxidase, 33 kDa small Heat Shock Protein, and a NOMT (41kDa)	Torres et al. (2007)

10.5.1 Effects of ozone stress on the proteome of different crop plants

Rice (*Oryza sativa* L.) has been most studied for its response to O_3 stress, perhaps due to the protein sequence availability in a wide range of databases. Agrawal et al. (2002a) detected marked reductions in major photosynthetic proteins in rice, including Ru Bis CO, and the induction of various defense and stress-related proteins due to O_3 in leaves (Table 10.1). Large and small subunits of enzyme RuBisCO are the major abundant proteins vulnerable to environmental influences. They perform key roles in carbon assimilation in photosynthesis and take part in certain signaling cascades (Kang et al. 2010). Feng et al. (2008) later exposed rice seedlings to elevated O_3 and reported a significant reduction in the abundance of photosynthetic proteins and energy metabolism-related proteins (Table 10.1), which indicates that O_3 severely affects energy metabolism, disturbing the Calvin cycle, and decreasing photosynthetic rate and production, and hence affecting the final grain yield (Feng et al. 2008). However, the findings of Sawada et al. (2012), dealing with proteomic analysis of flag leaves in different rice cultivars exposed to O_3 stress suggested that it is not only degradation of RuBisCO (which is usually considered as a reason of yield reduction) but also other factors that control the productivity of rice under elevated O_3 conditions. Moreover, accumulation of defense-related proteins including L-APX, PBZ, PR10, PR5, etc., to high levels in response to O_3 treatment indicates a defensive role of these proteins as well as a connection between O_3 stress and responses due to pathogen stress (Agrawal et al. 2002a; Cho et al. 2008; Singh and Jwa 2013). Identification of these candidate proteins may reveal that some of them have functions that are noticeably consistent with the stress-tolerance trait and, therefore, may be recorded as potential biomarker proteins (Komatsu 2008).

Chaperones are known to act as disaggregates, repairing the damaged proteins, and assisting in their renaturation, as well as protecting them from stress. In addition to heat stress, the expression of molecular chaperones is also altered in response to various other stresses (Kang et al. 2010). Heat shock proteins (HSPs) of molecular weight, approximately 24 and 30 kDa in maize and 33 kDa in bean, are found to be strongly induced due to O_3 stress (Torres et al. 2007) (Table 10.1). An enhanced expression of HSPs, which are the molecular chaperones, assists in refolding misfolded proteins and stabilizes protein structure and function, therefore maintaining cellular homeostasis (Hossain et al. 2013). Induction of heat shock proteins due to O_3 exposure in Nipponbare rice seedlings pointed towards the probable reduction of oxidative damage and the prevention of photosynthetic machinery disruption (Cho et al. 2008). Gottardini et al. (2016) have also proposed that induction of HSPs due to O_3 exposure may help in increasing the resistance of the photosynthetic apparatus to photoinhibition.

Furthermore, to investigate the correlation between the transcriptomics and proteomics results derived from 24h of O_3 exposure, the authors checked the transcript levels of genes corresponding to the identified proteins in two-week-old rice seedlings. Out of the identified differentially expressed protein spots, 12 protein spots up- or down-regulatingat 24 h were derived from a respective increase or decrease at their transcript levels at 12 h of O_3 exposure (Cho et al. 2008). Correspondingly, some specific and rapidly accumulating PR proteins, antioxidants such as APX, Mn-SOD, and ATP-dependent caseinolytic proteases (Clp), could have helped in the recovery process in rice post the 24 h O_3 treatment (Cho et al. 2008; Kosová et al. 2011) and possibly may function as potential marker proteins to overview the O_3-linked damage in rice, besides other plants in general (Kang et al. 2010). The O_3 response in two rice cultivars (Malviya dhan 36 and Shivani) in open-top chambers mimicking near-natural field conditions was studied. Rice leaves were exposed to ambient+10 and 20 ppb elevated O_3. Twenty-five differential protein spots were observed in cultivar Malviya dhan 36, while thirty-six were observed in cultivar Shivani compared to controls with an O_3 filtered chamber. Moreover, protein spots of molecular weight ranging between 96 and 43 kDa displayed reduced abundance besides large and small RuBisCO subunits in both test cultivars under the elevated O_3 environment (Sarkar et al. 2015). Reductions in the accumulation of Ru Bis CO's larger and smaller subunits have also been affirmed in maize (Singh et al. 2014 a,b), mung bean (Mishra and Agrawal 2015), and linseed (Tripathi et al. 2011). Additionally, Singh et al. (2014a) observed a lowered expression of primary CO_2 acceptor protein PEP carboxylase (PEPC) in maize and recommended that RuBisCO LSU, SSU, and PEPC can serve as marker proteins for O_3 stressed maize plants.

Analysis of soybean leaf exposed to O_3 showed that differentially expressed proteins were involved in signaling, antioxidant pathways, photosynthesis, carbon metabolism, biosynthesis of amino acid, and secondary metabolites (Galant et al. 2012) (Table 10.1). Hossain and associates (2013) have proposed that proteins related to glycolysis and the citric acid cycle involved in carbon metabolism are largely altered to meet the energy demand of the cells required under stress. Moreover, it was suggested that expressions and/or oxidations of RuBisCO subunits and its linked proteins, photosynthetic enzymes, and chlorophyll metabolism associated proteins might have helped the soybean plants to keep the normal pace of photosynthesis level at the early growth stages before the progression of senescence induced due to O_3 exposure (Galant et al. 2012). Ahsan et al. (2010) supplied acute O_3 exposure to soybean and identified differentially expressed proteins in leaf and chloroplasts (Table 10.1) and highlighted that proteins associated with photosynthetic pathways were affected the most, which could have been a probable cause for the decline in photosynthesis under O_3 treatment. Therefore, key proteins involved in the response to O_3 stress appear to be antioxidative and metabolic proteins, photosynthesis-related proteins, glycolytic pathway, electron transport chain, and stress-responsive proteins like HSPs, or PR proteins, and others (see Table 10.1). Nevertheless, the proteomics approach for the differential-expression of protein (identification and quantitation) itself cannot give considerable information regarding the functional aspect of protein since it also depends on its interacting partners or related post-translational modifications (Kosová et al. 2011). Furthermore, the diversity of plant species (specifical crops) for O_3-linked proteomic responses shall be increased due to hitherto limited report availability (Table 10.1).

10.5.2 Analysis of two-dimensional gel electrophoresis of proteins from ozone-treated maize plants

Apart from published data that have been reviewed in this chapter (section 10.5.1) relating to proteome analysis, a two-dimensional (2D) separation of maize leaf proteins was performed in our lab (data unpublished). Foliar samples from maize cultivar HQPM1 were isolated from the two populations, one from those grown in the filtered chambers (FC)/control and another from the plants treated with ambient O_3 concentration+30 ppb O_3 (AO+30) [average ambient O_3 concentration 64 ppb for the experimental period] grown under field conditions (Fig. 10.3).

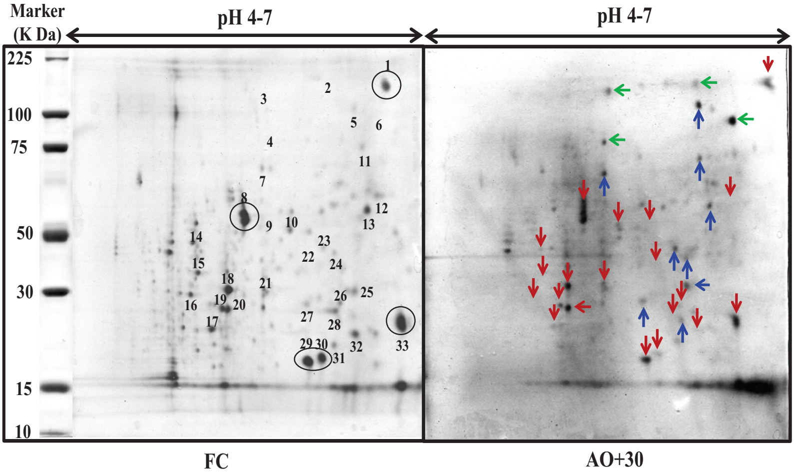

Fig. 10.3 Two-dimensional gel electrophoresis of maize leaf protein. FC represents the filtered chambers (served as control), while AO+30 represents the +30 ppb O_3 exposure above the ambient level. Red arrows show the decreased abundance of protein spots, blue arrows show the increased abundance of protein spots and green arrows show the newly formed protein spots in AO+30 samples, which were not observed in control samples

Total protein content was reduced in O_3 treated foliar samples, and two-dimensional gel electrophoresis showed that maize leaf proteins were negatively affected due to O_3 treatment with most of the protein spots showing reduced abundance. A differential response in leaf total proteome was observed at elevated O_3 exposure of AO+30 as compared to the control FC (Fig 10.4). Spot numbers have been assigned to each differential spot. A decreased abundance of protein spots is shown by the red arrows, blue arrows show the increased abundance of spots and green arrows show the newly appeared spots in AO+30 samples, which were not detected in FC samples. Out of the total thirty-three differential spots, twenty spots showed decreased abundance in AO+30, while nine spots showed an increase in their abundance. Four new spots (2, 3, 4, and 6) of high molecular weight were observed at the AO+30 O_3 dose but not detected in control samples. Five spots (1, 8, 39, 30, and 33) manifested pronounced differences between the control and the O_3 treated samples (Fig. 10.4). Therefore, the results showed that O_3 treatment applied to maize led to alterations in the leaf proteome and the changes were apparent.

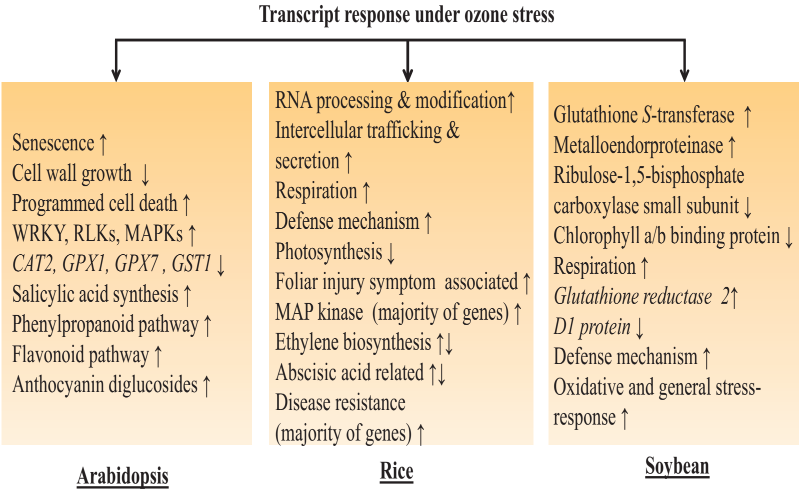

Fig. 10.4 A summary of genes encoding various cellular processes, pathways, transcription factors, and enzymes that are up- or down-regulated due to ozone exposure in Arabidopsis, rice, and soybean (refer to section 10.4 for details) ↑ Up-regulated, ↓ Down-regulated

10.6 Metabolic responses due to ozone exposure

10.6.1 Low molecular weight metabolites serve as potent non-enzymatic antioxidants

Metabolomics has become an instrumental tool to study the metabolic changes in plants in response to various abiotic stresses. The metabolome is the collection of metabolites as a whole in a biological sample whose quantification can be done to identify and quantify the metabolites, which usually aims to discover metabolic markers (Renaut et al. 2009). The role of stress-associated metabolites in abiotic stress response has been found in the context that metabolites are not only functional players in stress tolerance but also act as signaling molecules (Genga et al. 2011). Low molecular weight metabolites, which are chiefly non-enzymatic antioxidants, viz. ascorbic acid/ascorbate (AA), flavonoids, vitamin E (tocopherol), thiols (most abundantly glutathione), carotenoids, polyamines, phenolics, etc., counteract the stress imposed by ROS burst generated due to O_3 (Ashmore 2005; Castagna and Ranieri 2009; Nadgorska-Socha et al. 2013). Usually, a high ratio reduced to an oxidized ratio of ascorbate and glutathione is needed for proper scavenging of cellular ROS, and this ratio is maintained by the enzymes, glutathione reductase, monodehydroascorbate reductase, and dehydroascorbate reductase, utilizing NADPH as a reducing power (Noctor and Foyer 1998; Mittler 2002). Besides, ascorbic acid and thiols have been identified as potential biomarkers for O_3 stress in plants (Fatima et al. 2019), which will be elaborately discussed in this review.

Antioxidant activities in the apoplast are crucial for protection from O_3 damage (Kangasjarvi et al. 2005), and AA is proposed to scavenge the significant amounts of ROS resulting from O_3 exposure in the leaf apoplast (Ranieri et al. 1999); this is a critical metabolite in the ascorbate-glutathione pathway that protects chloroplasts from oxidative stress during photosynthesis (Noctor and Foyer 1998) and plays an essential role in maintaining cellular homeostasis (Mittler 2017). It has been proposed that AA plays a decisive role in O_3 tolerance in many plants. This is supported by the enhanced O_3 sensitivity of *Arabidopsis* mutants deficient in ascorbate biosynthesis (Conklin et al. 1996). The oxidative stress response of a rice TOS17 insertion mutant (ND6172) for a GDP-D-mannose-3',5'-epimerase gene involved in AA biosynthesis, which had an approximate 20–30% lower AA level, was tested with respect to the wild type rice variety Nipponbare. It was conferred that a moderately higher level of leaf symptoms induced by O_3 fumigation in ND6172 was correlated with the lower concentration of foliar ascorbate (Frei et al. 2012). Two sensitive wheat cultivars Columbo and Sculptur had differential manifestations of O_3 injury symptoms. At the same time, Sculptur did not show any symptoms weeks after O_3 exposure but Columbo displayed symptoms within a few weeks. One of the underlying mechanisms was the higher content of the constitutive ascorbate pool in Sculptur (Picchi et al. 2017). Continuous O_3 exposure resulted in a less efficient metabolism of the AsA–GSH cycle in the SY63 (O_3-sensitive) compared to the WXJ14 (O_3-resistant) rice cultivar (Wang et al. 2013). Recently, the findings of Fatima et al. (2019) also supported that tolerant wheat cultivars showed a higher increment in AA content in response to elevated O_3 exposure. Similarly, Jiang et al. (2018) reported that the constitutive level of AA in leaves was the most important parameter to assess O_3 sensitivity in soybean cultivars. However, reductions in the AA pool have also been detected due to O_3 treatment. Iglesias et al. (2006) analyzed *Citrus clementina* under ambient and elevated O_3 treatments (30 and 65 ppb) and observed that AA levels in citrus leaves showed a significant decline (75–80%) under ambient and elevated O_3 treatments compared to controlled, filtered air. Biswas et al. (2008) assessed the O_3 sensitivity of twenty wheat cultivars and found that modern cultivars, on average, displayed 30% reductions in the level of ascorbate (Table 10.2).

Table 10.2 List of studies showing changes in non-enzymatic antioxidant metabolite ascorbic acid in response to O_3 stress in different crop species

S.No.	**Plant species/ cultivar/genotype/accession**	**Ozone Treatment**	**Non-enzymatic antioxidant metabolite ascorbic acid (AA)**	**Reference**
1.	*Oryza sativa* L., cultivars SY63 (O_3-sensitive) and WXJ14 (O_3-resistant)	1.5 times ambient O_3	AA content in WXJ14 was 35.6% higher than SY63	Wang et al. (2013)
2.	*O. sativa* L., cultivars Malviya dhan 36 and Shivani	10 and 20 ppb above ambient O_3	Malviya dhan 36 (35.3%and 62.1%↑) Shivani (19.6% and 31.3%↑)	Sarkar et al. (2015)
3.	*Triticum aestivum* L., twenty cultivars	82±5 ppb	Modern cultivars (30%↓) Old cultivars (11%↑)	Biswas et al. (2008)
4.	*T. aestivum* L., varieties: Yangfumai 2 (O_3-sensitive) and Yangmai 16 (O_3-tolerant)	27 ppb higher than ambient O_3	12.0% higher AA in Yangmai 16 than Yangfumai 2	Feng et al. (2010)
5.	*T. aestivum* L., cultivars Sonalika and HUW 510	10 and 20 ppb above ambient O_3	Sonalika (23.13% and 34.8%↑) HUW 510 (14.1% and 21.8%↑)	Sarkar et al. (2010)
6.	*T. aestivum* L., cultivars M 533 and PBW 343	50.2 ppb in the first year 53.2 ppb in the second year	Higher increase in AA in M 533 than PBW 343 at the vegetative stage	Rai and Agrawal (2014)
7.	*T. aestivum* L., fourteen cultivars	Ambient+30 ppb (±5 ppb) O_3	Tolerant cultivar KH65 (55.9% at vegetative phase and 40.3% at reproductive phase) and sensitive	Fatima et al. (2019)

			cultivar HD28 (11.5% at vegetative phase and 6.3% at reproductive phase)	
8.	*T. aestivum* L., cultivars HUW468 and HD3086 (early sown) and HUW234 and HD3118 (late sown)	20 ppb above ambient O_3	Increase in AA in both early and late sown cultivars	Yadav et al. (2019)
9.	*Zea mays* L., cultivars DHM117 and HQPM1	15 and 30 ppb above ambient O_3	DHM117 (3.8% and 12.2%↑) HQPM1 (16.8% and 22.6%↑)	Singh et al. (2014b)
10.	*Glycine max* L.	1.5 times ambient O_3	Total AA was increased in upper canopy leaves Total AA was decreased in lower canopy leaves	Booker and Fiscus (2005)
11.	*G. max* L., cultivars PK 472 (O_3-sensitive) and Bragg (O_3-tolerant)	70 and 100 ppb of O_3	Reduction in AA content more in PK 472 compared to Bragg	Singh et al. (2010)
12.	*G. max* L., cultivar Pioneer 93B15	90 ppb	42%↑	Gillespie et al. (2011)
13.	*G. max* L., cultivars Sambaíba (O_3-sensitive) and Tracajá (O_3-tolerant)	40 and 80 ppb	Sambaíba (85%↑ and 73%↓) Tracajá (27%↑ and 9%↑)	Bulbovas et al. (2014)
14.	*G. max* L. genotypes Mandarin (Ottawa) (O_3-sensitive) and Fiskeby III (O_3-tolerant)	70 ppb	Higher AA level in Fiskeby III than Mandarin (Ottawa)	Roth (2017)

15.	*Phaseolus vulgaris* L., cultivars S156 (O_3-sensitive) and Provider (O_3-tolerant)	71 ppb	28% higher AA content in Provider compared to S156	Burkey et al. (2003)
16.	*P. vulgaris* L., cultivars Fepagro 26 and Irai	One week of O_3 treatment daily for six hours	Fepagro 26 (50%↑) Irai (No change)	Caregnato et al. (2013)
17.	*Vigna radiata* L., cultivars HUM-2 and HUM-6	Ambient+10 ppb	HUM-2 (5.7 %↑) HUM-6 (3.8 %↑)	Mishra and Agrawal (2015)
18.	*Trifolium alexandrinum* L., cultivars Bundel, Wardan, JHB-146, Saidi, Fahli, and Mescavi	10 ppb above ambient O_3	Wardan (sensitive):26 %↑ Fahli (resistant): 71.2%↑	Chaudhary and Agrawal (2013)
19.	*Medicago truncatula* Marrakech (sensitive accession) and JE154 (resistant accession)	300 ppb O_3	Two-fold increase in JE154	Puckette et al. (2008)
20.	*Linumu sitatissimum* L. cultivar Padmini	10 ppb above ambient O_3	22.4%↑	Tripathi et al. (2011)

(↑) = increase, (↓) = decrease

Moreover, a higher reduction in ascorbate level was observed in sensitive soybean cultivar PK 472 concerning tolerant cultivar Bragg at 70 and 100 ppb O_3 exposure four hours from germination to maturity (Singh et al. 2010) (Table 10.2). However, no significant change was reported for AA concentration in *Arabidopsis* (Col 0) leaf tissue fumigated with 5, 125, or 175 ppb O_3 in controlled environment chambers (Booker et al. 2012). Apoplastic AA did not show any significant differences between the knock-out and overexpression line for the apoplastic localized OsORAP1 protein involved in O_3-induced cell death in rice (Ueda et al. 2015).

Thiols are important metabolites present as GSH (reduced glutathione) or GSSG (oxidized glutathione) or bound specifically to proteins acting as antioxidants and playing significant roles in the detoxification of ROS and redox homeostasis (Mungli et al. 2009; Noctor et al. 2012). High levels of total glutathione and its precursor amino acids, such as glutamic acid and glycine, were accumulated in O_3-exposed rice leaves compared to those in the control plants (Cho et al. 2008). These results suggest that the antioxidant molecule glutathione may play crucial roles in reducing oxidative damages occurring due to O_3. Two sensitive wheat cultivars Sculptur and Columbo had higher levels of reduced glutathione under a 60% elevated O_3 concentration than ambient air compared to control filtered-chamber plants (Picchi et al. 2017). A study by Sarkar et al. (2015) highlighted that variable thiol content was responsible for the differential response of rice cultivars under elevated O_3. Nonetheless, Pandey et al. (2015) have also proposed reduced glutathione to screen the O_3 tolerance/sensitivity in rice cultivars effectively. *Medicago truncatula* plants were given 300 ppb O_3 for 6 h, and it was observed that resistant JE154 showed significant increases in GSSG and GSH levels, whereas sensitive Marrakech plants had four-fold increases in the levels of GSSG (Puckette et al. 2008). Significant increases in glutathione content in tolerant and sensitive soybean cultivars were found due to O_3 stress (Roth, 2017). In an investigation across fourteen wheat cultivars, Fatima et al. (2019) detected that percentage increment of thiols varied between 9.6% and 14.8% in sensitive wheat cultivars, 11.2 and 20.7% in intermediate sensitive cultivars, and 7.8 and 15.7% in tolerant cultivars at the reproductive stage. Singh et al. (2014b), Mishra and Agrawal (2015), and Tripathi et al. (2011) also reported increased thiol contents in maize, mung bean, and linseed, respectively, under O_3 stress. Glutathione amounts were induced in the eighth leaf, and the fourteenth leaf in soybean when it was treated with 1.5 times the ambient O_3 concentration (Booker and Fiscus 2005), whereas, no significant changes in total thiol levels were observed in the two tested *P. vulgaris* cultivars Fepagro 26 and Irai, after one week of O_3 fumigation (Caregnato et al. 2013). Reduced (GSH) and oxidized (GSSG) glutathione quantities were affected by O_3 treatment in an O_3-tolerant soybean cultivar Tracajá (Bulbovas et al. 2014). *Capsicum baccatum* L. var. pendulum grown in OTCs at mean O_3 concentrations of 171.6 ppb for six hours had an approximately75% lower content of total reduced thiol groups in leaves than that of the control plants (Bortolin et al. 2014). It is therefore conferred that enhanced ROS formation, in general, induces low molecular weight non-enzymatic antioxidants like ascorbic acid and thiols as potent ROS scavenging molecules.

10.6.2 Secondary metabolites as antioxidants

Apart from detoxification through a non-enzymatic antioxidative defense system, secondary metabolism is an important physiological process in plants that plays an important role in plants' response to environmental stresses. Plants' secondary metabolites such as the phenolic compounds act as non-enzymatic antioxidants in response to oxidative stress caused by O_3. Long-term O_3 exposure stimulates the carbon fluxes from the primary towards the secondary metabolic pathways, largely inducing a shift of available resources in favor of the synthesis of secondary metabolites (Iriti and Faoro 2009). Secondary metabolites are derived chiefly from three pathways: the phenylpropanoid, isoprenoid, and alkaloid pathways that act as potent antioxidants able to counteract oxidative stress (Iriti and Faoro 2009). Phenylpropanoid metabolism is induced as a general response to stress in plants, and enhancement of key enzyme activities and accumulation of secondary metabolites occur early on, in an encounter with O_3 to provide tolerance or resistance. PAL is an important enzyme of the phenylpropanoid pathway and is considered to be a biochemical marker involved in the activation of plant defense, including the synthesis of structural barriers and protective compounds. In *Arabidopsis thaliana*, expression of the PAL encoding gene was rapidly induced within three hours of O_3 treatment (300 ppb daily for 6 h), reaching thrice the level detected in control plants (Sharma and Davis, 1994). Leaf total polyphenol content was decreased by approximately 38% in O_3 treated plants. In comparison, leaf anthocyanin content was 63.5% higher in O_3 treated plants than in control plants in *Capsicum baccatum* L. var. pendulum accession "*Pimenta-dedo-de-moça*" grown in OTCs at a mean O_3 concentration of 171.6 ppb for six hours (Bortolin et al. 2014). The raised concentration of anthocyanin was reported in the leaf, and the leaf sheath of two maize cultivars DHM117 and HQPM1 in an elevated O_3 environment of 15 and 30 ppb above the ambient level supplied five hours daily (Singh et al. 2014b). Francini et al. (2008) found that anthocyanins were rapidly utilized by a resistant clone as antioxidant compounds for O_3 detoxification.

Increases in gene transcription and the activity of a large number of enzymes of the phenylpropanoid pathway and the chloroplast shikimate pathway due to O_3 induction led to the production of phenolic compounds, flavonoids, and monolignols (Toumainen et al. 1996; Booker and Miller, 1998; Betz et al. 2009). Likewise, induced foliar production of total phenolics has been reported under O_3 stress in rice, wheat, maize, mung bean, and linseed (Sarkar et al. 2015; Rai and Agrawal 2014; Singh et al. 2014b; Mishra and Agrawal 2015; Tripathi et al. 2011). Flavonoids are a diverse group of polyphenolic compounds that are effective antioxidants (Pérez 2014). The maximum rise in flavonoid content was found in HD 2987 (O_3-sensitive) followed by PBW 502 (intermediate O_3-sensitive) and Kharchiya 65 (O_3-tolerant) wheat cultivars under 30 ppb elevated O_3 fumigation given for six hours daily, and a similar trend was detected for total phenol content in wheat cultivars displaying differential O_3 sensitivity. Besides, the enzymes related to the phenylpropanoid pathway showed significantly increased activity in sensitive cultivar HD 2987 under elevated O_3 (Fatima et al. 2018). Elevated O_3 can also increase secondary metabolites from polyamine biosynthesis pathways such as GABA, from the phenylpropanoid metabolic pathway, and stress hormones such as abscisic acid and ethylene (Ludwikow and Sadowski 2008). Cho et al. (2008) found that most amino acids, particularly phenylalanine, tyrosine, and tryptophan increased in rice leaves exposed to O_3 for 12 and 24 h, suggesting the biosynthesis of secondary metabolites derived from phenolic amino acids. Ozone treatment increased the apoplastic concentration of sinapoyl malate, a hydroxycinnamic acid in the Col 0 accession of *Arabidopsis thaliana*, although the concentrations were too low to act as effective protectants (Booker et al. 2012). However, in a SoyFACE experiment, Betzelberger et al. (2010) found no significant effect of O_3 treatment on total phenolic content at twice the concentrations of ambient O_3. Furthermore, no significant change in phenolic content was recorded in *Glycine max* L.; cultivar Pioneer 93B15 was treated with a chronic O_3 concentration of 90 ppb (Gillespie et al. 2011). Phenol content was reduced significantly by 21.8 and 38%, respectively, at 70 and 100 ppb of O_3 in soybean cultivar PK 472 (O_3-sensitive) and by 16.7% at 100 ppb in cultivar Bragg (O_3-sensitive) compared to the untreated control plants (Singh et al. 2010).

10.7 Conclusion

Increased anthropogenic activities, industrialization, over-exploitation of natural resources, and unsustainable development have made tropospheric O_3 a prominent secondary air pollutant formed by the reactions between primary pollutants during favorable atmospheric conditions. Ozone exposure in plants leads to an oxidative burst by the generation of ROS and downstream signal perception and switching on of the related transduction involving various kinases, proteins, and hormones. Susceptibility, tolerance, or resistance towards O_3 encompasses a diverse array of responses in which the transcript expression of genes, translated proteins, and antioxidants play significant roles, particularly in defense. Usually, the genes and proteins involved in signaling, transport, defense pathways and redox control, including stress-related proteins and metabolites, are induced by oxidative stress. At the same time, those related to photosynthesis and energy metabolism are suppressed. Transcriptomics and proteomics techniques have thus generated information regarding the vast number of genes and proteins that respond to O_3 stress adaptation and tolerance, in line with which various marker genes and proteins have been identified. Furthermore, metabolites like ascorbic acid and thiols can prove to be important metabolic markers for O_3 stressed plants. So, investigations having a holistic approach, considering the integration of transcript, protein, and metabolite profiling could give a better insight into O_3 responses in plants that should be encouraged. The diversity of plant species taken up for the sesorts of studies, including wild species, needs to be promoted. Identification of principal regulator genes and key proteins could help to develop genetically engineered O_3 stress-tolerant/-resistant crops that may have better signaling network proteins for early stress perception, enhanced ROS detoxification, proteostasis maintenance, efficient photosynthetic performance, and ATP turnover for repair mechanisms under stress.

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CHAPTER ELEVEN

RESPONSE OF CROP PLANTS TO TROPOSPHERIC OZONE UNDER DIFFERENT AGRONOMIC PRACTICES: AN INSIGHT INTO THE MECHANISMS AND SIGNALING PATHWAY(S) UNDERLYING SUCH INTERACTIONS

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Abstract

Tropospheric ozone (O_3) precursors, and consequently O_3, are predicted to rise globally by 40–60% of the current level by 2100 due to rapid economic development, suggesting a critical O_3 pollution scenario; thus, agricultural sectors and food security are facing a major threat. Considering such a state of affairs, it is the need of the hour to adopt appropriate agronomic practices in farming to ameliorate the detrimental impacts of O_3 on agricultural productivity. The effectiveness of different agronomic practices in controlling the O_3 stress on crop plants is gaining utmost importance by various research groups worldwide. Studies suggested that soil amendments with N fertilization enhanced the efficiency in resource partitioning and activation of the antioxidant defense system to help the crop to combat O_3 toxicity. Besides, the application of different plant protectants such as suitable fungicides, growth regulators, antioxidants, natural plant extracts, and ethylene diurea displayed different natures and modes of action to ameliorate the O_3-induced injuries in plants.

Furthermore, a few investigations have also been carried out to assess O_3 pollution's impact on crop growth and performance under weeds' competition. It was observed that weeds developed a stronger antioxidative defense system against O_3 as compared to crop plants and compete them out, resulting in an enhanced loss of the crop yield. Seed treatment with radiation before sowing induces activation of the antioxidative enzyme system, which acts as an effective quencher for reactive oxygen species. Moreover, the management of other abiotic stresses such as drought and salinity must also be considered as critical agronomic practices to reduce the O_3-induced crop yield losses. However, interactive studies on O_3 and drought are very complex, and responses can be additive, synergistic, and antagonistic. In this review, an attempt has been made to explain various mechanisms and signaling pathway(s) associated with the application of such agronomic practices. This knowledge will be beneficial for a broad group of workers to determine the efficacy of implementing such agronomic practices under present and future O_3 concentration scenarios.

Keywords: Agronomic practices; Crops; Ozone; Reactive oxygen species; Signaling

11.1 Introduction

The rising environmental problems can be intrinsically linked to the phenomenon of global climate change and atmospheric pollution (Han et al. 2019). At present, tropospheric ozone (O_3) induced crop yield loss has been discussed and gained much limelight in tropical/subtropical countries. Mostly, countries of Southeast Asia and Central America are deemed to be more vulnerable to the rising concentration of the O_3 precursors (Ghosh et al. 2018, Cooper et al. 2014). Emission of O_3 precursor gases (hydrocarbons, nitrogen oxides, carbon mono oxides) from anthropogenic activities concomitant with high-temperature regimes and sunshine hours has been observed to inflict high O_3 concentration via photochemical oxidative reactions (Lou et al. 2015; Unger 2012). Such a prevalence of high background O_3 concentration has been a matter of global concern for the agricultural sector owing to its phytotoxic nature.

As per the highest emission scenario, Representative Concentration Pathway 8.5 of the Intergovernmental Panel on Climate Change (IPCC), the projected risk of O_3-induced damages in global vegetation increased by 70% between 2000 and 2100 (Sicard et al. 2017).

Ozone-mediated reduction in crop yield is mainly attributed to the suppression of photosynthetic activity and early senescence of leaves owing to cellular dysfunction and membrane destabilization. The reproductive stage of plants seems to be more vulnerable to O_3 injury risk as a plant prioritizes its energy investment towards developing its reproductive parts and its overall nourishment (Leisner and Ainsworth 2012). Uptake of O_3 via leaf stomata prompts the apoplastic generation of reactive oxygen species (ROS), mostly active free radicals such as singlet oxygen, hydrogen peroxide, superoxide radicals, and hydroxyl radicals, etc. (Ainsworth 2017). Such a ROS build-up in the apoplast activates costly defense metabolism in plants, which causes a resource and energy diversion from growth and yield maintenance, promoting leaf senescence (Ainsworth 2017).

Adopting different agronomic practices can be a promising solution to deal with various environmental stresses in the plants, as reported from other published studies (Ghosh et al. 2018; Cui et al. 2014; Singh and Agrawal 2009; Wang et al. 2007; McKee and Long 2001). Agronomic practices are basic scientific methods integrated into farming and agriculture to protect plants from biotic and abiotic stresses. These include soil amendment practices, shifting crop calendars, utilization of plant protectants, weed management, and other abiotic management stresses in the area experiencing a high background O_3 level. However, there are certain limitations to such applications, as reported by Teixeira et al. (2011). For example, predicting such practices' success may vary with the crop/or cultivars. It is not evident whether such practices minimize O_3-induced damage equally in every O_3 stressed region around the globe. Integration of these practices with biotechnological knowledge, appropriate economic analysis, and other environmentally sustainable farming methods such as integrating livestock and adaptation of agroforestry can broaden the farming scope and bring immense success to different environmental stresses in crops and vegetation.

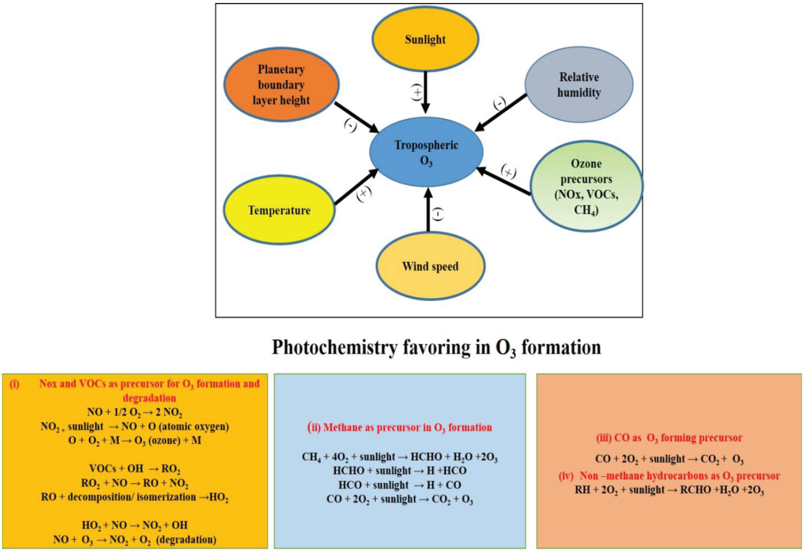
In this review, we attempted to provide an insight into the underlying mechanism(s) associated with different agronomic practices under high O_3 concentration. Under these practices, understanding such mechanism(s) is essential to apprehend the application of a particular agronomic practice in a specific existing situation, considering different environmental and agro-ecological scenarios for the same. This compilation may find its relevance for broader groups of workers such as agriculturalists, environmentalists, researchers, policymakers, and farmers to consider implementing different agronomic practices under existing and future O_3 levels.

11.2. Ozone formation, its fate and cellular response

Meteorological parameters play a crucial role in forming, transporting, and dispersing pollutants in the atmosphere (Hůnová et al. 2019; Garrido-perez et al. 2019; Pyrgou et al. 2018). As a result, alteration in the meteorological conditions viz., temperature/solar radiation, wind speed/direction, relative humidity, and boundary layer height can affect tropospheric O_3 concentration enormously, exhibiting both spatial and temporal variations (Ghosh et al. 2018). Numerous observational studies have evidenced a positive correlation between solar radiation/temperature and O_3 concentration and this is observed to be the initiating factor for driving the photochemical reactions (Pyrgou et al. 2018) for O_3 formation. Li et al. (2017) observed that the summertime concentration of O_3 was enhanced during high temperatures in China's Hangzhou district. Another critical factor is wind speed, controlling air pollutant dispersion in the atmosphere (Garrido-perez et al. 2018; Fujibe 2003). Low wind speed and high temperatures are often associated with static meteorological conditions, conducive to high O_3 concentration (Khiem et al. 2010).

Additionally, humidity is an essential factor in determining the photochemical processes for O_3 formation; however, studies' availability is limited, dealing with relative humidity on O_3 formation. Observations with high relative humidity associated with low surface O_3 concentration are generally common in data sets related to atmospheric quality; however, past models have under-predicted the weightage of such a correlation (Kavassalis and Murphy 2017) and failed to provide an explanation for the same. The statistical analysis performed by Kavassalis and Murphy (2017) of 28 years of meteorological observations and O_3 across the United States concluded that vapor pressure deficit (VPD) is the strongest indicator regulating seasonal O_3 loss via stomatal conductance and thereby dry deposition. Inclusion of VPD in the modeling study of tropospheric O_3 loss and the production process might help to provide a plausible explanation of the negative humidity–O_3 slope, which remained unexplained in the modeling studies performed in the past.

The planetary boundary layer (PBL) is the lowest part of the atmosphere through which the Earth exchanges mass, momentum, and heat, and exhibits its vital role in O_3 formation. When the height of PBL is high, it allows more vertical mixing of air, which dilutes the pollutant concentration, while inhibition of vertical mixing and stagnation of pollutants is a common feature under low PBL height, leading to increased O_3 concentration (Levi et al. 2020; Dey et al. 2018; Mbululo et al. 2018). Several studies have also reported a positive correlation between O_3 and its precursors' gases such as NO_x, VOCs, etc. (Feng et al. 2019; Wu et al. 2019; de Souza et al. 2017). Precursors mediating photochemical reactions for the O_3 production process are detailed below and schematically presented in Figure 11.1.



Photochemistry favoring in O_3 formation

(i) Nox and VOCs as precursor for O_3 formation and degradation

$NO + 1/2\ O_2 \rightarrow 2\ NO_2$

$NO_2 +$ sunlight $\rightarrow NO + O$ (atomic oxygen)

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

$VOCs + OH \rightarrow RO_2$

$RO_2 + NO \rightarrow RO + NO_2$

$RO +$ decomposition/ isomerization $\rightarrow HO_2$

$HO_2 + NO \rightarrow NO_2 + OH$

$NO + O_3 \rightarrow NO_2 + O_2$ (degradation)

(ii) Methane as precursor in O_3 formation

$CH_4 + 4O_2 +$ sunlight $\rightarrow HCHO + H_2O + 2O_3$

$HCHO +$ sunlight $\rightarrow H + HCO$

$HCO +$ sunlight $\rightarrow H + CO$

$CO + 2O_2 +$ sunlight $\rightarrow CO_2 + O_3$

(iii) CO as O_3 forming precursor

$CO + 2O_2 +$ sunlight $\rightarrow CO_2 + O_3$

(iv) Non –methane hydrocarbons as O_3 precursor

$RH + 2O_2 +$ sunlight $\rightarrow RCHO + H_2O + 2O_3$

Fig. 11.1. Illustration displaying factors regulating the formation of O_3 and the related photochemistry involved in its formation and degradation. Meteorological factors such as temperature/sunlight and O_3 precursor gases showed a positive correlation influencing the concentration of O_3 in the troposphere (shown by +), whereas, negative correlations have been reported between other factors (boundary layer height, wind speed, and relative humidity) and O_3

Furthermore, the transboundary movement of these precursor gases also determines the regional and local concentration of O_3. Despite its short chemical lifespan on Earth's surface, it exhibits an extended chemical longevity ranging from a few weeks to months once released into the tropospheric region of Earth's atmosphere (Dentener et al. 2010). Such long-distance transport is mainly regulated by wind speed and direction (Zhang et al. 2015).

The fundamental photochemistry driving the destruction and production of O_3 has been elucidated in earlier reports by Rai and Agrawal (2012), Monk (2005), Jenkin and Clemitshaw (2000), etc. In brief, the process of O_3 production in the troposphere of the atmosphere is mainly determined by the photolytic reaction of nitrogen dioxide (NO_2) to produce atomic oxygen (O) and nitric oxide (NO). The atomic oxygen thus produced consequently gets associated with a molecular oxygen atom (O_2) via thermo molecular reactions in third co-reactants (M). However, the complex inter-conversion of NO and NO_2 remains an important factor in regulating the O_3 concentration at a particular place and a given point. In other words, high NO_2 concentration in the atmosphere favors more O_3 formation while the peaking of NO with a consequent low NO_2 concentration mediates tropospheric titration of O_3 (Monks et al. 2015).

The emission of carbon monoxide (CO) resulting from the incomplete combustion of carbon from the vehicular exhaust also propagates the formation of O_3. However, the presence of oxides of nitrogen (NO_x) along with other volatile organic carbons (VOCs), both of biogenic and non-biogenic origin, triggers the formation of hydroxyl radicals (OH^-), which in turn facilitate O_3 formation via photolysis of NO_2 as shown schematically in Figure 11.1.

As we know, tropospheric O_3 is a major secondary air pollutant across the globe, which adversely affects crop production because of its phytotoxic nature and its prevalence in rural areas, mostly dominated by crops (Agathokleous et al. 2015a). As cuticular uptake of O_3 has been reported to be insignificant, stomatal flux contributed to the maximum O_3 entry to the apoplastic space, and this was largely controlled by stomatal opening and the dimension of stomata (Castagna and Ranieri 2009). Hormonal and oxidative redox signaling in the guard cell mainly drives O_3 stomatal flux (Fig. 11.2; Kangasjärvi et al. 2005). However, a reduction of stomatal conductance in O_3 fumigated plants has been observed as a protective mechanism to limit the entry of O_3 in the cellular spaces (Yadav et al. 2020; Hoshika et al. 2019; Kinose et al. 2019). Immediate diffusion of O_3 into the sub-stomatal space triggers the spontaneous oxidation of O_3 molecules to produce ROS via series of reactions occurring in the cell wall, plasma membrane and apoplast, inducing ROS production (Fig. 11.2; Ainsworth 2017; Castagna and Raneiri 2009).

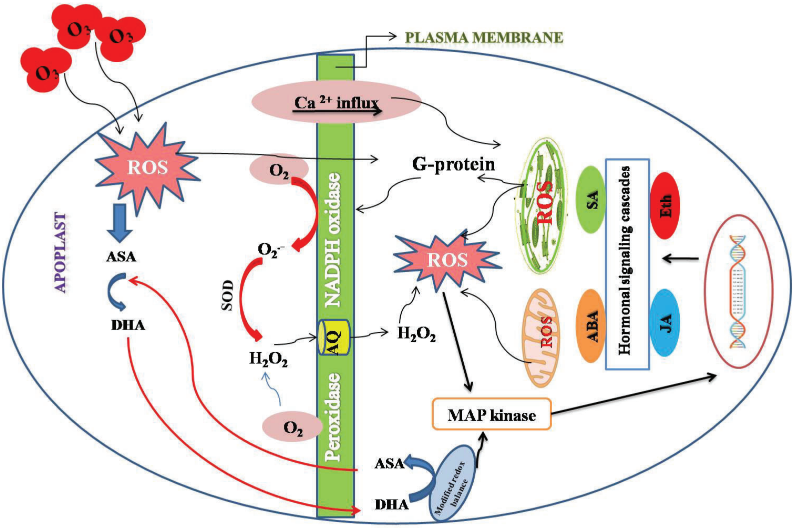


Fig. 11.2. A schematic diagram displaying O_3-induced signaling cascades as generalized cellular responses in plants (modified after Ainsworth 2017). Diffusion of O_3 into the apoplastic space induces the production of reactive oxygen species (ROS). Ascorbate in the apoplast (ASA) scavenges ROS; however, if ROS accumulation overreaches the detoxification capacity, an array of the signal is induced. NADPH oxidase and peroxidase specific to the plasma membrane promulgate ROS in the symplastic region. Heterotrimeric G-protein causes a biphasic pattern of ROS burst attributed to the activation of plasma membrane-bound NADPH oxidase. Aquaporins (AQ) are proteins involved in propagating ROS (hydrogen peroxide H_2O_2) into the symplastic region and modifying the intercellular redox balance. Such a changed redox balance results in mitogen-activated protein (MAP) kinase signaling, which alters the biosynthesis of hormones via transcriptional responses

#SOD superoxide dismutase; ABA abscisic acid; SA salicylic acid; Eth ethylene; JA jasmonic acid

The extent of ROS accumulation in the cell depends on the potentiality of the apoplastic antioxidative scavenging system, which in turn triggers a signaling cascade both in the apoplast and intercellular space (Vainonen and Kangasjärvi 2015), giving rise to a biphasic pattern of the oxidative burst with consequent H_2O_2 accumulation in the cell wall, plasma membrane, cytoplasm, mitochondria and peroxisomes (Pellinen et al. 1999). Failure of the apoplastic ROS scavenging pathway targets the bilipid membrane layer, resulting in lipid peroxidation and membrane damage (Tiwari and Agrawal 2018). Numerous ROS-forming mechanisms have been reported so far by previous reports on O_3-induced oxidative stress such as extracellular dependent peroxidases, membrane NADPH oxidase, oxalate oxidase, polyamine oxidase, etc. (Pazarlar et al. 2017; Ainsworth 2017; Vainonen and Kangasjärvi 2015; Castagna and Raineri 2009; Šebela et al. 2001). Following O_3 fumigations, evidence showed activation of plasma membrane-bound NADPH oxidase by kinase protein OST1, which is attributed to the further activation of the ion channel in the guard cells, regulating the process of stomatal opening (Ainsworth 2017). Joo et al. (2005) postulated the role of heterotrimeric G-proteins in inducing a biphasic pattern of ROS burst and further activation of plasma membrane-bound NADPH oxidase under acute O_3 exposure; however, such a mechanism may not be found to be activated under the chronic level of O_3 (Fig. 11.2; Booker et al. 2012).

The role of aquaporins (extracted from Arabidopsis) in ROS (H_2O_2) transportation from apoplast to symplast has been identified by Dynowski et al. (2008). This transportation contributed to the alteration of the cellular redox balance, which in turn induced an array of signals for the activation of mitogen-activated protein (MAP) kinase pathway (Ainsworth 2017; Ahlfors et al. 2004), resulting in the modulation of cellular hormonal signaling cascades (ethylene, jasmonic acid, salicylic acid, and ABA) under an acute O_3 dose (Fig. 11.2; Kangasjärvi et al. 2005)

11.3. Importance of different agronomic practices in controlling ozone phytotoxicity

Phytotoxicity induced under O_3 stress adversely affects the agricultural sectors incurring high economic losses world-wide. To cope with the increasing food demand, we are in urgent need of the implication of certain agronomic practices to minimize the foliar injury and yield losses occurring due to O_3. Agronomic practices are scientific methods interrelated with agriculture to reduce biotic and abiotic stresses on crops (Ghosh et al. 2018). Such practices can be shifting the crop growing period, soil amendment practices, use of plant protectants, weed management in the crop system, seed treatment with radiation, and management of other abiotic stress factors generally co-occurring in areas with high O_3 concentration. The previous work of Ghosh et al. (2018) has documented the importance of such practices against high O_3 concentration. For example, soil amendment practices by altering NPK levels could reduce O_3-induced adverse effects on crops. Nitrogen affects the plant's dry matter content by developing the leaf area and increasing the photosynthetic efficiency, thus delaying leaf senescence. Besides N, potassium's role in controlling O_3-mediated damage in plants has also been reported by Dunning et al. (1974).

Plant protectants like fungicides are also effective against O_3 injury. Applications of different fungicides have been reported to delay senescence by inhibiting ethylene synthesis and activating enzyme-mediated free radical detoxification (Saitanis et al. 2015; Wu and Tiedemann 2002). Ascorbic acid (ASA), due to its potential role as an electron donor in various enzymatic and non-enzymatic reactions, proves to be a potent antioxidant (Chaudhary and Agrawal 2014a). The studies of Agrawal et al. (2004) documented the beneficial role of foliar application of ASA in reducing O_3-induced leaf injury and the promotion of yield and biomass in three wheat cultivars. An antioxidant like ethylene diurea (EDU) is also a commonly used plant protectant against O_3 damage. Its potential role as an antiozonant has been reported in numerous past studies (Singh et al. 2015; Singh et al. 2009; Wang et al. 2007; Carnahan et al. 1978) and some recent studies (Singh et al. 2018a; Fatima et al. 2019). Syntheses of enzymes (superoxide dismutase, catalase, GSH, GSSG) involved in antioxidative defense mechanisms have been found to be up-regulated in EDU treated plants (Singh et al. 2018a; Gupta et al. 2018; Jiang et al. 2018).

Besides such applications, priming seeds with radiations like gamma (ɣ) and ultraviolet (UV B/C) before sowing which amplifies the inherent potential for stress tolerance in crop plants, has been gaining popularity in the field of agronomy. Chaudhary and Agrawal (2014b) postulated the importance of seed exposure to ɣ radiation (5 krad) as a priming technique before sowing to induce higher antioxidative potential, suggesting the stimulation of a stress tolerance ability to counter oxidative damage against elevated $O_{3.}$

Additionally, weed management in the crop system is also an important agronomic practice to combat an O_3-induced negative impact on plants. Weeds act as competitors in the crop fields and have been reported to intensify the O_3-induced detrimental effects on crop plants (Li et al. 2016). Hence, the management of such species must be given the utmost importance to prevent the pressure of multiple stress factors on crop plants. At the same time, the shifting of planting dates for crops has been a common practice often employed by farmers with an expectation to procure maximum productivity. Optimization of the sowing period is an absolute non-monetary strategy in contingent agricultural planning that can be regulated to avert deleterious conditions due to the changing global climate pattern, gain a noticeable crop yield, and minimize the production expenditure. Through a modeling study, Teixeira et al. (2011) emphasized the significance of altering planting dates with respect to peaking regional O_3 pollution. However, both positive and negative impacts have been reported under such practices due to their crop-area specificity. A study of Ferrise et al. (2010) highlighted that the adverse effect of the delayed sowing of planting dates relative to their optimum timing might expedite the phenological development, and thereby the exposure of different growth stages to altered environmental conditions with a consequent crop yield reduction. Therefore, such information warrants a further inquisition about the pros and cons of practicing alterations of the crop calendar for different crops with particular relevance to the agro-ecological conditions influencing such a practice.

Plant responses to O_3 stress get altered when encountering other stresses such as drought and salinity compared to when a single stress factor dominates. Similar meteorological conditions naturally favor the co-occurrence of high background O_3 concentration and a water deficit condition. Moreover, areas experiencing severe drought periods often pose the problem of high salinity. Cumulative effects on plant growth and productivity under the co-prevalence of both these stresses have been reported earlier (Bohler et al. 2015). However, studies related to such an interaction exhibited varying responses: synergistic, additive, and antagonistic. Stomatal regulation of O_3 uptake is a significant factor determining the plant responses under O_3/drought or O_3/salinity interactions. Closing of stomata under a water deficit/saline condition has been proposed as a defensive measure against high O_3 concentrations under co-occurrence of both stresses (O_3/drought or O_3/salinity) (Pellegrini et al. 2019; Hassan et al. 2004; Khan and Soja 2003; Herbinger et al. 2002). However, this theory can be modulated with several factors, especially the order in which the stresses have been initiated (Bohler et al. 2013; Le Thiec et al. 1994). For example, an early water deficit condition could result in a reduction of stomatal conductance with consequent protection against later exposure to a high O_3 dose. In contrast, the pre-existence of O_3 stress followed by the later appearance of a water deficit condition might exacerbate the effect of stomatal sluggishness initially caused by high O_3 exposure. Therefore, proper attention must be paid in investigating such interactions as such management can be a promising technique to regulate O_3-induced responses in crop plants.

11.4. Interactive effects of O_3 and agronomic practices

11.4.1 Soil amendments by fertilizer management

Nutrient contents in soil play an important role in determining the growth and productivity of plants. By altering the input of the fertilizer level in soil, possibilities to minimize the adverse effects induced by O_3 have been reported by Pandey et al. (2018), Singh et al. (2015; 2011), Calvo et al. (2009), and Dunning et al. (1974). Nitrogen (N) has been reported to positively impact plant photochemistry, leading to an increased assimilation rate in *Carthamus tinctorious* (Dordas and Sioulas 2008). Application of higher amounts of N in the soil seemed to contribute to delaying leaf senescence via the accumulation of N in leaves (Schulte auf'm Erley et al. 2007).

Information related to the interaction of N fertilization and O_3 pollution on crop plants is still very limited (Pandey et al. 2018). Highlighted results from the previous studies suggested a dynamic nature of such interaction, altering throughout the crop growing season (Pandey et al. 2018). Here, we have briefed different possible signaling pathways to better understand the aspect of such interaction. Firstly, the mechanisms underlying such interaction may be correlated with N's investment in protein syntheses, which plays a significant role in stimulating the photosynthetic efficiency (Palmroth et al. 2014). Moreover, the positive impact of high N on O_3-exposed plants' functionality may be attributed to the up-regulation of the enzymatic and non-enzymatic antioxidative response affected by N fertilization (Pandey et al. 2018). The role of nitrogen in the up-regulation of the synthesis of metabolites of the Halliwell-Asada cycle may have contributed to limit O_3-induced damage, as reported by Podda et al. (2019). Furthermore, N-induced stimulation of glutathione-dependent dehydroascorbate reductase activity has promoted more ascorbic acid formation, attributed to a larger ascorbate pool for ROS detoxification (Fig. 11.3; Podda et al. 2019).

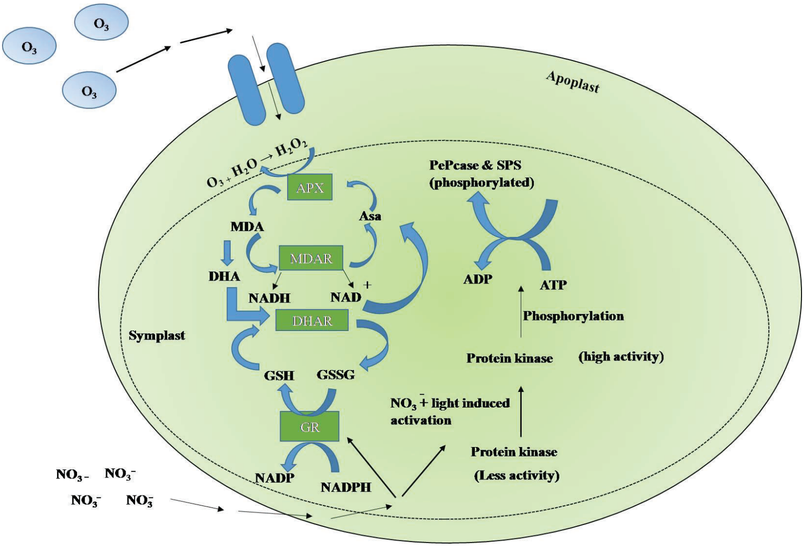


Fig. 11.3. Schematic diagram showing nitrogen's role in the form of nitrate (NO_3^-) in stimulating the enzymes involved in the Halliwell-Asada pathway to up-regulate the detoxification mechanism against O_3-induced oxidative burst. On the other hand, NO_3^- acts as signal molecules in partitioning the carbon pool away from sucrose metabolism and being directed towards amino acid synthesis via phosphorylation of phosphoenolpyruvate carboxylase (PePcase) and sucrose phosphate synthase (SPS). Phosphorylation of PePcase and SPS results in the activation of the PePcase and deactivation of the SPS

Secondly, the mechanism involving the partitioning of the carbon pool between the carbohydrate pathway and the metabolism of nitrogen via modulation of cytosolic protein kinases has been documented earlier by Champigny and Foyer (1992). Nitrogen fertilization has modulated the activities of two important enzymes involved in the process of protein phosphorylation (sucrose phosphate synthase (SPS) mediating synthesis of sucrose and phosphophenylpyruvate carboxylase (PePcase) inducing amino acid synthesis) in wheat; however, an inverse relationship has been observed for these two. The role of nitrate as a signal molecule in the cytosolic activation of protein kinase is attributed to altering the activities of these enzymes (sucrose phosphate synthase and phosphophenylpyruvate carboxylase) in the partitioning of the carbon pool, resulting in its diversion towards amino acids rather than sucrose biosynthesis (Fig. 11.3; Champigny and Foyer, 1992). This information was corroborated by the work of Landolt et al. (1997) on a tree species (*Betula pendula* Roth), where it is observed that the O_3-induced up-regulation of the sucrose pool was higher under a low N dose and reduced with N fertilization. However, the activity of PePcase remained high, irrespective of the N dose in the plants, suggesting a redirection of assimilates' flow towards the process of anaplerotic reactions mounting the process of ROS detoxification induced under high O_3 concentration. However, to our knowledge, work emphasizing such a carbon partitioning mechanism has not been reported in crop plants hitherto, encouraging future investigations in this direction. Besides several studies reporting positive feedback of N fertilization against O_3-induced phytotoxicity, a recent meta-analysis carried out by Broberg et al. (2017) reported a reduction of N, P, and K fertilization efficiency under high O_3 concentration which in turn exhibited loss of N from the agro-ecosystem via leaching and emission in the form of nitrous oxide, challenging the effectiveness of the application of a high dose of N fertilizer in countering the O_3-induced phytotoxicity.

11.4.2 Plant protectants against ozone injury

Several researches evaluated the effectiveness of different agrochemicals e.g., fungicides (azoxystrobin, epioxyconazole, benomyl, hexaconazole, Penconazole, Trifloxistrobin), antioxidants (ascorbic acid), growth regulators (kinetin and abscisic acid), and plant extracts (extract from fresh leaves of *Salvia sclarea* L., *Ocimum basilicum* L., *Tagetes patula* L.) to minimize O_3-induced crop yield loss (Saitanis et al. 2015; Chaudhary and Agrawal 2014a; Blum and Didyk 2007; Wu and Tidemann 2002). Amongst these antioxidants, a well-known chemical compound used for plant protection against O_3 phytotoxicity is ethylene diurea –N-[2-(2-oxo-1-imidazolidinyl) ethyl]-N' phenylurea (also known as EDU) which has also been used as an O_3 biomonitoring indicator (Fatima et al. 2019; Singh et al. 2018(a); Singh et al. 2018b; Singh et al. 2015; Agathokleous et al. 2015b; Oksanen et al. 2013). It is a synthetically derived cytokinin-like compound, belonging to the group of non-purine cytokinins. EDU has been observed to exhibit cytokinin-like activity and reported to inhibit chlorophyll and protein breakdown in the leaves of an O_3-susceptible tobacco cultivar (Zwack and Rashotte 2013; Lee and Chen 1982). The cytokinin mediated signaling system also delays leaf senescence via the up-regulation of extracellular invertase activity, which in turn protects chlorophyll degradation (Kieber and Schaller 2014). Another recent report by Singh et al. (2018a) depicted increments of two photosynthetic mediated proteins (PePcase and Ribulose-1,5-biphopshate carboxylase/RuBisCo) under EDU treatment in maize with a concomitant increase of foliar starch content, suggesting EDU mediated protection against an O_3-induced disruptive effect on plants' assimilative capacity.

Although EDU's structural moiety poses four nitrogen atoms, accounting for 22% of the total molecule, it has been revealed that EDU does not protect O_3-induced phytotoxicity via nitrogen addition (Manning et al. 2011). However, the realistic mechanism underpinning EDU protection against O_3 injury has remained ambiguous until now. A predictable mode of action has been attributed to its potential in inducing detoxification mechanisms via acting as a stimulant for antioxidative metabolites (Singh et al. 2018; Pandey et al. 2015; Paoletti et al. 2009; Singh et al. 2009). A recent review carried out by Agathokleous (2017) added a new dimension of understanding towards the mode of protection offered by EDU against O_3 toxicity. The work outlines the possibility of EDU mediated hormesis, explaining its mode of action (Fig. 11.4). A dose-response assessment on *Lemna minor* L. by Agathokleous et al. (2016) has reported a hormonal effect at low EDU doses indicating biological plasticity. It may exhibit overcompensations due to homeostatic disruption. However, such overcompensation may up-regulate the plant defense potential to counteract O_3 stress, thereby, extending its protective possibilities against O_3-induced phyto injuries. Moreover, an EDU-induced hormetic effect has also been reported earlier in wheat by Archambault and Li (2002).

Several plant species contain high antioxidative potentials (carotenoids, flavonoids, phenolics, etc.), which help them inhibit chain reactions (Blum and Didyk 2007). Although the utilization of such naturally extracted antioxidants is an eco-friendly plant-protecting approach against O_3 stress, limitation of their applications has been recognized owing to their low stability in the natural conditions and the requirement for application in a highly concentrated form compared to chemically manufactured antioxidants such as ethylene diurea (Blum and Didyk 2007). Other agrochemical compounds such as piperonyl butaoxide, ascorbic acid, abscisic acid, and kinetin are also known to provide protection against O_3 injury in crops (Chaudhary and Agrawal 2014a; Didyk and Blum 2011; Agrawal et al. 2004; Lin et al. 2001). Piperonyl butaoxide (PB), a commonly used insecticide synergist, is a derivative of benzimidazole reported to be a powerful protectant against O_3-induced toxicity (Nouchi 2003). Initial experiments reported that foliar treatment with 0.1% PB suppressed O_3-induced visible foliar damage by 70-100% (Fukuda et al. 1975). Numerous studies have elucidated the role of O_3-induced ROS (especially, superoxides (O_2^-) in chlorophyll decomposition (Al-Rawahy et al. 2013; Caregnato et al. 2013; Thwe et al. 2014). Therefore, it seems that the ROS scavenging prospect of these chemicals could suppress the adverse effect of O_3 on plant physiological mechanisms. Inhibition of photoreduction activity of DCPIP (2, 6-dichlorophenol indophenol) stimulated foliar injury against O_3 pollution, has been reported by the classical experiment of Koiwai and Kissaki (1976). They demonstrated that tobacco leaves pre-treated with PB partially protected the photosynthetic pigments from O_3-induced negative impacts and this was highly effective in protecting the Hill reaction activity against O_3 stress. Additionally, Koiwai et al. (1977) considered that the possible mode of PB action in preventing O_3-induced damage to membranes' permeability results from protection induced to avoid the peroxidation of the unsaturated lipid pool.

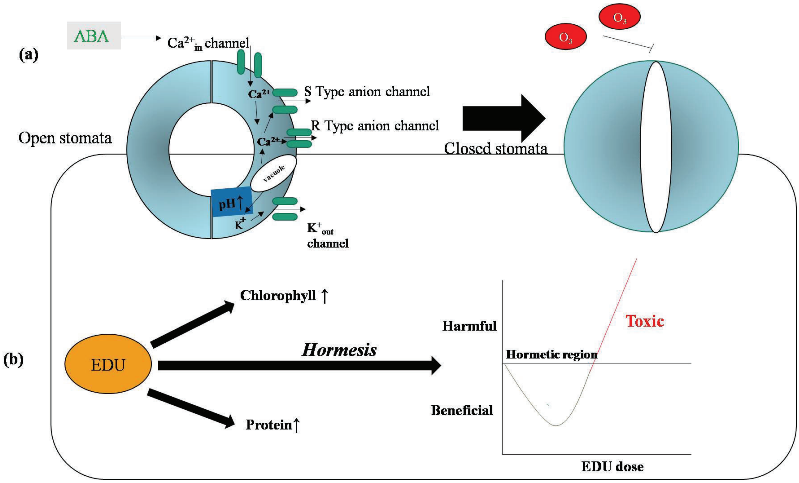


Fig. 11.4. Schematic diagram showing both abscisic acid (ABA) induced stomatal closure and EDU protection via hormesis under O_3 stress. (a) ABA activates Ca^{2+} ion influx via the Ca^{2+} influx channel and releases Ca^{2+} from voltage-dependent ion channels into the vacuolar space leading to increased Ca^{2+} concentration in the stomatal cell. Such an increase may affect anion channels (both the S-type and R-type), which leads to depolarization of the plasma membrane due to high cellular alkalization and ultimately induces stomatal closure. (b) The hormetic effect induced in plants might protect against O_3 injury on EDU application. A low dose of EDU may stimulate a better detoxification mechanism against high O_3 concentration

Recent studies have substantiated the fact of stomatal closure under abscisic acid (ABA) signaling as a promising strategy to protect plants against O_3 toxicity (McAdam et al. 2017; Munemasa et al. 2015). In particular, abscisic acid (ABA), a phytohormone, induces stomatal closure as an avoidance mechanism, thereby limiting the entry of O_3 via the reduction of stomatal uptake (Ghosh et al. 2018; McAdam et al. 2017). Complex signaling of ABA evoked anion effluxes along with potassium through ion channels present in the plasma membrane of guard cells, thereby reducing turgor pressure in guard cells and inducing stomatal closure (Munemasa et al. 2015). ABA signaling activates two different anion channels in the plasma membrane (one called slow-sustained/S-type and another rapid-transient/R-type), which stimulates the depolarization of the plasma membrane's consequent efflux of K^+ via the voltage-controlled K^+ channel (Fig. 11.4; Christmann et al. 2006). Although, activation of the slow-sustained anion channel/S-type mostly plays a crucial role in the ABA signaling stomatal closure (Brandt et al. 2015). Another important ROS role as a second messenger in guard cell ABA signaling has been documented by Wang and Song (2008). Exogenous application of ABA triggers the production of hydrogen peroxide (H_2O_2), which was first elucidated in guard cells of *Vicia faba* by Miao et al. (2000).

Additionally, ROS production is a prevalent manifestation of O_3-induced toxicity in plants, which acts as a signal for the actuation of the defense pathway resulting from stress response (Bellini and De Tullio 2019; Dai et al. 2019; Fatima et al. 2018a). However, potential stimulation of the ascorbate-glutathione pathway facilitates the scavenging of H_2O_2, which reverses the action of H_2O_2/ABA signaling stomatal closure (Chen and Gallie 2004) and might expedite the O_3 flux via stomata. Chen and Gallie (2004) documented that guard cells with potent ascorbate redox status showed less responsiveness towards ABA or H_2O_2 stimulation. Therefore, the efficacy of utilizing ABA as an O_3 protectant seems to have limitations and it might not always prove to be effective in protecting plants against $O_{3.}$ Furthermore, the concept of hormesis must be considered regarding using such agrochemicals to reveal the appropriate doses with their maximum efficacy in preventing O_3 photodamage. Therefore, it is suggested that interest should be taken for future prospects to screen the effectiveness of such agrochemicals along the dose-response continuum on different biological models followed by a subsequent selection of these doses in the range of triggering the defense mechanism to be applied to cope with O_3 toxicity in a proper scientific manner.

11.4.3 Weed management in the crop system

In general, weeds can be considered as complex ecotypes existing both as native and invasive species, naturally possessing the potential for adaptability under the pressure of a changing climate (Neve et al. 2009). Previous studies have reported that several weeds emerged to be more competitive than the crop under changing climate scenarios such as extreme heat and water deficit stress (Clements and DiTommaso 2012; 2011). Competition due to weeds' presence in the field is among the leading important biological factors inducing crop yield reduction (Gallandt and Weiner 2015; Miller et al. 2012). Primarily, such competition exists because weeds utilize resources (in terms of water, light, nutrients, etc.) that otherwise would have been available to the crop species (Booker et al. 2009). The magnitude of crop yield reduction depends on numerous other environmental factors; out of all of these, O_3 has been reported to attenuate interspecific competition's adverse effect relative to the crop species (Li et al. 2016; 2013; Fuhrer 2003). However, data related to such crop-weed interaction under changing O_3 concentrations are still rare. Li et al. (2016) observed that flixweed competition pronounced the adverse effect of elevated O_3 in winter wheat and caused a higher yield reduction than that grown under elevated O_3 alone. The presence of a more developed and stronger antioxidative system in flaxseed favored its growth under elevated O_3 concentration as compared to winter wheat. It emerged to be more competitive under the future climate change scenario. Moreover, plant exposure to elevated O_3 can lead to a shift in the competitive pattern under crop-weed interaction (Ghosh et al. 2020c; Kaciene et al. 2019; Li et al. 2013; Shrestha and Grantz 2005). Kaciene et al. (2019) observed an interaction between summer rape (*Brassica napus* L.) and wild mustard (*Sinapis arvensis* L.) under elevated O_3. They observed that in monoculture conditions (grown singly), rape emerged to be more competitive than wild mustard under O_3 stress alone; however, when grown under mixed culture conditions, weed competition interferes strongly with rape growth, and this effect was intensified under the combination of both factors (O_3 + competition).

Interestingly, the concept of allelopathy cannot be ignored when it comes to plant/plant interaction. However, to our knowledge, no such studies have been performed to evaluate allelopathy's role in crop-weed interaction under O_3 stress. Several studies revealed O_3-induced stimulation of phenolic synthesis in both crops (Fatima et al. 2018a; Saviranta et al. 2010; Li et al., 2008) and weeds (Kaciene et al. 2019; Cotrozzi and Nali 2014), playing an important role in plant defense against stress. However, such phenolic compounds in allelopathic interaction have also been reported earlier (Li et al. 2010; Belz 2007; Inderjit 1996). A recent study conducted by Ghosh et al. (2020c) documented that an O_3-induced increase of phenolic acids has been observed in both wheat and fat-hen; however, when grown together, a higher rise of phenolic compounds and flavonoids in the roots of fat-hen might have interfered with the growth of wheat which resulted in higher yield losses under combined stress factors. These findings allow us to assume that the presence of a high phenolic content in the root exudates of the plants grown under stress conditions may interfere in the growth reduction of the neighboring plants (Fig. 11.5; Li et al. 2010). O_3-induced reduction of microbial biomass has been reported in the recent studies of Fatima et al. (2018b) and Dong et al. (2019), suggesting a decline of the microbial population, which may slow down the degradation of phenolic compounds in the soil. As a result, the accumulation of these phenolic compounds in the soil may reach its phytotoxic level (Blum 1998). Therefore, the potential mechanism in such crop-weed studies based on phenolics' allelopathic interaction should be investigated under O_3 stress. A clear picture of biochemical signaling can be established to understand such an interaction changing the climate scenario.

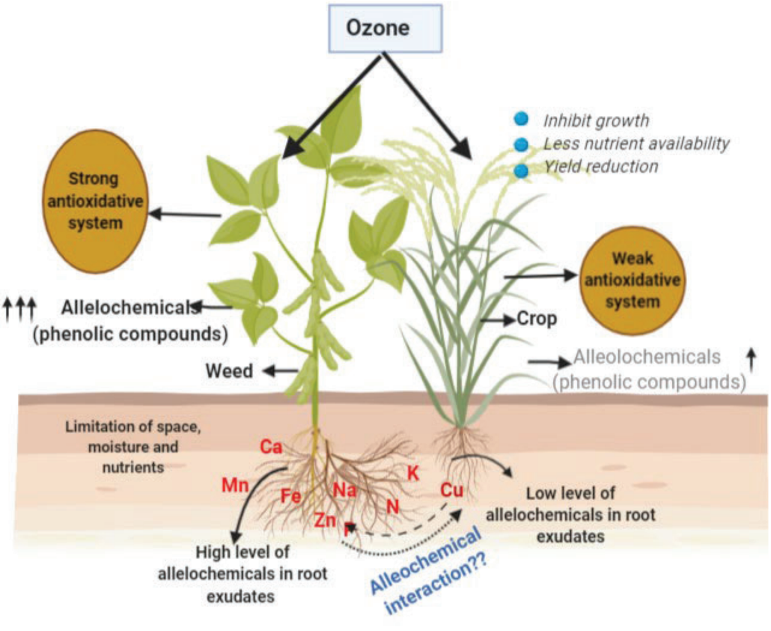


Fig. 11.5. Diagram showing an interactive effect of O_3 and weed competition in the crop field. In such an inter-competitive situation, crop growth is altered based on the comparable responses of the species exposed to O_3. Weeds have emerged to be more potent and competitive under high O_3 concentration (Kaciene et al. 2019; Li et al. 2015, 2013) relative to the crop species. The association between O_3 fumigation and weed species has displayed a significant O_3 resistance capacity in

weeds due to the more developed antioxidant system. A hypothesis has been framed based on O_3-induced up-regulation of phenolic compounds in weeds, which may affect the allelopathic interaction via root exudates. Such allelochemicals in the soil chelate nutrients and limit their uptake availability, affecting plant growth and productivity. However, question marks have been placed before the allelopathic interaction as studies associated with O_3 and allelopathy are still rare and lack much evidence

11.4.4 Seed treatment with radiation

The enhancement of crops' stress tolerance capacity has channelized thoughts towards utilizing radiation for priming seeds and/seedlings to counterbalance oxidative stress-induced stress. Such seed exposure to a low dose of radiation is generally observed to be safe concerning plant productivity and yield with the concomitant stimulation of tolerance in plants against biotic and abiotic stresses (Delibaltova and Ivanova 2006). Priming seeds with ultraviolet (UV)/gamma (γ) radiation prior to sowing has already been reported to impart natural resistance to plants against different abiotic stress factors such as O_3 (Chaudhary and Agrawal 2014b), water deficit stress (Moussa 2011), and salinity (Aboul Fotouh et al. 2014; Helaly and Hanan El-Hosieny 2011) which involves various physiological and biochemical changes, resulting in such positive feedback (Mazza et al. 2013). Prior exposure of 5 krad γ radiations to clover seeds improved the performance against O_3 concentration of 47.4–55.6 ppb due to pre-activation of the antioxidant system under treatment of γ radiation before germination (Chaudhary and Agrawal 2014b).

Although studies related to such a priming effect of radiation have been performed by various research groups in different crop plants, the possible mechanism underlying the action of radiation in the up-regulation of such metabolic activities is still not well understood. However, the mode of action of UV-B radiation at the molecular level in the model plants of *Arabidopsis thaliana* and *Zea mays* (maize) has been explored. Binkert et al. (2016) observed that the UV-B photoreceptor, UV RESISTANCE LOCUS8 (UVR8), is responsible for regulating the UV-B signaling in plants. UV-B photoreception up-regulates the accumulation of UVR8 in the nucleus, which stimulates chalcone synthase (CHS), a UV target gene, and the primary gene involved in the regulation of the flavonoid biosynthetic pathway (TT and Puthur 2017). Furthermore, Jenkins (2014) observed UVR8 dimer dissociation into monomers via UV-B absorption, which initiated the transduction of signals in combination with constitutively photomorphogenic1 protein (COP 1). Yin et al. (2016) reported the dual role of COP1 in regulating UVR8 accumulation and its signaling. Various UVR8-related genes reported encoding for chloroplast proteins, suggesting its involvement in photosynthesis due to UV-B radiation (Davey et al. 2012).

11.4.5 Management of other abiotic stresses such as water deficit stress and salinity

High O_3 concentration and water deficit stress often coincide when the temperature is high during summer (Wilkinson and Davies 2010). The increasing pattern in the background O_3 concentration, mostly in tropical and sub-tropical countries, along with the rise in the severity of drought stress periods (Bates et al. 2008), directed the attention of various studies involving interactions of O_3 and other concurrent stress factors such as drought and salinity, to more accurately evaluate the impacts of present and future climates on different crop plants (Wittig et al. 2009). Evidence displaying the high tolerance of crop species to O_3 stress under water deficit conditions was mainly correlated to reducing stomatal O_3 uptake due to drought-induced stomatal closure (Harmens et al. 2019; Gao et al. 2017; Herbinger et al. 2002). The study of Harmen et al. (2019) concluded that a reduced water supply could be practiced as a useful management tool to provide partial or full mitigation against high O_3 concentration. The study circumscribed stomatal regulation in controlling the uptake of O_3, resulting from reduced stomatal conductance under low irrigated conditions, thereby limiting the stomatal flux of O_3. However, studies related to such interactions are very complicated in nature, and variable responses have been reported hitherto (Pellegrini et al. 2019; Ghosh et al. 2018; Pollastrini et al. 2014; Khan and Soja 2003; Grulke et al. 2002). The interactive effect of drought/salinity with O_3 in different crop species since 2000 has been presented in Table 11.1.

Table 11.1: Interactive effect of some abiotic stresses (water deficit/salinity) with O_3 in crop species (2000 – 2020)

Species / cultivars/varieties	**Ozone dose**	**Water deficit /Salinity stress (dose/level/ concentration)**	**Abiotic stress**	**Type of interactions**	**References**
Triticum aestivum cv. HD 2967	65.9±1.1 ppb (seasonal mean)	50% s.w.c	Water deficit	(-)	Ghosh et al. (2020)
Punica granatum L. cv. Dente di cavallo	58.7 ppm h (AOT 40 throughout the experimental period)	50mM NaCl	Salinity	(-)	Calzone et al. (2019)
Triticum aestivum L. Korongo	36.5 ppb, (24 h mean)	10% soil water content	Water deficit	(-)	Harmens et al., 2019
Vigna unguiculata (L.) Walp	120±10 ppb (12 h d^{-1})	Three week water deprivation	Water deficit	(+)	Rebouças et al. (2017)

Triticum aestivum L. cv. BAW1059 and Shatabdi	24 and 34 nL L^{-1} (24 h seasonal mean)	4 and 8 dSm^{-1}	Salinity	(-)	Kamal et al. (2015)
Triticum durum Desf. cv. Virgilio Neodur	21ppm, seasonal mean	75mM NaCl	Salinity	Both (-) and (+)	Gerosa et al., (2014)
Triticum aestivum L. cv. Dekang961 and Lumai15	80±5 ppb (8 h d^{-1}) for 30 days	100mM NaCl	Salinity	(-)	Zheng et al., (2014)
Medicago truncatula cv. Jemalong	70 nmol mol^{-1} (6 h d^{-1})	Withholding water supply for 10 days	Water deficit	(+)	Iyer et al. (2012)
Triticum aestivum (L.) cv. Dekang961 Lumai15	80±5 ppb (8 h d^{-1}) for 30 days	100mM NaCl	Salinity	(-)	Zheng et al., (2014)
Triticum aestivum L. Xiaoyan 22 *Triticum turgidum ssp durum*	83 ppb, (7 h d^{-1})	42% s.w.c	Water deficit	(-)	Biswas and Jiang (2011)

Medicago sativa L.var. Garisenda	14.7 ppm h (AOT value, 2005)	1.06dSm^{-1}	Salinity	(+)	Maggio et al., 2009
Glycine max	10 ppm h (AOT, at the end of the soyabean crop cycle)	Water stressed (-0.8MPa< leaf water potential< -0.4MPa)	Water deficit	(+)	Jaoude′ et al. (2008)
Lycopersicon esculentum Mill.cv.Paco	38(filtered)- 62ppb (non- filtered) (ambient)	7.0 dsm^{-1}(osmolarity)	Salinity	(-)	Maggio et al., 2007
Triticum aestivum L. cv. Giza 63	50 mm3 m-3 (8 h d^{-1})	50mM NaCl	Salinity	(+)	Hassan (2004)
Triticum aestivum L. cv. Perlo	80 ±6 nL L^{-1} (8 h d^{-1})	Water deficit stress (35% s.w.c)	Water deficit	(+)	Khan and Soja (2003)
Triticum aestivum Desf.cv. Nandu and Extradur	Ambient+50 ppb	40% s.w.c.	Water deficit	(+)	Herbinger et al. (2002)
Cicer arietinum L. Var. CSG8890 CSG88101	85nmol mol^{-1} (6 h d^{-1})	30mM NaCl	Salinity	(-)	Welfare et al. (2002)

Triticum aestivum L. cv. JN17	80±5 ppb (8 h d^{-1}) for 30 days	100mM NaCl	Salinity	(+)	Zheng et al. (2011)

s.w.c soil water capacity; AOT 40 accumulate O_3 dose threshold above 40 ppb; (+) additive/synergistic interaction; (-) antagonistic interaction

Presently the challenge is to manage the supply of water to such an extent that it can sustainably reduce the chances of causing drought stress without elevating the O_3 phytodamage. Besides the concept of drought-induced protection against O_3 uptake, additive and synergistic responses under their interaction have also been observed due to the reduction of carbon fixation and its limited availability towards the overall plant defense mechanism (Ghosh et al. 2020a; Cotrozzi et al. 2016; Alonso et al. 2014; Matyssek et al. 2006; Grulke et al. 2002). A recent study by Ghosh et al. (2020a) elucidated an additive interaction of water deficit stress and O_3 on the grain yield of an Indian wheat cultivar HD 2967. The reduction of water availability might have checked the uptake of nutrients and its translocation to various parts, causing an unusual shift in the plant's normal physiological functions. Such nutrient limitation and low photosynthetic rate under combined stress might have lowered the plants' ROS detoxification potential, resulting in high yield loss.

Somewhat similar views exist concerning the responses under the combined effect of O_3 and salt stress. Observations are sometimes contradictory; either salinity stress counterbalanced the O_3-induced adverse effect by declining stomatal opening (Gerosa et al. 2014; Maggio et al. 2007; Hassan 2004) or aggravated the impact of the O_3-triggered impact (Calzone et al. 2019; Guidi et al. 2017, Zheng et al. 2014). According to Deinlein et al. (2014), the carbon reserves and energy investment policy and the osmoregulating mechanism played a significant role in plant tolerance to saline conditions regulating salt stress interaction with other stress factors. Besides the plants' physiological mechanism, observations related to the cellular and metabolic status provided supplementing evidence to correlate the role(s) of several antioxidants and metabolites in plant acclimatization under stress conditions.

The O_3-drought/-salinity responsive signaling mechanism on stomatal conductance is generally regulated through the cross-talk of ethylene signaling with abscisic acid (ABA). ABA's primary role in stomatal closure under water deficit/salinity conditions has been observed in past studies (Wilkinson and Davies 2010). Similarly, ethylene production has been found to be up-regulated under both O_3 stress (Rao et al. 2002) and water deficit stress (Voisin et al. 2006; Sobeih et al. 2004), individually. However, an antagonistic interaction has been found between ethylene and ABA signaling regulating stomatal sensitivity under both stresses. The sensitivity of stomata to ABA declined under O_3-induced ethylene stimulation due to the inhibition of stomatal closure in the presence of ABA induced under water deficit stress (Fig. 11.6; Mills et al. 2009).

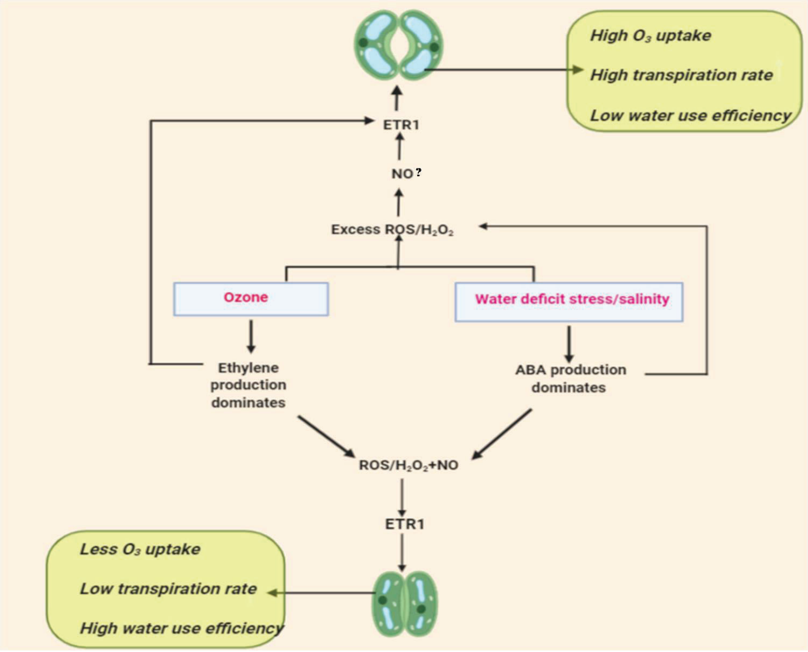


Fig. 11.6. The signaling pathway induced in response to O_3 and other abiotic stresses (water deficit/salinity) mediating stomatal closure and non-closure. Production of abscisic acid (ABA) and ethylene dominates under water deficit stress/salinity and a high O_3 dose, resulting in consequent stomatal closure via H_2O_2 signaling. However, under the interaction of both stresses (O_3 + water deficit/salinity), high H_2O_2 accumulation in the presence of nitric oxide (NO) inhibits stomatal closure. Another alternative mechanism proposed by Wilkinson and Davies (2010), postulating ethylene's preferential role on ethylene receptor gene 1 (ETR1), hinders ABA-induced H_2O_2 signaling in stomatal closure. The role of NO in this signaling pathway is still not clearly understood relative to ETR1, and hence a question mark has been placed before NO (modified from Wilkinson and Davies 2010)

Such a condition impedes stomatal closure, resulting in higher stomatal conductance under a water deficit and elevated O_3 state, and water loss continued to be high in the form of transpiration. A hypothetical mechanism suggested by Wilkinson and Davies (2010) showed that in the absence of either of the hormones, stomatal closure was induced via H_2O_2 signaling. However, under both hormones' dominance, the authors predicted the complete failure of stomatal closure due to much higher H_2O_2 accumulation and nitric oxide (NO) (Fig. 11.6). Alternatively, ethylene might act at the ethylene receptor gene ETR1 to prevent ABA-induced H_2O_2 signaling in stomatal closure. However, the role of NO in such signaling cascades is still very uncertain. Such results challenged the concept of the stomatal O_3 exclusion principle (Harmens et al. 2019; Reboucas et al. 2017; Gerosa et al. 2014; Herbinger et al. 2002), predicting the partial protection offered against O_3 uptake under water deficit stress/salinity. These findings reflected the importance of proper water management in agricultural systems under prevailing changing climatic conditions. Other climate change variables were inducing ABA-mediated stomatal closure such as high PAR (Devireddy et al. 2018; Haworth et al. 2018), temperature (Killi et al. 2017), VPD (Yaaran et al. 2017), and salinity (Xu et al. 2018), which can also be suppressed under O_3 stress. The studies performed by Yu et al. (2019) and Wilkinson and Davies (2009) help us to infer that O_3-induced stomatal closure in response to any stress stimulating the ABA-mediated regulatory mechanism exhibited antagonistic cross-talk as a normal signal and caused a reduction of ABA-mediated stomatal closure.

11.4.6 Shifting the crop calendar

Shifting the crop calendar may be an effective adaptation strategy for farmers to combat climate change (Sacks et al. 2010). The crop sowing time is an important component of agronomic practices that determines the different seasonal conditions to which plants get exposed, affecting crop growth and productivity (Caliskan et al. 2008). Interestingly, a modeling study by Teixeira et al. (2011) postulated the possible advantages of adjusting crop calendars to help crops escape high O_3 concentration. However, such adaptation may not exhibit uniformity for all the crops/ cultivars and may not be validated as useful for all O_3 affected regions. To date, the individual effect of delayed sowing on crop productivity has been documented for a different number of crop species; however, to our knowledge, no studies in the past have reported the interactive effect of shifting calendar practices under high O_3 concentration.

A recent experiment performed in India assessing the effectiveness of delayed sowing under high O_3 concentrations in wheat cultivar HD 2967 displayed that this practice did not imply any effectiveness for wheat cultivation against O_3 triggered yield losses under the prevailing environmental conditions of the Indo-Gangetic plain (Ghosh et al. 2020b). In delayed sowing by 20 days, plants were exposed to higher AOT40 O_3 concentration during their reproductive period, resulting in an O_3-induced lowering of photosynthetic rate, thereby causing limitation in the availability of assimilates for proper grain development. The study also documented that wheat plants displayed growth reductions under the combined treatment of elevated O_3 and timely sowing conditions. Simultaneously, synergistic interactions were observed for the grain yield when plants were exposed to a combined treatment of a high O_3 dose and late sowing conditions. Another option for such an adjustment is selecting appropriate crop varieties with different growth cycles to cope with adverse environmental factors (Teixeira et al. 2011). In another study, Yadav et al. (2019) showed the high vulnerability of early sown wheat cultivars to O_3 relative to the late sown cultivars based on their detoxification potential via the antioxidative defensive mechanism, concluding with the preference for selecting late sown cultivars in areas of Northern India experiencing high background concentrations of O_3. More such experiments are needed in different parts of the globe using other crop plants to determine the flexibility of adopting such practices to deal with the rising concern of O_3-induced crop losses.

11.5 Conclusions

The rise in background concentrations of O_3 and its consequent deleterious effect on crop productivity and quality of products across the globe have been a remarkable challenge to global food security for a rapidly growing population. The importance of numerous agricultural practices has been identified, which could counter O_3-induced injuries in crops and prevent economic losses. Adaptation to certain agronomic practices such as soil amendment via fertilizer management, agrochemicals, weed management, a shifting crop calendar, and seed priming with radiation might help the crop trigger its self-defense capability either by stress avoidance or the stress tolerance pathway. However, complex mechanisms and modes of action under such interactions between agronomic practices and O_3 determine the possibilities of success to some extent. For example, applying agrochemicals such as fungicides, natural antioxidants, and the most compelling, EDU could prove important in ameliorating O_3-induced crop injuries. However, the concept of hormesis should be considered in understanding their mode of action and selecting appropriate doses in a range, causing a stimulating effect, capable of counteracting O_3 phytotoxicity.

Soil amendments via N fertilization proved to effectively reduce yield loss under high O_3 concentration by triggering the potential antioxidative pathway. Diversion of the carbon pool away from sucrose synthesis towards anapleuretic reactions favored the antioxidative stimulatory effect of N. On the other hand, O_3 pollution has been reported to reduce applied fertilizers' efficiency resulting in its loss via leaching or gaseous soil emission. Adjustment in the crop sowing calendar could protect plants against the O_3 peaking period during different critical growth stages. A recent report documented that late sowing of wheat plants by 20 days has not proved to be feasible (in terms of economic yield) under high O_3 concentration in the agro-ecological scenario of the Indo-Gangetic plains. Therefore, such a hypothesis may not be accurate for all crop types and proved to be of regional importance.

Consideration of weed management practices seems to ensure a less competitive environment for crop growth corresponding to indispensable resources, affecting crop productivity. Ozone has been reported to trigger weeds' competitive nature via a strong antioxidative system and more adverse conditions for crops growing under these stress factors. Nonetheless, allelopathic interaction should also be considered for future investigation to understand the apparent mode of action under weed competition and O_3 pollution on crop plants. Additionally, seed priming with radiation before sowing could impart natural resistance to crops to cope with O_3 triggered phytotoxicity via up-regulating UVR8-related genes encoding for chloroplast proteins' stimulation of plant photosynthetic capacity. Furthermore, management of other abiotic stresses exhibiting high co-occurrence with O_3 peaking conditions, such as drought and salinity, must be considered to avoid possible synergistic or additive effects, resulting in higher adversity in crop responses. To establish a better assessment of the effectiveness of such agricultural practices in combating the menace of O_3 on crop productivity and the modulation of plant responses under their interactions, undertaking future investigations tracing out the underpinning mechanistic pathways should be a priority.

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CHAPTER TWELVE

IMPLICATIONS OF OZONE ON ECOSYSTEM SERVICES

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Abstract

Anthropogenic activities trigger the emission of a wide array of pollutants in the atmosphere, including the secondary pollutant tropospheric ozone (O_3), a damaging phytotoxicant. However, the ecosystem's responses to O_3 will feed back directly or indirectly on the meteorology and atmospheric composition, global productivity, different ecosystem services, and global biodiversity. Here we tried to review the influence of past, future, and current trends of O_3 concentration on various ecological services and related processes that are interlinked in nature. Experimental studies exhibit the negative impacts of O_3 on plant growth, reproductive structures, primary productivity, timber production, water economy, species composition, nutrient cycling, below-ground processes, carbon sequestration, pollination, aesthetic values, and human health. Such processes are categorized into four major ecosystem services: supporting, provisioning, regulating, and cultural. This chapter marks the compilation of these processes based on information related to the ecological system's services. It thereby highlights the potential overall implications of O_3 on such services and biodiversity. It shows the necessity for adopting a multi-sectoral approach to decision- and policymaking systems related to air pollutants for humanity's future well-being.

Keywords: Biodiversity; Ecosystem services; Ozone; Productivity

12.1 Introduction

The simple notion of ecosystem services is widely attributed to the advantages that humans resort to for the functioning of healthy ecological systems (Jeffers et al. 2015), basically representing a unidirectional service flow from the environmental system in favor of humankind (Comberti et al. 2015). However, this conceptualization simply ignores the reality of mankind, contributing to its destruction and over-exploitation, as frequently evidenced, either directly or indirectly. Population bloom concomitant with endless human desires in raising living standards, accompanied by the constant up-gradation of technicalities and several societal changes, has caused intense derogatory impacts on the ecological systems' service quality. An increase in global pollution mostly due to anthropogenic influence has directly led towards ecosystem degradation and other causes such as demographical, socio-economic, political, cultural, spiritual, scientific, and technical advancements, being categorized as indirect drivers.

As per the classification provided by the Millennium Ecosystem Assessment (2005), eco-services have been simply categorized into regulating, provisioning, cultural, and supporting services (Table 12.1). It is usually evident that impacts on eco-services are interconnected, i.e., a change caused by a driver in one service may affect other eco-services and vice-versa. For example, tropospheric O_3-induced negative impact on plant photosynthesis would impact the provisioning services in terms of yield loss, low-quality timber production, etc. Other benefits would also get affected, as stated by alteration in water use (regulating services), alteration in nutrient cycling, lowering of primary production, etc. (supporting services) and loss of the aesthetic appearance of the ecosystem (cultural services) (Mills et al. 2013). Moreover, changes in the ecosystem have been mainly attributed to the underlying myriads of interactive processes amongst the drivers, determining the combined impact of multiple factors, amplified via reciprocal feedbacks (Nelson et al. 2006).

Table 12.1. Type of ecosystem services and benefits obtained for human welfare and the healthy functioning of services (Millennium Ecosystem Assessment 2005)

Type of services	Definition	Benefits derived from the services
Supporting services	Services that promote the unhindered functioning of other ecosystem services	Nutrient cycling, soil formation processes, hydrological cycle, primary production
Provisioning services	Services that depict the energy flow and material outputs from the ecological system	Food, timber, fiber, genetic resources, water
Regulating services	Service is derived from natural systems that aid in determining environmental conditions	Climate regulation, air quality, pollination, flowering
Cultural services	Services which are non-materialistic benefits derived from nature via intellect, spiritual beneficiation, recreation, etc.	Education, inspiration, aesthetic pleasure, spiritual peace

Tropospheric O_3 has been considered to be a critical phytotoxic air pollutant, mainly formed at the surface level when precursor compounds such as oxides of nitrogen (NO_x) and volatile organic compounds (VOCs) undergo photochemical reactions (Hayes et al. 2020). With the continuous rise in global population, one can observe the budding of the limitless requirement for resources such as fossil fuels, food, space, etc., which is likely to trigger the emissions of O_3 precursor gases (Oltmans et al. 2006). As variations in background O_3 concentration displayed spatial and temporal differences, it is therefore regarded as a serious concern on a global scale (Van Dingenen et al. 2009). Several studies conducted in different parts of the globe documented an increasing trend of O_3 in regions such as South Korea (Shin et al. 2017), China (Wang et al. 2018), India (Ghosh et al. 2020c; Kumari et al. 2020), Africa (Huang et al. 2018), the United States (Choquette et al. 2019; Jiang et al. 2018) and Europe (Sicard et al. 2020).

In reference to the ecosystem services, concentrations of O_3 have usually been observed to be much higher in the suburbs and upland regions, mostly considered as the leeward side of urban establishments, whereas in cities, the presence of other pollutants causes O_3 titration, resulting in the prevalence of a much lower background concentration of O_3 (Mills et al. 2013). Such sub-urban regions are mainly dominated by natural (forests) or human-made ecosystems (agricultural land, water reservoirs), which are mostly recognized as the major suppliers of essential services for mankind. The impacts of O_3 on different ecosystems will vary between direct and indirect effects, marked with induced toxicity, alteration of ecological interactions between organisms, and variations in the rate of various biological and chemical processes (Ainsworth et al. 2012). The entry of O_3 into vegetation mainly occurs through the leaves' stomata, later reacting in the apoplast to generate reactive oxygen species (ROS) (Wilkinson et al. 2012). This production of ROS can induce damage to the cell structure and membrane and trigger an array of biochemical reactions to damage photosynthetic structures (Caregnato et al. 2013), resulting in an alteration of plant physiological functions, growth reduction, an alteration in carbon stock, water availability and species composition, etc. Therefore, the impact of O_3 can be recognized on the functioning processes of the ecosystems, while a reductionist scenario in steps deriving benefits from such services is often observed.

The present chapter highlights the interference of existing and predicted O_3 concentrations in the provision of ecosystem services via vegetation and biodiversity. It also emphasizes the impacts of O_3 on the different processes and benefits associated with ecological systems, including supporting, provisioning, regulating, and finally, the most neglected, cultural services. The role of biodiversity in maintaining ecosystem stability is also discussed to provide a deep insight into understanding how environmental changes directly impact the ecosystem’s functioning. This compilation draws attention to adopting a more comprehensive approach in making decisions and policies to assess the unwanted alteration in the ecosystem along with the gap between supply and demand and the prevailing scenario of each ecosystem service in a changing environment.

12.2 Effect of ozone on the supporting ecosystem

12.2.1 Change in soil processes, micro-flora, and associated nutrient cycling

Although the below-ground processes are not directly exposed to O_3 toxicity, translation of the O_3-induced detrimental effect on above-ground processes is evidenced, resulting in the consequent alteration of nutrient cycling in association with changes caused in the microbial community (Lindroth 2010). The existing concentration of O_3 is very much capable of altering the rhizospheric interactions (Ghosh et al. 2020a; Fatima et al. 2018) and soil carbon flux. Generally, most of the research findings in relation to the O_3-induced carbon allocation documented a preferential allocation pattern of biomass towards shoots compared to the roots (Grantz et al. 2006), thereby causing a reduction of root litter input, which may subsequently pose major consequences to the rate of soil C formation on the prolonged period (Fuhrer et al. 2016). Furthermore, retardation in the rate of litter decomposition could be due to the alteration of soil microbial biota in consequence of O_3-induced modification in the litter quality (Aneja et al. 2007). Detritus generated from O_3 fumigated plants has been reported to be less prone to degradation (Kim et al. 1998), usually as a result of a higher C/N ratio (Wittig et al. 2009), and an increased level of tannins, lignins (Richet et al. 2012) and other phenolic compounds (Liu et al. 2005).

Microorganisms thriving in the rhizospheric zone typically contribute to recycling organic matter added to the soil and other nutrients and form a major part of the soil carbon pool (Chen et al. 2009). The root substrate derived from the O_3 exposed plants would impact the structure and function of soil microorganisms (Zhang et al. 2016, 2019) due to a steep reduction in the carbon flux in soil via plants and alteration in the availability of nutrients (Li et al. 2013; Booker et al. 2005). To be more specific, O_3 has been reported to cause a reduction in soil microbial biomass (both carbon and nitrogen), the diversity of bacteria, fungi, and another microfauna, especially those involved in the fixation of soil N and C, and also an induced shift in the structure of the microbial community (Ghosh et al. 2020a; Fatima et al. 2018; Huang and Zhong 2015; Pritsch et al. 2009) which could further slow down the litter decomposition. Eventually, such a downturn in the litter decomposition process will, in turn, impede the movement of C stock in the soil fractions along with other nutrients (Fig. 12.1).

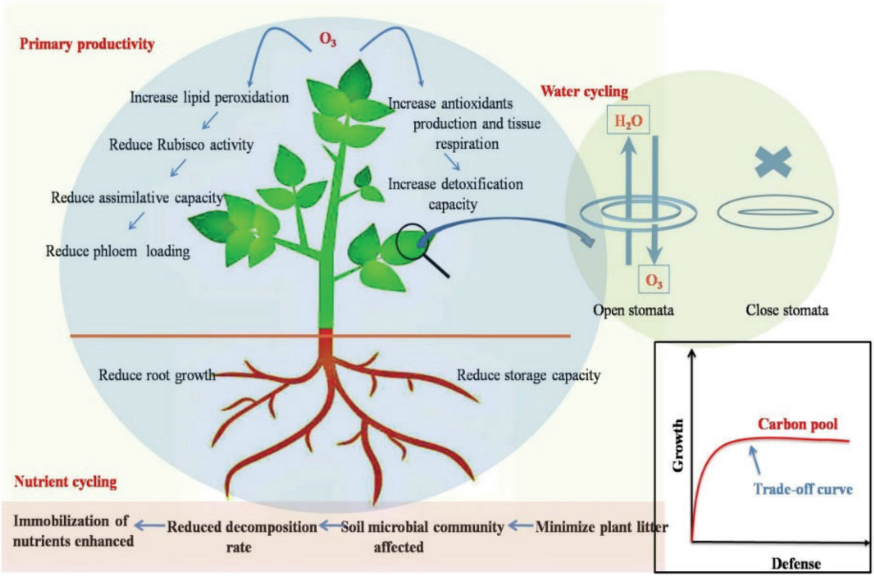

Fig.12.1: Pictorial illustration of the O_3-induced alteration of primary productivity, nutrients, and water cycling. (A) Primary productivity: Reduction in assimilative capacity owing to foliar damage and premature senescence caused under elevated O_3 concentration. Enhanced demand of C for detoxification and repair will, in turn, alter the allocation pattern, resulting in less availability of C for plant growth. The subset in the figure depicts a trade-off between growth and defense. Less of a carbon pool will be available for plant growth and its allocation to below-ground processes. More C is required for secondary plant metabolism to detoxify O_3 and/or repair O_3-induced cell damage, thereby regulating the source-sink balance. (B) Water cycling: Elevated O_3 can induce both stomatal closure and stomatal opening. Stomatal closure will eventually aid the preservation of soil water while water loss from both soil and vegetation results under O_3-induced stomatal opening conditions, thereby influencing the water availability and stock. (C) Nutrient cycling: O_3-induced alteration of C allocation towards shoots compared to roots reduces root litter input, which may pose a significant consequence to the rate of soil C formation. Furthermore, retardation in the rate of litter decomposition could alter soil microbial biota as a result of O_3-induced modification in the litter quality. Eventually, such a downturn in the litter decomposition process will, in turn, impede the movement of C stock in the soil fractions along with other nutrients

As evidenced from the past literature, plants grown under O_3 exposure displayed a limitation of C allocation to the below-ground plant parts (roots) as their growth gets circumscribed by the impairment of carbon assimilation and an increased demand of C towards chemical and physical defense mechanisms (Andersen 2003). Such a process further negatively influences the symbionts' colonization of roots as under a C limited condition, plants reduced the transfer of C to fungus and, in turn, raised the competitive possibilities between the host and fungus for the available C stock (Bassin et al. 2017). However, the inconsistencies of such outcomes have also been observed. For example, Duckmanton and Widden (1994) have reported a subtle O_3-induced effect on arbuscular mycorrhizal colonization in sugar maple accompanied by improved internal mycelia, vesicles, and hyphal colonization. In contrast, Wang et al. (2011) observed an overall detrimental effect of O_3 on mycorrhizal and hyphal colonization in snap bean. Furthermore, a meta-analysis conducted by Wang et al. (2017) documented that the degree of impairment of mycorrhizal colonization will depend on the concentration of O_3, the duration of exposure, plant types, types of mycorrhizal species, and the presence of additional environmental stress factors, etc.

12.2.2 Changes in carbon stock via mediated O_3 induced impact on primary productivity

Numerous meta-analyses have evaluated O_3 stimulated phytotoxicity along with the significant reduction of light-saturated photosynthesis (A_{sat}) in both trees and crop varieties, including soybean, wheat, and rice (Ainsworth 2008). Eventually, non-structural carbohydrates such as starch and sucrose also showed a reduction, crucial for plant growth, thereby negatively influencing the primary metabolism, interlinked to CO_2 fixation and photosynthetic machinery as chlorophyll and Rubisco activity (Ainsworth et al. 2012). Additional studies on the molecular level investigating the O_3-induced proteomic alteration in wheat and rice have revealed changes in Rubisco, enzymes involved in the Calvin-Benson cycle, such as ATP synthase, Rubiscoactivase, NADP-glyceraldehyde-3-phosphate dehydrogenase, and phosphoglycerate kinase, etc. (Sarkar et al. 2010). Such implications at the cellular level are attributed to reduced leaf area, resulting in the decline of net primary productivity. Additionally, O_3 fumigated plants commonly exhibited high mitochondrial respiration rates, potentially required for detoxification to counter the O_3 triggered reactive oxygen species (ROS) and to repair the cellular injuries due to O_3 phytotoxicity (Ainsworth et al. 2012) (Fig. 12.1). Plant physiological studies in the context of O_3 toxicity have also reported early senescence of leaves under elevated O_3, indicating the limited possibilities for carbon yield. Such metabolic alterations can modify the source-sink association, conventionally a manifestation of reduced C allocation towards the root system following O_3 fumigation (Fiscus et al. 2005).

The global flux of carbon between the atmosphere and forest determines the global carbon cycle via the rate of carbon being assimilated, and the subsequent establishment of C stock in plant living tissue (Wittig et al. 2009) being returned to nature. Vegetation such as deciduous forests, grasslands, and agriculture displayed larger O_3 deposition rates per year and higher stomatal fluxes of O_3 compared to forests of tundra and boreal coniferous forests (Hardacre et al.2015). A simulation study performed by Oliver et al. (2018) exhibited that higher O_3 concentration in Europe between 1901 and 2001 caused a reduction of gross primary productivity by 3–9%, with a reduction of up to 30% being observed in some European provinces. Similarly, another recent modeling study conducted by Park et al. (2018) documented a reduction of about 68.1 tC/km^2/year of the Korean forest's net primary productivity. The study further estimated that the net primary productivity has been lowered by 8.3% under the prevailing O_3 concentration in the Korean forest and is predicted to reduce by 8.5 to 10.6% in 2050, which may rise to 11.2% in the 2090s as per the existing scenarios.

12.2.3 Modification in water economy via stomatal functioning

Water cycling at the plant level and exchange of gases between the environment and vegetation are completely interconnected (Domec et al. 2010); therefore, any factor influencing the water absorption process via roots tends to determine the environment's water balance. The entry of O_3 mainly occurs via stomata, small pores on the leaf surfaces, which function as an interface for the exchange of water and gases. Additionally, stomatal closure has usually been observed in plants growing under high O_3 concentration (Hoshika et al. 2015), which generally lowers the uptake of O_3 and has also emerged to be a water-saving mechanism owing to lowering of the transpiration rate (Hoshika et al. 2020). In contrast to this mechanism, O_3 is also known to cause stomatal sluggishness or leaky stomata due to the disruption of stomatal control and induce inefficient closure of stomata, e.g., under the high light condition as reported in snap beans (Hoshika et al. 2016). This may cause a higher stomatal influx of O_3 and also the consumption of water (Fig. 12.1). Ozone-induced sluggishness of stomatal cells has been documented in various tree species

(Hoshika et al. 2012a; 2012b; 2014). A modeling simulation by Hoshika et al. (2015) on temperate deciduous forest suggested that O_3 profusely impacted water use efficiency (WUE) of the tree species of temperate deciduous forest and recorded a reduction of WUE by 20% under the O_3-induced sluggish condition of stomata and by 5% under negligible stomatal sluggishness, respectively. A recent study by Paoletti et al. (2020) reported that O_3 magnified the water loss rate in mature beech trees under a short period of fumigation owing to the impedance of stomatal dynamics. However, such impact is nullified and even reduced in the long run due to the reduction of steady-state conductance of stomata.

Evidence of recent and past findings reported a different pattern of responses under the presence of multiple stress factors. For instance, the lowering of stomatal conductance of the underwater deficit stress condition has further reduced the stomatal flux of O_3 and also the transpiration rate in wheat (Ghosh et al. 2020b; Harmens et al. 2019) and Mediterranean tree species *Phillyrea angustifolia, Quercus ilex, Q. pubescens*, and *Q. robur* (Hoshika et al. 2015). However, the study of Hoshika et al. (2015) reported species-specific responses in exerting an avoidance strategy to check the water loss via transpiration and cope with O_3 phytotoxicity. Regardless of this, the efficiency to counter O_3 toxicity was eventually reduced with leaf aging, and as a result, the assimilation rate was affected, and failure of stomatal closure to limit water loss was also observed. Similarly, evidence has suggested elevated CO_2 to ameliorate the phytotoxicity of O_3 by lowering stomatal conductance and hence lessen the potential flux of O_3 and check transpiration linked water losses (Paoletti and Grulke 2005). Accordingly, such responses significantly contribute to the resultant alteration of the water balance and carbon stock of forest and vegetation under changing climatic conditions.

12.3 Effect of O_3 on the ecosystem's provisioning services

12.3.1 Crop yield losses

With the prediction of the world population approaching 9 billion by 2050, the assurance of food supplies is becoming the biggest question of this century. The co-occurrence of high background O_3 concentration over rural/agricultural regions results in the substantial decline of crop yields globally (Ainsworth et al. 2012). Ozone-induced damage in crops plants is well evidenced from past studies, which are manifested in the form of visible foliar injury, reduction of photosynthetic rate, changes in the pattern of carbon allocation, and lowering of yield quantity and deterioration of its quality (Singh and Agrawal 2017; Ainsworth 2017; Ainsworth et al. 2012; Fuhrer 2009; Fiscus et al. 2005). Stomatal pores provide access to O_3 inside the apoplast, where it generates ROS (hydrogen peroxide, and hydroxyl radical and superoxide ions) (Heath 2008) on reaction with the hydrous layers within the apoplast. On encountering ROS, apoplastic antioxidant generation gets stimulated to counter these toxic free radial ions' detrimental impact. An array of mechanisms potentially influences the production process of apoplastic antioxidants both enzymatic and non-enzymatic, such as the activity of specific enzymes (e.g., ascorbate peroxidase, catalase, glutathione reductase, superoxide dismutase (Chernikova et al. 2000)) and the production of certain non-enzymatic antioxidative compounds viz., ascorbic acid, thiols, phenolic compounds, polyamines, β-carotene, and glutathione (Chernikova et al. 2000; Baier et al. 2005; Dizengremel et al. 2008). However, such a beneficial process operating such mechanisms comes against the extra expenditure incurred as the demand for carbon to sustain such defense operations. An increase in mitochondrial respiration rates has been evidenced in various crops such as wheat, soybean, common bean, and rice as a defense strategy to amplify detoxification processes (Ainsworth et al. 2012). In the case of over-empowerment of apoplastic defensive capacity either due to a limitation of resource allocation towards a defense mechanism or higher flux of O_3, the ROS derivates or O_3 itself can easily reach the subsequent phase of the cellular attack, i.e., the plasma membrane (Emberson et al. 2018) and can also penetrate much deeper inside the cell resulting in metabolic disturbances. Overall, these reactions will provoke rapid response surges that may trigger O_3-induced damage on crops via alteration in physiological processes and modification in growth, which interfere with the quantity and/or quality of crop yield.

Some of the crops which hold global significance for being consumed as staple food such as soybean, wheat, and some leguminous crops emerged as being severely O_3 sensitive along with maize, potato, and rice as moderately sensitive (Mills et al. 2013) and the impact on the productivity of these crops tends to pose a severe threat to food security for billions. In recent times, numerous modeling studies have evaluated the concentration of present and future O_3 posed on crop productivity and established several statistical relationships, both on a regional and global scale (Tang et al. 2013; Avnery et al. 2011). The estimated yield reduction of 4–15% in wheat, 2–5% for maize, 3–4% for rice, and 5–15% for soybean has been attributed to O_3 pollution (Avnery et al. 2011; Van Dingenen et al. 2009). Recent modeling by Mills et al. (2018) accounted for the prevalence of multiple stress factor in influencing the impact of O_3 on wheat yield and exhibited that the impact of O_3 was more severe in the rain-fed and well-irrigated regions of primary wheat-producing nations such as India, Russia, China, the United States, and France. The model quantified around a mean of 9.9% O_3-induced reduction in wheat productivity in northern hemispheric regions while reducing 6.2% in the southern part of the globe, which is analogous to a grain loss of around 85 Tg (million tonnes), estimated for the period 2010-2012. The study also documented the nation-wide mean wheat yield loss which ranked India to be at the top with a 12.2% loss, followed by China (11.7%), the United States (10.1%) and Russia (10.8%), corresponding to around 45 Tg (million tonnes) of wheat productivity loss. In China, economic losses were estimated at around 10.3 billion $ and 10.7 billion $ for wheat yield losses annually for the calculated AOT40 in 2015 and 2016, respectively (Feng et al. 2019a). Furthermore, Tai et al. (2014) concluded that enhanced O_3 pollution in South Asian countries could minimize wheat production by approximately 40% as predicted in 2050, which could further raise the need for land acreage for crop cultivations by 8.9% in tropical regions like Asia. Another recent study performed by Sharma et al. (2019) presented the Indian economy's economic losses for the O_3-induced yield loss for the major grown crops in India – wheat and rice. The relative yield losses estimated for wheat (~21-26%) were much higher than that of rice (~6%), translating into an economic estimate of ~$5 billion for wheat and $1.5 billion for rice.

Elevated O_3 has also been reported to regulate the quality of crop output (Booker et al., 2009). Visible damage caused in the leafy crop plants, growing under high O_3 concentration reduces the economic value of the crop productivity (Ashmore 2005). Besides, the grain quality has also been impacted due to O_3 influence. Broberg et al. (2015) conducted a meta-analysis considering 42 experiments and concluded that elevated O_3 reduced the starch content while protein and other nutrients such as P, Mg, K, Ca, Fe, and Zn concentrations displayed increments in wheat. Similarly, Frei’s (2015) study was in agreement with the former study in the case of rice. Such an increment has been hypothesized to be because of early senescence and reduction of the grain filling time, which patronage the remobilization of nutrients towards the sink (Frei 2015). In addition to these alterations in grain quality, implications on baking properties and dough texture have been documented in wheat, which can influence the crops' consumer consumption preferences (Broberg et al. 2015).

12.3.2 Ozone regulating timber production

The noxious impact of O_3 on forest ecosystems has sometimes been discussed (Ashmore, 2005), and such evidence has been backed up by the results drawn from experiments performed under controlled environments (Wittig et al. 2009). Numerous species-specific responses (foliar injuries, physiological and morphological alterations) have been observed to date; however, attention is more drawn towards the substantial effects on growth and productivity (assimilative rate, biomass production) because of the implications for the resultant global carbon stock and carbon sequestration (Wang et al. 2016). An earlier meta-analysis conducted by Wittig et al. (2007) documented an approximate 11% reduction of photosynthesis in trees, which is attributed to ~7% minimized tree productivity due to rising O_3 pollution since the industrial era. Past literature reviews suggested that deciduous trees are more sensitive to O_3 pollution than the coniferous tree species (Mills et al. 2013; Girgždienė et al. 2009; Felzer et al. 2004; Fuhrer et al. 1997).

Many O_3 susceptible tree species are commonly found, covering vast areas of European countries (birch, beech, deciduous oaks, spruce, etc.). The presence of C stock (as biomass) for ten major European countries has been presented in Fig. 12.2, and data have been obtained from the Food and Agriculture Organisation, FAO statistic, State of the World Forest (2009). Numerous observational studies related to the influence of O_3 on forest health have been performed across Europe (Ferretti et al. 2018; Braun et al. 2017, 2014; Proietti et al. 2016; Harmens and Mills, 2012; Šrámek et al. 2012; Karlsson et al. 2006). According to the derived remote sensing dataset for the period 2000-2010, Proietti et al. (2016) quantified the adverse impact of O_3 on the gross primary productivity of European forest to be ranging between 0.4 and 30%. Furthermore, Braun et al. (2014, 2017) estimatedan O_3-induced declination of annual growth of 19.5% for deciduous forests and 6.6% for conifers, with a mean reduction of 11% for the Swiss forest (1991-2011). Besides Europe, another remote sensing-based technique evaluated the O_3 damage in terms of net primary productivity reduction in the Kanto area of Japan for the year 2013 to be around 4–39% (Shimizu et al. 2019). However, an interesting report from China exhibited that the annual tree biomass in the evergreen broadleaf forest of sub-tropical Chinese provinces showed a 13% O_3-induced reduction while a deciduous forest of the north-central part showed an 11% declination, which translated to $52.3 billion economic losses for the nation (Feng et al. 2019b).

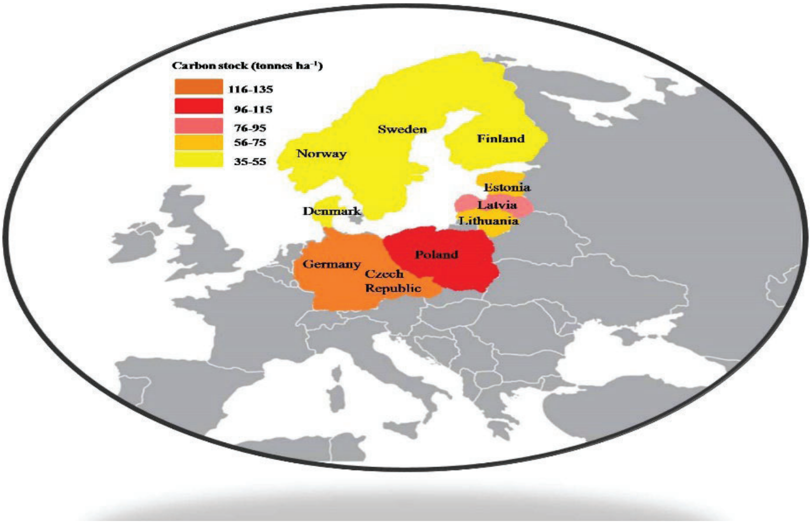

Fig.12.2. Heat map demonstrating C stock (as biomass) for ten major European countries

12.3.3 O_3 affecting the biodiversity pattern and species composition

Tropospheric O_3 has been reviewed as the most common phytotoxicant, resulting in substantial long-term stress across large areas of vegetation (Ashmore 2005). Enormous O_3-induced detrimental impacts on the diversified forest ecosystem are in full swing yearly, with considerable consequences to the existing biodiversity (Bergmann et al. 2017). Most of the research examining such threats has mainly concentrated on the pattern of species richness and its diversity. Typical O_3 triggered impacts on sensitive species comprehend a modification in carbon allocation patterns, visible injuries over different parts and enhanced premature senescence; minimize development and productivity; and influence the time of flowering and seed maturation (Singh and Agrawal 2017; Fuhrer et al. 2016; Rai and Agrawal 2012; Kangasjärvi et al. 2005). Such consequences are believed to pose much more significant implications for influencing the pattern of biodiversity as a whole. Such a shift in community patterns resulting in modifying ecosystems' diversity could be manifested differently within the eco-regions.

(a) Shift due to species-specific sensitivity towards O_3

The sensitivity of species to O_3 pollution depends on numerous factors starting from the diffusive properties of leaves, detoxification, and repair capacity, to the carbon allocation pattern for the sustenance of various cascades of defense mechanisms (Fuhrer et al. 2016). Although the genetic make-up in the regulation of O_3 sensitivity remains unclear, limitations also exist to understand the functional pattern of such responses. A vast literature survey by Bergmann et al. (2017) documented the higher sensitivity of herbs and deciduous tree species to O_3 in comparison to conifers and grasses. Observation-based study through field survey along a variable gradient of O_3 concentration to evaluate the ecosystem effect in North America and Europe displayed visibly apparent O_3-induced foliar injuries for 258 species of the herbaceous family (Bergmann et al. 2017). Interestingly, the study also categorized O_3-sensitive families, revealing that most O_3-sensitive species were observed in the families of Myrtaceae, Onagraceae, and Salicaceae whereas, moderate and less sensitive species were found mostly in the families of Brassicaceae, Plantaginaceae, and Boraginaceae. However, species of Leguminosae comprising herb, shrub, and tree species have been observed with the consistent report of high O_3 sensitivity with its prevalence in both tropical and temperate eco-regions (Fuhrer et al. 2016). Furthermore, a meta-analysis comprised of a compilation of experimental observations on the heathlands, grasslands, and wetlands of European temperate regions, exhibited that threophytic species tend to show higher susceptibility to O_3 pollution (Hayes et al. 2007). On the other hand, another study of a similar database revealed the higher O_3 sensitivity of heliophytic life forms relative to those of sciophytes; life forms thriving in dry sites showed higher sensitivity than those in the moist zones, whereas halophytes are more sensitive to O_3 than the non-halophytes (Jones et al. 2007).

Community shift can also be determined by species-specific O_3 sensitivity in regulating the relative growth rate (Davison and Barnes 1998). In other words, the ecosystems showing a dominance of slower growing species will be less responsive to O_3 phytotoxicity, whereas ecological regions comprising of fast-growing species will pose higher adversities. Other factors that might influence community shift patterns are alteration in biomass allocation patterns and modifications in reproductive success (Wang et al. 2015). For example, a recent study of Dolker and Agrawal (2019) displayed the O_3-induced impact on two herbaceous species, *Ischaemum rugosum,* and *Malvastrum coromandelianum*, dominating the open areas and grassland of the Indo-

Gangetic Plains of Northern India. The study highlighted the higher sensitivity of the C_3 forb species *M. coromandelianum* towardsO_3 compared to the C_4 grass, *I. rugosum*as consequences of the more detrimental impact of O_3 pollution on the biomass production and growth of the forb species.

(b) Interactive effect of O_3 and inter-specific competitions

In reality, mostly in natural or semi-natural communities, a highly competitive environment is prevalent for the growth of vegetations (Gallandt and Weiner 2015). In a competitive environment, growth is regulated by the relative O_3 responses of the multiple species growing together. However, most wild species have been observed to be less O_3-sensitive than agricultural crops. Hence, evaluating the risk posed under the interaction of both these factors on sensitive species is relevant to understanding the community's competitive shift. A recent study performed by Ghosh et al. (2020a) elucidated that the presence of *Chenopodium album* can change the adversity of O_3 and intensify the productivity loss in wheat compared to being under O_3 exposure alone (without a competitive environment). Another experimental analysis by Kacienė et al. (2019) exhibited a strong intervention of inter-specific competition by wild mustard (*Sinapis arvensis* L.) onthe growth of summer rape (*Brassica napus* L.), which intensified under O_3 pollution. Similarly, numerous O_3-sensitive grasses and leguminous species have been noted to accomplish a shift in the ratio of grass and legume (Rebbeck et al. 1998), promoting grasses' growth.

12.4 Effect on regulating services

12.4.2 Impact of O_3 on vegetation in modulating air quality

Vegetations can directly or indirectly regulate the regional or local air quality to a great extent. Trees with large canopies can predominantly minimize the local atmospheric temperature, but as a consequence of the limitation imposed on the exchange of turbulence with the canopy air, the temperature tends to increase below or within the canopy areas (Heisler et al. 1994). As the formation of many secondary air pollutants is temperature-driven, reducing atmospheric temperature can improve the quality of air around us. Generally, the considerable scientific manifestations demonstrated that the vegetations' positive feedback in mitigation of most of the environmental issues has consequently resulted in a normative belief of expansion of urban vegetations to be most desirable (Escobedo et al. 2011). However, the relationship between tropospheric O_3 and vegetation establishments tends to be a complicated one. Although trees predominantly act as sinks for O_3 dry deposition (Fitzky et al. 2019), their contribution to the formation of O_3 has also been evidenced (Jenkin et al. 2015).

Under usual conditions, the primary share of an individual tree in the removal of O_3 or the emission of biogenic volatile organic compounds (BVOC) (O_3 precursor gases) is primarily species-specific, and the process of gaseous exchange is equivalent to the canopy size, as determined by the mass of the leaves or leaf surface area (Vos et al. 2013). Dry deposition over the urban land is 40 times less efficient than over the vegetation covers, as per some parameterizations derived from the atmospheric models (Wesely 2007). Ozone tends to diffuse into the apoplastic space from the atmosphere via stomatal pores and is deposited by reactions inside the intercellular space. In partial stomatal closure, deposition occurs on foliar surfaces via reactions with moisture, waxes, and salts (Altimir et al. 2006). Therefore, vegetations featuring large foliar areas, hairy leaf surfaces, wax coating, and moist surfaces, concomitant with less stomatal sensitivity, have shown a better potential for an O_3 removal strategy (Fitzky et al. 2019; Cape et al. 2009).

The emission and production of BVOCs can either function to safeguard the plants against O_3-induced phytotoxicity by enhancing thermo tolerance, possibly to combat the surplus energy via the release of physiological by-products (Niinemets et al. 2013) or by serving as a precursor to O_3 formation (Calfapietra et al. 2013). Isoprene is a well-known BVOC, and trees such as *Quercus robur, Q. pubescens, Populus nigra*, etc., are some major isoprene emitters, posing the highest potential for the formation of tropospheric O_3 (Xie et al. 2008). Monoterpenes are emitted mostly by coniferous and broad-leaved tree species, dominating the forests of central Europe (Kramer et al. 2010).

Meteorological and other prevailing stress factors largely control the BVOC production and emission by vegetations. High temperatures and light intensities often tend to favor the formation of O_3 (Chameides et al. 1988). Although the emission of BVOC exhibits a positive correlation with temperature (Guidolotti et al. 2019) as plants utilize isoprene for the stabilization of membrane integrity under high-temperature stress conditions (Behnke et al. 2013), this trait seems to be species-specific. However, the direct impacts of high temperature on the deposition of O_3 emerge as being negligible. High temperature enhances evapotranspiration, thereby inducing water-deficit stress conditions, with eventual stomatal closure (Morani et al. 2014), indirectly affecting the O_3 deposition mechanism. With the elevation of the drought/water deficit condition, partial or complete stomatal closure limits the stomatal uptake of O_3 (Livesley et al. 2016), resulting in the rise of tropospheric O_3 levels by ~10% (Anav et al. 2018). However, trees have evolved to exploit different strategies to combat reduced water availability. Some anisohydric tree species such as *Fagus sp.*and *Quercus sp*. allow stomatal opening under moderate drought conditions and eventually maintain O_3 deposition during summertime when its concentration usually remains high (Grote et al. 2016). In addition to drought conditions, limited nutrient availability can also negatively affect O_3 deposition as the consequences of growth reduction and induced sensitivity towards pest attacks, resulting in a relatively smaller leaf area (Hu et al. 2018).

12.4.3 Interactive impacts of O_3 with other environmental factors on carbon sequestration

Responses of the plant to O_3 are exceptionally reliant on other ecological factors. An illustration presented in Fig. 12.3 shows the interactive effects of O_3 stress with other climate change factors such as elevated CO_2, temperature rise, and water deficit conditions on vegetations that promote reduced carbon sequestration. Enhanced environmental CO_2 fixations may diminish stomatal O_3 flux in some tree species in certain natural conditions. Changes in carbon stock availability and its allocation pattern inside plants under raised CO_2 may present a compensative limit against O_3 stress (Watanabe et al. 2017). As opposed to the impacts of O_3, increased CO_2 may improve tree development if different factors, for example, nutrient supplements and water accessibility, do not compel growth (Norby et al. 2010). Whether the current CO_2 increasing trend can minimize the O_3 promoted phytotoxicity in real field conditions is still a subject of consistent discussion (Matyssek and Sandermann 2003). In general, high CO_2 concentration in the atmosphere instigates stomata's closing and thus diminishes stomatal O_3 flux (Ainsworth et al. 2008). A more prominent carbohydrate level is evident because of a higher photosynthetic rate under raised CO_2 concentration, which may translate to better detoxification and repair efficiencies against O_3-induced stress (Riikonen et al. 2004). However, if O_3 levels are sufficiently high to lessen the photosynthesis as well as plant growth, at that point, less CO_2 and O_3 will be consumed by the leaves of vegetation, prompting positive feedback to the background concentration of CO_2 and O_3, leading to more warming of atmosphere (Mills et al. 2013).

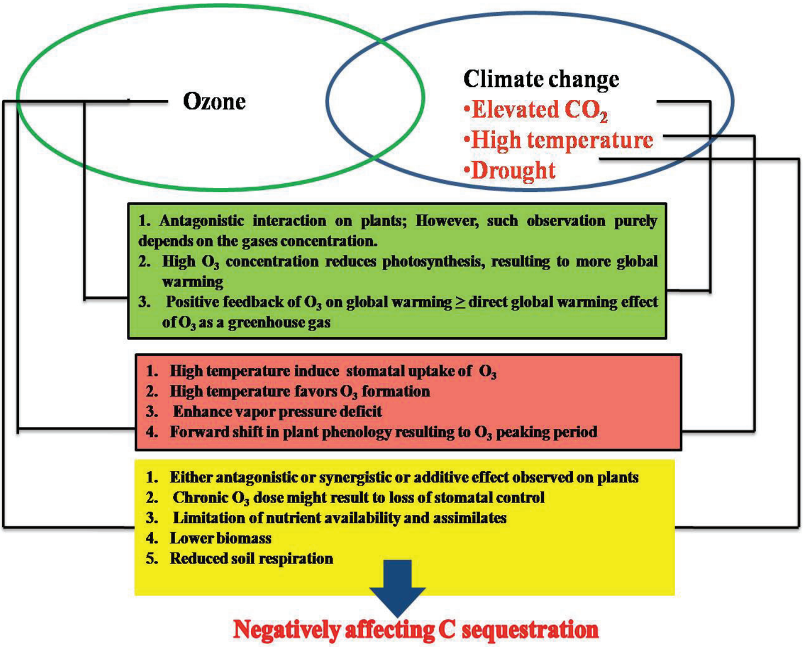

Fig.12.3. Interactive effects of O_3 stress with other climate change factors such as elevated CO_2, temperature rise, and water deficit conditions on vegetations that affect the reduction in carbon sequestration

In a recent analysis, Feng et al. (2019b) concluded that the prevailing concentration of O_3 for the year 2015 in China declined the yield of wheat by 6%, rice by 8%, and yearly forest biomass (tree growth) by 11–13%, which translated to 7% of the O_3 incurred cost of the gross Chinese product. Another simulation study by Yue and Unger (2014) displayed that O_3 induced a reduction of average gross primary productivity by 4–8% in the eastern part of the United States, which anticipated a maximum decrease of 11–17% in provinces along the east coast, thereby translating into the minimization of land carbon stock.

The intricacy of the interactive effects of the multiple stress factors on vegetation is still elusive to establish a clear understanding. When a single factor is considered, the high temperature probably enhances the stomatal flux for O_3 (Emberson et al. 2000). However, the impacts of warming will likewise be influenced by the accompanying indirect impacts of triggering the formation of tropospheric O_3, reduction in soil water potential and advanced plant growth prior to its usual timing, thereby exposing plants to O_3 peaking periods. Therefore, predictions regarding the interactive impact of multiple stress factors are complicated and based on the timing, duration of exposure, occurrences of seasonal trends in O_3 peaking periods, alteration in precipitation, etc.

The rise in temperature is often succeeded by the drying up of soil, resulting in a changing water status in the soil and plants. Consequently, periods with high O_3 levels in the atmosphere often coincide with water deficit conditions in the summertime, resulting in coincidental vegetation exposure to both drought and high O_3. Reduction in water availability usually limits the plant production process, leading to a lower carbon stock (Ghosh et al. 2020b). However, the interaction of the water deficit condition with O_3 commonly manifests either a synergistic, additive, or antagonistic impact. However, all these impacts will translate to lowering carbon sequestration due to the closing of stomata, limiting the assimilate productions (Mills et al. 2013).

12.4.4 O_3-induced impact on flowering and pollination

The reproductive phase is considered to be a primary stage in the plants' growth cycle and has often emerged to be sensitive toO_3 stress (Leisner and Ainsworth 2012). Early meta-analyses and reviews have established the direct and indirect impacts of O_3 on the reproductive structures (Leisner and Ainsworth 2012, Black et al. 2000). Alteration of the flower blooming time, regulation in pollen and ovule development processes and increased abortion of seeds, are some of the impacts noted under O_3 pollution (Rai and Agrawal 2012). Injury to vegetative tissues eventually minimizes the assimilative capacity and its allocation to the reproductive structures (Davison and Barnes 1998). Black et al. (2000) demonstrated that O_3 could directly impact the surface geology of the style and stigma, influence pollen and anther maturation and development, damage floral sites, and negatively affect seed and fruit productions. The uptake of O_3 into the intercellular space inside the reproductive structures triggers ROS productions, which further influences the integrity of cell membranes and stigmata surfaces (Feder and Shrier 1990), which affects the germination processes of pollen on such surfaces. Leisner and Ainsworth (2012) reported an alteration in pollen tubes' growth and guidance for fertilization of the embryo under O_3 stress. Furthermore, the study of Hayes et al. (2012) has reported the O_3-induced triggering of flower initiation processes, resulting in the advancement of maximum flowering by six days with an increase of O_3 concentration from 30 to 70 ppb. Another study by Black et al. (2012) documented an increase in ovule abortion and premature germination of seeds concomitant with a reduction in the number of mature seeds per pod in *Brassica campestris* L., which could confer the impairment of seed vigor.

Volatile organic hydrocarbons emitted by flowers are considered to be crucial in attracting pollinators and plant defense mechanisms against insects' herbivory. Some studies have established that O_3 interferes with these floral scents and influences the plant-insect interactions (Fuentes et al. 2013; Blande et al. 2010; McFrederick et al. 2008). Ozone regulates plant emission and their physiology in either way: (a) by impacting plant physiological processes and inducing modifications in emission profiles (Holopainen and Gershenzon 2010) or (b) by interfering with the emitted compounds after their release into the atmosphere (Blande et al. 2014). The O_3-induced oxidative degradations of such floral volatile hydrocarbons may minimize their concentration in the odor plume and also reduce the distance traveled by the compounds and could not be detected by the target pollinators (McFrederick et al. 2008). The oxidative reactions between O_3 and floral volatile compounds result in the productions of new organic species that may not be part of the original compounds, which may trigger confusion in the signal receivers (Farré-Armengol et al. 2016).

12.5 Effect on cultural services

Cultural services represent the nonmaterial advantages that humans receive from ecosystems through cognitive development, recreation, reflection, spiritual enrichment, and aesthetic experiences (Millennium Ecosystems Assessment 2003). Measuring the potential effects of O_3 on cultural services has attracted very little consideration so far, even though O_3 can produce both unpretentious and significant impacts over some parts of cultural services by affecting the visual appearance and quality of the natural environment (Gribacheva and Gecheva 2019). The effects of O_3 on the visual appearance and quality of the natural environment manifest as changes in the color and outward form of leaves, alterations of species' composition and lowering of the number, and the delayed timing of flowering. The ozone-induced effect on human health is also regulated by the uptake of O_3 via leaf stomata, thereby influencing the hazardous impact of O_3 on human society.

12.5.1 Aesthetic view

Vegetation creates an aesthetically pleasing landscape by creating various colors, shapes, and textures (Fig.12.4). Besides, vegetation is sometimes utilized as a screen and plays a significant role in blocking undesirable views, channeling people's sight toward pleasant scenes, and furnishing a natural frame for scenery (Chen and Jim 2008). These characteristics of flora can be affected by O_3 pollution (Yang et al. 2016). Elevated concentration of O_3 in ambient air may diminish the attractiveness of areas of excellent natural beauty or ornamental plants by reducing photosynthesis and accelerating leaf senescence (Yang et al. 2018). Such impacts of O_3 might affect the tourism industry (Keiser et al. 2018). In addition to the above-mentioned effects, O_3 episodes can further cause visible foliar injury of a broad range of species as O_3 is a major air pollutant in the United States, inducing foliar injury inmany agronomic and horticultural crops, deciduous trees, and conifers (Frey 2011). Various plant species of natural vegetation have been recorded with symptoms like stippling ascribed to O_3. Moderate O_3 levels typically result in chlorotic stippling, while excessive exposures bring about red or purplish stippling on the leaf's upper surface. Furthermore, O_3 exposure can also increase vegetation's susceptibility to pathogen attack and can spoil the aesthetics of vegetation (Mills et al. 2013). Ozone exposure can impact the species balance in natural ecosystems, with less sensitive species potentially outcompeting O_3-sensitive species. The effects of such competition can cause a decrease in the visual attractiveness of sites where fewer attractive flowering forbs are present. Besides, O_3-induced changes in flowering time affect the visible appearance of natural locations. A deferral or reduction in the flowering of iconic species in moderate-high O_3 areas could potentially reduce the enjoyment of visiting natural places (Hayes et al. 2012).

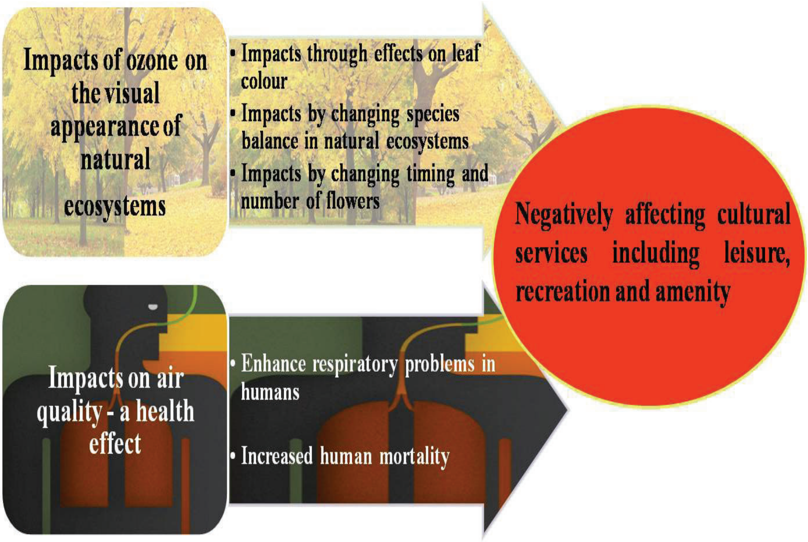

Fig. 12.4. Illustration showing the O_3-induced impacts on aesthetic ecosystem services

12.5.2 Impacts on human health via air quality regulation

The harmful impact of O_3 has been evidenced in humans, which usually manifests as respiratory problems, promoting oxidative stress once it enters the air passage (WHO 2008) (Fig.12.4). The underlying impacts of O_3 mainly depend on the exposure concentration and duration, resulting in both chronic and acute respiratory problems. Vegetation is considered to be a significant sink for atmospheric O_3 deposition, minimizing around 20% of its annual concentrations (Royal Society 2008). Premature senescence and stomatal closure can diminish the absorption rate of O_3, thereby positively feeding back on the atmospheric O_3 concentration, which will enhance the risk of exposure possibility. Such impacts have been evidenced to be more intensified in rural or suburban areas, especially if O_3 stress is concurrent with drought or high temperature, with substantial impacts on recreation.

12.6 Conclusion

The present study highlights how O_3 poses threats to every ecosystem service sphere, either directly or indirectly. Although each ecosystem service has been reviewed separately, all of these services and the related underlying mechanisms are interconnected, thereby producing holistic implications for the ecosystem on a global scale. Ozone-induced negative impacts on nutrient cycling, water economy, and carbon stock (primary productivity) vitiate the supportive services of the ecosystem while crop productivity losses concomitant with damage to timber stock and genetic resources quantify the effects on ecological provisioning services. Moreover, the interactive effects of O_3 with the factors of climate change trigger the adversity on carbon sequestration, resulting in a reduction of gross primary productivity, primarily determining the implications of O_3 on the regulating services. In addition to this, O_3-induced alteration of the aesthetic characteristics of nature and air quality, impacting human health marks the harmful effects of O_3 on the cultural ecosystem services. Therefore, this evidence-based compilation will deepen our insight to better understand how rising O_3 pollution impacts the ecosystem services and their related functioning processes as a whole. However, further research must be conducted to improve knowledge of these services where information is still not sufficient to derive response functions.

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CHAPTER THIRTEEN

PROTECTING PLANTS AGAINST OZONE TOXICITY BY APPLYING ETHYLENEDIUREA: AN UPDATE

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Abstract

In a world with persisting ozone pollution, it is essential to find ways to protect vegetation. The most widely studied chemical as a protectant of plants against ozone toxicity is ethylenediurea (EDU), a synthetic antiozonant used in the rubber industry for many decades. As a plant protectant, EDU has been used successfully in research programs over the last four decades. It has significantly reduced ozone-induced injuries in cultivated agricultural crop plants, trees, and other forms of natural vegetation. Although its mode of action in protecting plants against ozone-induced toxicity remains unknown, newer studies provide novel insights at molecular, nutritional, and physiological levels, which are discussed in this chapter.

Furthermore, a direct reaction with ozone occurs when applied to foliage, thus directly reducing the ozone at the leaf surface. This suggests that EDU can protect plants both indirectly, via a systemic action, and directly, via reaction with ozone. The protection against ozone injury occurs at EDU concentrations below the traditional toxicological threshold, in full agreement with the concept of hormesis. Before its potential agricultural application, pharmacological/toxicological studies should test for EDU effects on animals fed with agricultural products obtained from EDU-treated plants.

Keywords: Ethylenediurea; Hormesis; Ozone; Plant protection

13.1 Introduction

Ground-level ozone (O_3) is a greenhouse gas and a significant air pollutant that is widespread across the globe (Sicard et al. 2017; Lefohn et al. 2018). Industrial development in the previous century, and currently in Asia, has led to multi-fold increases in the concentrations of O_3 relative to the pre-industrial era (Lelieveld and Dentener 2000; Akimoto 2003; Takigawa et al. 2009; Li et al. 2019), and predictions suggest that O_3 levels will remain elevated throughout the 21st century (Sicard et al. 2017). Control of O_3 is challenging because it is not a directly - emitted pollutant. It is formed through chemical reactions in the atmosphere, depending upon other pollutant precursors (e.g., volatile organic compounds, NOx) and environmental factors (e.g., UV, weather conditions) (Krupa and Manning 1988; Wang et al. 2008; Sicard et al. 2016). Moreover, trans-regional, trans-national, and trans-continental transportation of O_3 and its precursors further complicates local, national, and regional efforts to decrease O_3 concentrations (World Health Organization (WHO) 2008; RoTAP 2012).

While low O_3 concentrations can act as an abiotic elicitor, enhancing plant defense capacity, high O_3 concentrations lead to adverse effects (Sandermann et al. 1998; Agathokleous et al. 2019b; Novriyanti et al. 2020). Increased accumulation of reactive oxygen species (ROS), impairment of gas exchange functioning, decreased photosynthesis, and inhibition of growth and productivity are characteristic effects of high O_3 exposures (Li et al. 2017; Bellini and De Tullio 2019; Dusart et al. 2019). Hence, chronic exposure of plants to elevated O_3 concentrations limits major crops' yields (Mills et al. 2018; Mukherjee et al. 2021). For example, China’s national relative yield losses due to O_3 were estimated to range between 10.2 and 18.1%, translating into economic losses of 5,554–10,745 USD, depending on the O_3 metric used for predicting the yield losses in 2015–2016 (Feng et al. 2019b). A different study also estimated China’s annual yield losses of rice and wheat, at approximately 8 and 6%, respectively (Feng et al. 2019a). This understanding suggests an enhanced threat to Asian food security, in the light of the highly increased and further increasing population, especially in India and China (United Nations 2019). To this end, there is a need for developing effective measures to protect plants against O_3 toxicity.

Ethylenediurea (EDU) is a synthetic chemical, which has been used as an antiozonant in the rubber industry for many decades (Manning et al. 2011). Four decades have passed since the first documentation that EDU can protect against O_3 phytotoxicity (Carnahan et al. 1978), and accumulated literature regarding EDU effects on plants is now available (Oksanen et al. 2013; Agathokleous et al. 2015d, b; Singh et al. 2015; Agathokleous 2017; Tiwari 2017). EDU has been proved effective in protecting a vast array of trees and cultivated crop species against O_3 toxicity (Contran et al. 2009; Singh et al. 2015; Agathokleous 2017; Tiwari 2017). EDU improves numerous plant functions like reducing O_3-induced visible foliar injury (76%) and enhancing photosynthetic rate (8%), above-ground biomass (7%), and crop yields (15%), compared to plants not treated with EDU (Feng et al. 2010). However, the EDU mode of action that protects plants against O_3 toxicity remains elusive (Agathokleous 2017; Tiwari 2017).

Here, we review the published literature to summarize the existing knowledge on EDU effects on plants and discuss newer developments, which can advance the current understanding of mechanisms via which EDU protects plants against O_3 toxicity.

13.2 Some Basics

Before dealing with the EDU mode of action, it is important to clarify some fundamentals regarding EDU, some historical foundations, and its use as a plant protectant against O_3 toxicity (Fig.13.1).

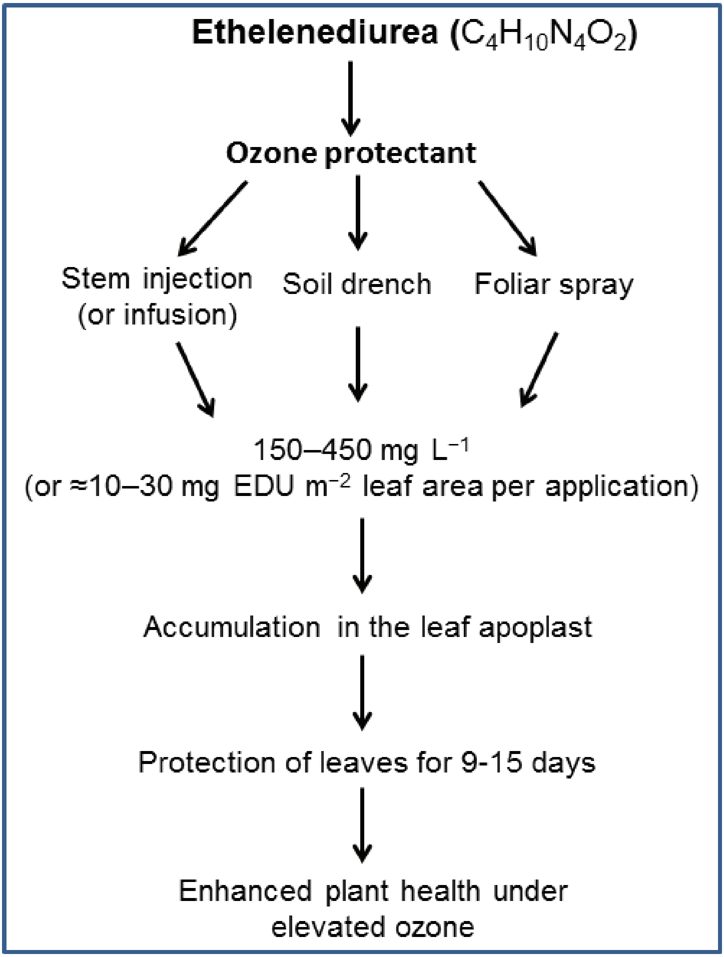

Fig.13.1. Diagram illustrating a basic understanding of the use of ethylenediurea (EDU) as protectant against ozone-induced phytotoxicity. The diagram is created based on the literature review by Agathokleous (2017)

13.3 Antiozonant versus O_3 protectant

First, before anything else, it is important to explain that a review of the relevant literature reveals confusion regarding the terminology used to describe EDU. It appears that the term antiozonant is used to refer to EDU because it protects plants against O_3 toxicity. However, the term antiozonant refers to chemical substances that protect abiotic materials against O_3 (Manning et al. 2011). Chemical substances that protect plants against O_3 are termed O_3 protectants (Manning et al. 2011). While EDU is indeed an antiozonant, it should be described as an ozone protectant (but not an antiozonant) as it protects plants against O_3 toxicity.

Antiozonants have no olefinic double bond and contain at least two nitrogen atoms; so EDU and Stantoflex, another antiozonant, are found to be O_3 protectants too (Manning et al. 2011). EDU's chemical formula is $C_4H_{10}N_4O_2$, and about 22% of the molecular weight (256) of EDU is nitrogen from urea and phenylurea (Carnahan et al. 1978). This is also the primary reason why it was believed that EDU is also an effective O_3 protectant, i.e., by contributing with nitrogen to plants, a hypothesis that seems to be false (Agathokleous 2017).

13.4 Historical foundations

Ozone was known to negatively affect plant physiology from the 1950s (Middleton et al. 1950; Bobrov 1955; Todd 1956; Freebairn 1957). Bearing this in mind, some research groups soon began investigating potential methods to protect plants against O_3 toxicity (Freebairn 1960). From the 1960s to the late 1970s, when EDU was first documented to protect against O_3 phytotoxicity (Carnahan et al. 1978), a vast array of chemical substances were tested as potential O_3 protectants, including vitamin C (Freebairn 1960), fungicides (Manning et al. 1973a,b; Saitanis et al. 2015), cytokinins (Runeckles and Resh 1975), and others (Agathokleous et al. 2015b). Many of these were found to act as O_3 protectants, although with high variability among chemical substances and species, as newer studies also shown (Saitanis et al. 2015; Agathokleous et al. 2016e; Tiwari 2017; Chen et al. 2018).

Following the report of Carnahan et al. (1978), EDU received wide attention from the research community evaluating O_3 effects on plants, with about ten papers being published in the decade of the 1980s alone, a number that was tripled in the next decade (Web of Knowledge, accessed on 13 February 2020). The relatively high interest in EDU was also because it could be used for O_3 biomonitoring, in an era when O_3 exposure infrastructures were limited, mostly to closed and semi-closed facilities, thus permitting the theoretical exposure of plants to O_3 in a completely open environment (Manning et al. 2011). However, this interest declined later due to some misconceptions regarding its phytotoxic potential and probably other reasons (Manning et al. 2011). Nonetheless, the late 2000s witnessed a resurgence of interest in the EDU research (Agathokleous et al. 2015b; Agathokleous 2017), and this was mainly due to the efforts of Dr. W.J. Manning, at that time Professor at the University of Massachusetts, Amherst, USA (Manning et al. 2011). His remarkable efforts to reproduce EDU and distribute it throughout the world (Manning et al. 2011) have led to a broad interest of numerous worldwide research groups in EDU research, especially in Asia (Agathokleous et al. 2015a; Agathokleous 2017; Tiwari 2017).

13.5 EDU as an O_3 protectant

It has been widely shown that EDU can be applied to plants via stem infusion or injection, as a foliar spray, or as a soil drench (Contran et al. 2009; Agathokleous 2017). Each application method has its advantages and disadvantages, and a detailed comparison of the methods can be found elsewhere (Agathokleous et al. 2015b, 2016b; Agathokleous 2017). This comparison suggests there is no "perfect" method. Although each technique has hitherto been applied exclusively, a more integrated approach that will include a set of two or more application methods may be considered for further research in the future. Such an approach could give more advantages by eliminating the degree of disadvantages of certain methods. For example, stem injection could be applied only in periods with prolonged rain, when the spray or soil drench application could be meaningless.

EDU accumulates in the leaf apoplast (but not the cytoplasm) and can protect leaves for up to 9–15 days, depending on the leaf development rate; it is measurable in the internal leaf tissues within one hour of spraying (Pasqualini et al. 2016; Agathokleous 2017). Hence, EDU's repeated applications on field-grown plants are needed for protecting plants against O_3 pytotoxicity throughout the active growing season. The first application is ideally conducted one week before exposing plants to O_3; this would permit maximum accumulation in the leaf before O_3 exposure and an efficient timing for the accumulation of the next EDU load before the first EDU load is degraded (Agathokleous 2017).

Based on empirical evidence from plenty of species, cultivars, and genotypes, the range of EDU concentrations sufficiently protecting against

O_3 phytotoxicity is 150–450 mg L^{-1} (Agathokleous 2017). Preliminary calculations also indicate that the maximum dose needed is ≈10–30 mg EDU m^{-2} leaf area per application (Agathokleous 2017). EDU, when applied at the typical concentrations used against O_3 toxicity (150–450 mg L^{-1}), does not cause toxicity to plants, but at higher concentrations (≥600 mg L^{-1}), it may be toxic to susceptible organisms (Agathokleous et al. 2016a; Agathokleous 2017; Agathokleous et al. 2018b; Agathokleous and Kitao 2018). Experiments with tree species, grown either under ambient air only (Carriero et al. 2015; Giovannelli et al. 2019) or FACE systems (Agathokleous, et al. 2021 a,b), have shown that the effectiveness of EDU as an O_3 protectant lasts for several years.

EDU is only available for research projects, and further research of the application methodology would also favor its potential commercialization and application in the agricultural practice in the future. However, the number of studies on the toxicology of EDU itself on various forms of life remains very limited; and assessments of agricultural products (e.g., grains) for potential EDU-induced chemical transformations, which can threaten the health of consuming humans and other animals, are entirely lacking (Manning et al. 2011; Agathokleous et al. 2016a; Agathokleous 2017). Before its potential agricultural application, pharmacological/ toxicological studies should test for EDU effects on animals fed with agricultural products obtained from EDU-treated plants.

13.6 A summary of EDU effects on plants under O_3 stress

The biological mechanisms of the mode of action of EDU as an O_3 protectant remain puzzling and have been reviewed in explicit detail up to 2017 (Contran et al. 2009; Feng et al. 2010; Manning et al. 2011; Oksanen et al. 2013; Agathokleous et al. 2015b; Singh et al. 2015; Agathokleous 2017; Tiwari 2017). Hence, this section summarizes the main understandings, up to 2017 (Fig.13.2), but does not enter into the details that can be found in earlier literature reviews.

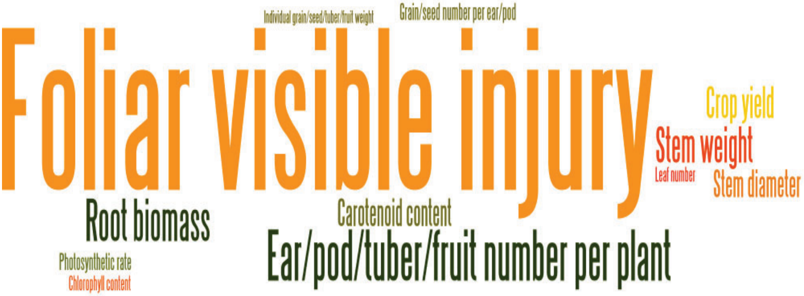

Fig.13.2. Effects of ethylenediurea (EDU) on plants under O_3 stress. All EDU effects are arithmetically positive (increased) except visible foliar injury that is negative (decreased), each of which is biologically positive (protection against O_3 stress). Each trait's size indicates the percentage of protection against O_3 stress relative to plants treated with 0 mg EDU L^{-1}. As an indicative, foliar visible injury exhibits 76% protection (the largest among all assessed traits), and individual grain/seed/tuber/fruit weight exhibits 6% protection (the smallest among all assessed traits). The effect's size is based on the results of a meta-analysis (Feng et al. 2010) rounded to the next integer (≥0.5). All the values are given in the text of this chapter. The effect sizes are based on the average effect across studies and experimental setups

Dozens of plant physiological traits have been assessed for EDU effects on plants under O_3 stress. The nature of the effects (positive, negative, or neutral) can be varying across studies or even within studies, depending on the concentrations used, sites, interacting environmental factors, the plant developmental phase, the stage of the stress response, and other factors (Agathokleous et al. 2015b; Pandey et al. 2019). The magnitude of the EDU effect also differs among species (Feng et al. 2010), considering that the effects of O_3, and thus of EDU, are genotype-specific and that many studies include different species and cultivars as well as other experimental setups (Oksanen et al. 2013; Agathokleous et al. 2015b; Singh et al. 2015; Agathokleous 2017; Tiwari 2017). It is challenging to identify common physiological mechanisms of plants treated with EDU under O_3 stress. Nonetheless, it is clear that EDU is confined to the apoplastic regions, affects the electron transport chain, primarily enhances biochemical defense (i.e., antioxidant defense machinery), and decelerates O_3-induced accelerated leaf senescence (Fig.13.2) (Singh et al. 2015; Tiwari 2017). These effects lead to a reduction of O_3-induced chlorophyll loss and maintenance of physiological efficiency and primary metabolites (Singh et al. 2015). Hence, EDU enhances the growth, biomass, and yield of plants under O_3 stress (Singh et al. 2015).

Quantitatively, based on the average effect across studies and experimental setups, EDU was found to reduce O_3-induced foliar visible injury by 76% and increase plant height by 8%, stem diameter by 13%, leaf number by 7%, stem weight by 17%, root biomass by 20%, and crop yield by 15% (Fig.13.2) (Feng et al. 2010). It was also found to increase photosynthetic rate, chlorophyll, and carotenoid content by 8, 7, and 13%, respectively (Feng et al. 2010). Furthermore, for an agricultural crops, EDU increased the ear/pod/tuber/fruit number per plant by 24%, the individual grain/seed/tuber/fruit weight by 6%, and the grain/seed number per ear/pod by 8% (Feng et al. 2010).

13.7 Newer research developments in the EDU mode of action

Since 2017, newer studies have been published, which offer fresh insights into the mode of action of EDU as an O_3 protectant.

13.7.1 Underpinning mechanisms of protection

In a study with 19 cultivars of Chinese soybean (*Glycine max*) with different degrees of O_3 susceptibility, grown in a field with ambient O_3, and screened for gas exchange, pigments, antioxidants, and biomass, the results suggest that the constitutional level of leaf total ascorbate was the most important trait affected by EDU (Jiang et al. 2018). This finding is supported by the earlier literature (Singh et al. 2015; Tiwari 2017). Further newer studies with a variety of experimental plants show that EDU improved O_3-affected plant morphology, foliar symptoms, chlorophyll levels, enzymatic and non-enzymatic antioxidant activity, lipid peroxidation, oxidized and reduced glutathione content, photosynthesis, minimal fluorescence yield of the dark-adapted state (F_o), maximal fluorescence yield of the dark-adapted state (F_m) and maximal quantum yield of PSII photochemistry (F_v/F_m) (Baqasi et al. 2018; Singh et al. 2018b, a; Zhang et al. 2018; Feng et al. 2018; Ashrafuzzaman et al. 2018; Gupta et al. 2018; Rathore and Chaudhary 2019; Pandey et al. 2019; Fatima et al. 2019; Xu et al. 2019). These commonly lead to increased biomass and economic yields.

Some of the new studies provide results for novel mechanisms by which EDU protects plants against O_3 toxicity (Fig.13.3):

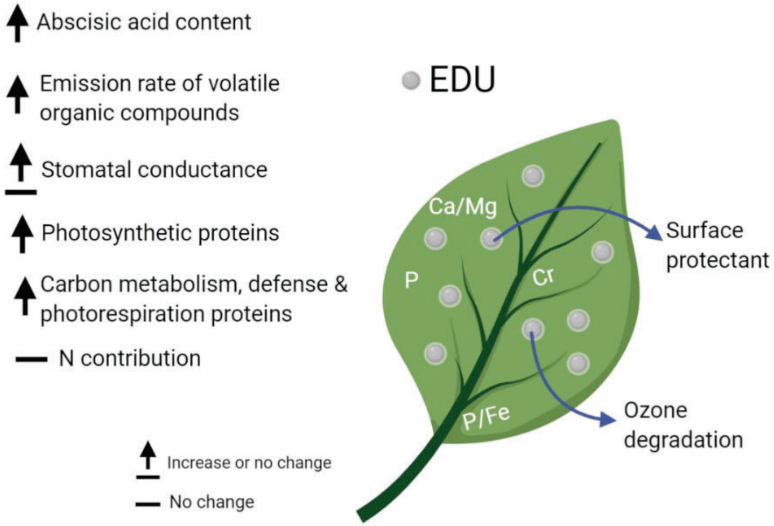

Fig.13.3. Effects of EDU on plants under O_3 stress based on research published later than the last literature reviews (Agathokleous 2017; Tiwari 2017). The mechanisms presented here are discussed in this chapter

1) EDU applied to willow (*Salix sachalinensis*) plants, in a free-air O_3-concentration enrichment (FACE) system, affected leaf Cr content, but had more profound effects on P content and affected Ca/Mg and P/Fe ratios across leaf types (depending on the EDU concentration) early in the O_3 stress (Agathokleous et al. 2018a). This study newly suggests that EDU can affect leaf stoichiometry and element cycling throughout plants.

2) New innovative experiments also document, for the first time, the O_3 degrading properties of EDU along with EDU deposits on leaf surfaces (EDU deposits on the leaf surface were also previously found by Pasqualini et al. 2016), which may suggest that EDU spray is a surface protectant that mitigates O_3 toxicity indirectly, with no direct interference inthe plant stress response system (Ashrafuzzaman et al. 2018).

3) It has previously been speculated that EDU may mediate abscisic acid (Agathokleous 2017). A new study with *Populus alba* "Berolinensis" saplings revealed that elevated O_3 decreased abscisic acid content (compared to unexposed controls), an effect that was partially reversed by EDU (Xu et al. 2019). Similarly, elevated O_3 decreased the emission rate of volatile organic compounds (VOCs) (compared to unexposed plants), and EDU induced recovery (Xu et al. 2019). This suggests that EDU can also mediate O_3 effects by VOCs-O_3 reactions near the leaf surface.

4) EDU increased the abundance of proteins related to carbon metabolism, defense, and photorespiration (Fig.13.3), which enhanced the performance of plants of Kundan, a double dwarf variety of wheat (*Triticum aestivum*), grown in ambient O_3 (Gupta et al. 2018). EDU also increased the photosynthetic proteins (PEP carboxylase and RuBisCO large and small subunits) of two Indian maize (*Zea mays*) cultivars (Buland and Prakash) grown in ambient or elevated O_3, thus enhancing biomass and economic yields (Singh et al. 2018a). These results agree with an earlier study showing that snap bean (*Phaseolus vulgaris*) plants protected by EDU against O_3 toxicity had levels of transcripts and enzymes similar to those in control plants (Paoletti et al. 2014). More recently, a study analyzing apoplast and chloroplast proteome in two Indian wheat varieties exposed to ambient O_3 also revealed that O_3-induced oxidative stress was minimized by EDU by regulating the abundance of chloroplast proteins [includingATP synthase, vacuolar proton ATPase, chaperonin, Ferredoxin NADP- reductase (FNR), fructose,1-6 bis phosphatase (FBPase), and RuBisCO] (Gupta et al. 2021).

5) Some of these newer studies show a cultivar-specific protective effect of EDU against O_3-induced decrease in g_s; i.e., EDU increases g_s in O_3-stressed plants (Ashrafuzzaman et al. 2017; Baqasi et al. 2018; Singh et al. 2018b; Zhang et al. 2018; Feng et al. 2018; Pandey et al. 2019; Fatima et al. 2019), which also depends on the plant developmental phase (Pandey et al. 2019); see also Agathokleous (2017) for a discussion of older literature regarding EDU effects on g_s. However, other studies, some of which were designed to study the effect of EDU on g_s, show that EDU does not significantly affect g_s, which also suggests that the protecting mode of action of EDU is not upon a decreased O_3 uptake through decreased g_s (Salvatori et al. 2017; Jiang et al. 2018; Xu et al. 2019). The latter non-significant effects of EDU on g_s agree with the older literature (Feng et al. 2010).

6) It is also documented that EDU does not contribute significantly to plants with N when applied at concentrations used for protection against O_3-induced toxicity (Agathokleous et al. 2018a, b), thus re-affirming the earlier discussion suggesting that the EDU mode of action against O_3 toxicity is not upon N contained in EDU (Manning et al. 2011; Agathokleous 2017).

13.7.2 EDU-induced hormesis

EDU effects are concentration-dependent, even within the range of concentrations typically applied to protect plants against O_3 toxicity

(Agathokleous 2017; Agathokleous et al. 2018a). Some new studies provide evidence for EDU-induced hormesis in plants, such as in maximum electron transport rate, plant growth, and biomass (Agathokleous et al. 2018a, b; Gupta et al. 2018; Jabeen and Ahmed 2021). Hormesis is a biphasic dose/concentration-response phenomenon (13.4), where low levels of xenobiotics induce stimulatory effects, and high levels induce adverse effects (Agathokleous et al. 2019d; Agathokleous and Calabrese 2019). In a standardized toxicological bioassay with EDU and duckweed (*Lemna minor*) in O_3-free air, it was demonstrated that EDU could induce hormesis in fluorescence parameters (Agathokleous et al. 2016a). Further discussions on the topic revealed that multiple published studies provide evidence of EDU-induced hormesis (Agathokleous 2017; Agathokleous and Kitao 2018), and the evidence is growing (Agathokleous et al. 2018a, b; Gupta et al. 2018; Jabeen and Ahmed 2021).

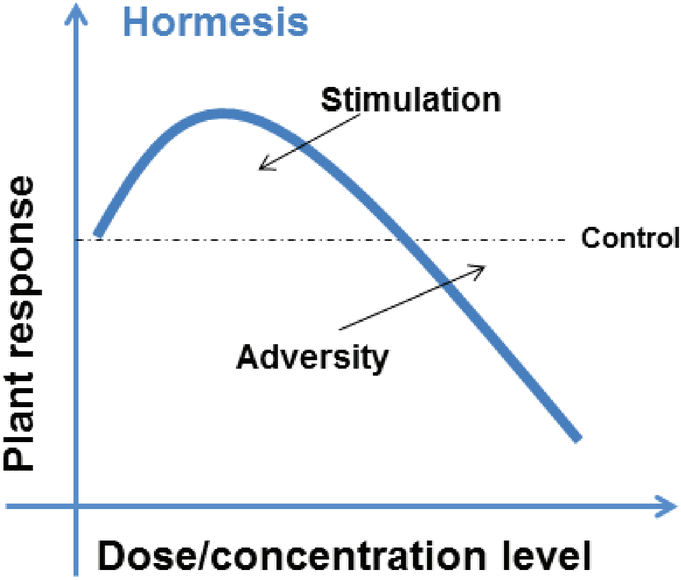

Fig.13.4. A hypothetical hormetic dose-response relationship. The traditional toxicological threshold is the dose level where the highest concentration crosses the baseline (control) and adverse effects begin

Hormesis has been induced by numerous xenobiotics in plants and animals and other forms of life, suggesting its general occurrence across systems (Agathokleous and Calabrese 2020). Its quantitative characteristics are also similar among forms of life and independent from the underpinning biological mechanisms, with the stimulatory response restricted below 200% and commonly within 120–160% of the control response; the limited stimulation also makes difficult its statistical identification (Agathokleous et al. 2019c, a, e; Calabrese et al. 2019). Its wide occurrence in plant systems is further supported by the fact that biostimulants and preconditioning (or priming) fall under the umbrella of hormesis (Agathokleous et al. 2019d).

EDU effects are more pronounced in O_3-susceptible genotypes than in O_3-tolerant genotypes of agricultural crops (Ashrafuzzaman et al. 2017, 2018; Pandey et al. 2019). EDU also has a higher effect on plants under higher O_3 stress than on plants under lower O_3 stress (Singh et al. 2018a; Xu et al. 2019). Furthermore, the EDU effects, especially at the plant level, when EDU is applied at the concentrations used against O_3 toxicity, are limited when the plants are under no O_3 stress (Agathokleous et al. 2018b). For instance, in a rice study (*Oryza sativa*), EDU had almost no effects on plants in theoretically O_3-free air (Ashrafuzzaman et al. 2017). However, it reduced O_3 adverse effects on the visible foliar injury, lipid peroxidation, leaf greenness, stomatal conductance (g_s), several spectral reflectance parameters, panicle number, grain yield, and spikelet sterility (Ashrafuzzaman et al. 2017). Similar results were obtained in different studies using low O_3 concentrations or theoretically O_3-free air and elevated O_3 concentrations as well as different concentrations of EDU (Agathokleous et al. 2014, 2016b, e, 2018a). A preliminary transcriptome profiling by RNA sequencing suggested that thousands of genes responded to O_3 in a susceptible variety of rice (*Oryza sativa*). However, almost none responded to EDU (Ashrafuzzaman et al. 2018). Interestingly, this newer study also found that significant O_3-EDU interactions occurred mostly in O_3-responsive genes, where EDU up-regulation was mitigated by EDU application (Ashrafuzzaman et al. 2018). It is important to note that these observations agree with the broader hormesis literature, where low-dose stimulatory effects are more pronounced in conditions with lower background response, i.e., under environmental stress, thus alleviating the adverse effects of stress.

In a study investigating the effects of EDU on Manna ash (*Fraxinus ornus*) plants under drought conditions in O_3-polluted ambient air, EDU improved plant functionality under water deficit stress (Salvatori et al. 2017). It suggests that EDU is not an O_3 protectant exclusively but potentially a general plant protectant against oxidative stress. Conversely, other experiments did not find mitigation of the effects of different abiotic stresses, including iron toxicity, zinc deficiency, and salinity, by EDU

(Ashrafuzzaman et al. 2018). However, such experiments are technically tricky because they require a multifactorial experimental design with an increased number of dose/concentration levels of each factor (EDU and stressor), which should also be eventually narrowly-spaced across the dose/concentration-response spectrum. In contrast, hormesis suggests that a hormetic agent would be capable of inducing stimulatory effects and protecting against various stressors. More studies are needed, and, especially, experiments that are designed to address the specific question of whether EDU is a general plant protectant and not an exclusive O_3 protectant (Agathokleous and Kitao 2018).

13.8 Conclusions

Some basics regarding EDU and its use as an O_3 protectant were discussed, including some definitional dimensions, historical foundations, and principles of its application to plants. A summary of the earlier knowledge regarding EDU effects on plants under O_3 stress was also presented. Finally, after the last literature review (2017 to date), research developments regarding the EDU mode of action were discussed. It is now known that EDU can affect leaf stoichiometry in the early stages of O_3-induced stress and mediate abscisic acid content in leaves and emission of VOCs from leaves. EDU also affects several proteins, such as increasing the abundance of proteins related to carbon metabolism, defense, and photorespiration, thus enhancing plant health under O_3-induced stress. It seems that EDU can also affect VOCs-O_3 reactions near the leaf surface.

Furthermore, EDU is deposited on leaf surfaces and seems to have O_3 degrading properties. Therefore, EDU also appears to have indirect effects on plant-O_3 interaction. EDU's direct impacts on plants under O_3 stress seem to perfectly fit the concept of hormesis, with several studies presenting empirical evidence showing that EDU is a hormetin. Newer studies with improved experimental studies, considering the discussion presented in this chapter, are highly encouraged.

Dedication

This chapter is dedicated to Dr. William J Manning, Emeritus Professor at the University of Massachusetts, Amherst, USA, for his leadership and stimulating role in O_3 protectant research from the early days in O_3-plant research to date.

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CHAPTER FOURTEEN

ISOPRENE EMISSION IN PLANTS: A TOOL TO HANDLE OZONE STRESS

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Abstract

Isoprene, a highly reactive and almost uncontrollable volatile organic compound, is emitted in large amounts by vegetation. In the troposphere, isoprene plays a significant role in the maintenance of the NO–NO_2–O_3 cycle, which is responsible for the formation and degradation of ozone (O_3). The functional aspects of isoprene emission in plants are still a matter of debate. It is hypothesized that the mechanism of isoprene biosynthesis evolved in plants during their water to land transition. Studies have shown that isoprene effectively preserves the thylakoid membrane's integrity, not only by interacting with lipid acyl chains but also by up-regulating the proteins associated with photosynthetic complexes. Tropospheric O_3, on the other hand, is a well-recognized air pollutant which can induce isoprene production in plants. Experiments have proved that exposure to high O_3 led to less cellular damage to the photosynthetic process in the presence of isoprene. It has been further observed that recovery of photosynthesis after relief from stress was more prominent in isoprene emitters than non-emitters. The present chapter aims to discuss the "how" and "why" of isoprene production in plants. Under the current climate change scenario, which promotes O_3 formation, plants are expected to face more severe O_3 stress in the near future. As such, the isoprene emitters will have a physiological advantage over the non-emitter lines. The present chapter also focuses on the role of isoprene in

atmospheric chemistry and the significance of isoprene emission under the future climate change scenario.

Keywords: Ozone stress; Vegetation; Isoprene; Emission; Tool

14.1 Introduction

Many biogenic volatile organic compounds (BVOCs) are emitted by terrestrial ecosystems, significantly affecting the atmosphere's chemistry and composition (Guenther 2013). Isoprene (C_5H_8; 2-methyl-1, 3-butadiene) represents an important class of BVOCs, comprising about half of the total BVOCs emitted globally (Zenone et al. 2016). The annual global BVOCs' flux is estimated to be about 1000 Tg, with isoprene forming 53% of the total emissions (Guenther et al. 2012). Isoprene is emitted from various terrestrial ecosystems, including foliage, flowers, stems, trunks, roots, leaf litter, soil microbes, etc. However, the most important source of isoprene emission is the foliage (Loreto and Schnitzler 2010; Guenther et al. 2012; Greenberg et al. 2012). Isoprene emission is considered to be a complex phenomenon as different processes control it. In some plants, isoprene is stored in the plant tissues isolated from the atmosphere and is emitted only if they are damaged (Yazaki et al. 2017). In other cases, isopreneis produced in structures that are in continuous contact with the atmosphere (Ciccioli et al. 1999). There are certainly other cases where isoprene is emitted only if the plant is under environmental stress (Dudareva et al. 2006).

Isoprene emission is not a universal feature and is confined to limited plant species, which are termed as "emitters." A compilation of isoprene-related studies indicates that about 20% of the world's perennial vegetation of tropical and temperate regions of the world are emitters (Loreto and Fineschi 2015). The energy cost of isoprene emission that emitters bear is relatively high, accounting for about 20 ATP and 14 NADPH per isoprene molecule (Sharkey and Yeh 2001). Under normal conditions, isoprene emission utilizes 2–5% of the total carbon fixed by photosynthesis; however, in stressed conditions, this amount reaches about 10–15%, or even more than that of photosynthesis (Brilli et al. 2014).

Isoprene emission should have a substantial physiological role in plants where it is emitted. It has been suggested that isoprene emission is an adaptive mechanism of emitters to overcome environmental stress like high temperature and oxidative pollutants (Sharkey et al. 2001; Monson et al. 2013). It has been shown that isoprene emission increases with temperature up to 40°C, even if carbon assimilation is declining (Brilli et al. 2014). This uncoupling of isoprene emission from the photosynthetic process strengthens the hypothesis that isoprene may protect plants against heat stress (Vickers et al. 2009). Similarly, a high concentration of ozone (100–300 ppb) for the short term is also known to stimulate isoprene emission (Calfapietra et al. 2009). The role of isoprene in providing tolerance against O_3 stress is well-cited (Loreto and Fineschi 2014). Experimental pieces of evidence have demonstrated that isoprene increases the resistance to O_3 stress by reacting with the reactive oxygen (ROS) and nitrogen species. It stabilizes the thylakoid membrane, not only through direct interaction with their lipid acyl chains but also by up-regulating proteins associated with photosynthetic complexes and enhancing the biosynthesis of relevant membrane components, such as mono- and di-galactosyl-diacylglycerols and unsaturated fatty acids (Velikova et al. 2015). Isoprene may additionally protect photosynthetic membranes by scavenging reactive oxygen species (ROS) (Velikova et al. 2014). Since isoprene is produced in the chloroplasts, the stabilization of the photosynthetic apparatus is expected to be the most important aspect of isoprene-induced tolerance in plants against O_3 stress. The hydrophobicity of the isoprene molecule allows the interaction with nonpolar components resulting in membrane stabilization. However, the increased ROS scavenging capacity of isoprene emitters also explains their O_3 tolerance. Therefore, it can be suggested that isoprene's positive effect against O_3 stress operates via multiple channels instead of working towards a single target.

14.2 Phylogeny of Isoprene Emission

It is still difficult to predict the phylogeny of isoprene emission, yet a few workers have attempted to categorize the emitters based on their biology, life forms, or ecology (Monson et al. 2013). A preliminary examination of the distribution of isoprene emission among different plant lineages does not show any particular correlation among the emitters and points towards independent origins (Monson et al. 2013). For instance, taxonomically distinct groups like mosses and oak trees emit isoprene but are closely allied groups like mosses and “hornworts” mosses are emitters, but “hornworts” are non-emitters (Hanson et al. 1999). Similarly, in closely related oaks and maple trees, oaks come under the emitter category, while maple is a non-emitter (Lerdan and Gray 2003). In a certain taxonomically diverse group such as Fabaceae, the occurrence of isoprene emitting genera is a frequent phenomenon. Still, in another high taxonomically diverse group, Poaceae, isoprene emission is rare (Monson et al. 2013). A detailed study of about 1200 plant speciesconcerning their isoprene emission ability was conducted by Loreto and Fineschi (2014), and the following patterns have been suggested,

1. Isoprene emission is mostly restricted to C_3 plants. CAM and C_4 plants rarely show the phenomenon of isoprene emissions. However, more studies and related data are required before conclusively ruling out emissions from CAM and C_4 species.
2. Isoprene emission is mostly observed in perennial plants (about 20% of the perennial plants come under the emitter category) and is almost absent in annuals. This pattern is observed in both dicots and monocots with annual and perennial families (Loreto and Fineschi 2014).
3. Isoprene emission is more common in deciduous than in evergreen plants. This pattern is more prominent in temperate regions where 30.7% of deciduous trees are isoprene emitters, whereas only 9% of evergreens come under this category. However, in the tropical area, the percentage of emitters is 16.3 and 20%, respectively, for deciduous and evergreen trees (Loreto and Fineschi 2014).
4. Isoprene emission is more common in fast-growing plants (Dani et al. 2014). Fast-growing plants such as *Populus* and *Salix* (dicots) and *Arundo donax* and *Phragmites australis* (monocots) are strong isoprene emitters (Loreto et al. 2014). Fast-growing species of oaks (*Quercus rubra* or *Q. robur*) are strong isoprene emitters, whereas slow-growing species (*Q. ilex* or *Q. suber*) are either non-emitters or isoprene emission is replaced by monoterpenes (Loreto et al. 2014; Dani et al. 2014).

14.3 Ozone-Vegetation Coupling, Role of Isoprene Emission in Air Quality

Tropospheric ozone (O_3), being a secondary pollutant, is mainly produced via the photochemical-oxidation of carbon monoxide (CO), methane (CH_4), and non-methane volatile organic compounds (VOCs) by hydroxyl radicals (OH), in the presence of nitrogen oxides (Gligorovski et al. 2015; Dunker et al. 2016; Sadiq et al. 2017). Vegetation cover plays a significant role in tropospheric O_3 chemistry as it is the largest source of natural biogenic VOCs (Guenther et al. 2012; Zenone et al. 2016; Fitzky et al. 2019). Isoprene is the single most abundant non-methane VOC, contributing significantly to O_3 formation. Isoprene, when present in the atmosphere, ensures the availability of OH and feeds the $NO–NO_2–O_3$

cycle that is responsible for the formation (Jardine et al. 2012) and degradation (Rivera-Rios et al. 2014) of O_3 in the troposphere. Isoprene chemistry has gained a lot of interest in recent years (Fuchs et al. 2013; Jacobs et al. 2013; Peters et al. 2014; Dunker et al. 2016).

The complexity of O_3 formation and the precursors' organic chemistry is still a subject of intensive research (Fittschen et al. 2017). The photolysis of NO_2 is always the initial step of O_3 formation. The recycling of NO to NO_2 occurs after CO, methane, or VOCs react with OH and generate hydroxyl and peroxy radicals, which readily react with NO, regenerating NO_2. This recycling of NOx is termed as the ROx–NOx cycle. The photochemical O_3 formation has a non-linear relationship with its precursors, VOCs, and NOx, depending on their relative concentrations (Liu et al. 2012; Ling et al. 2014). In the absence of isoprene, the formation of O_3 is governed by the total NOx levels in the atmosphere (Fig.14.1). However, in the presence of isoprene, the oxidation of isoprene by atmospheric hydroxyl radicals leads to the conversion of NO to NO_2, thus facilitating more O_3 production (Gilman et al. 2009; Sharkey et al. 2008) (Fig14.1). Therefore, at low NOx concentrations, O_3 formation becomes “NOx limited," but at high NOx concentrations, coupled with the presence of VOCs (isoprene), OH recycling becomes efficient to sustain high levels of VOCs (isoprene). O_3 formation thus becomes “VOCs limited” (Carter 1994) (Fig.14.1). Further, the RO_2 and NO formed by the isoprene oxidation of OH can lead to the formation of isoprene nitrate ($RONO_2$), which can be recycled to generate more NO_2 and O_3 (Paulot et al. 2012).

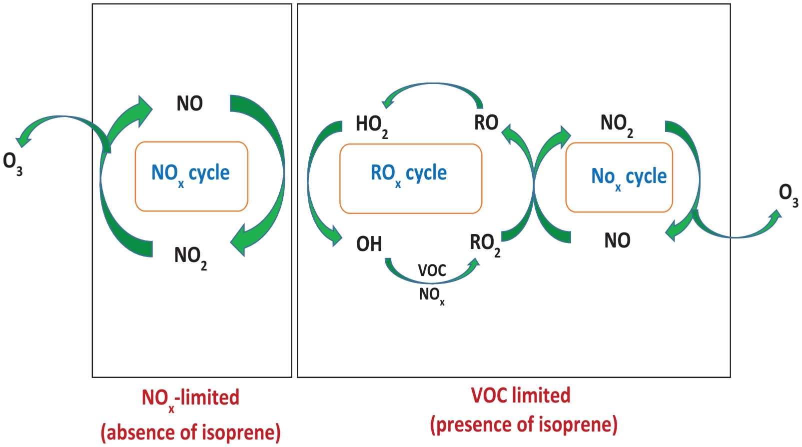

Fig. 14.1. Coupling of the RO_x–NO_x cycle in O_3 formation in the troposphere

There is prominent evidence that suggests the interdependence of O_3 formation and isoprene emission. Geng et al. (2011) used a regional dynamic/chemical model to study the effect of biogenic isoprene emission on O_3 production in the urban areas of Shanghai, as well as in the nearby forested areas. A high concentration of isoprene emission was recorded from a major forest region located in the south of Shanghai (Geng et al. 2011). As the concentration of NOx was low in the forest region, O_3 production due to oxidation of isoprene was lower. However, in Shanghai, situated downwind of the forest, HO_2 radicals produced by the isoprene oxidation reacted with the anthropogenic NOx, resulting in enhanced NO_2 concentration and increased O_3 production (Fig. 14.2).

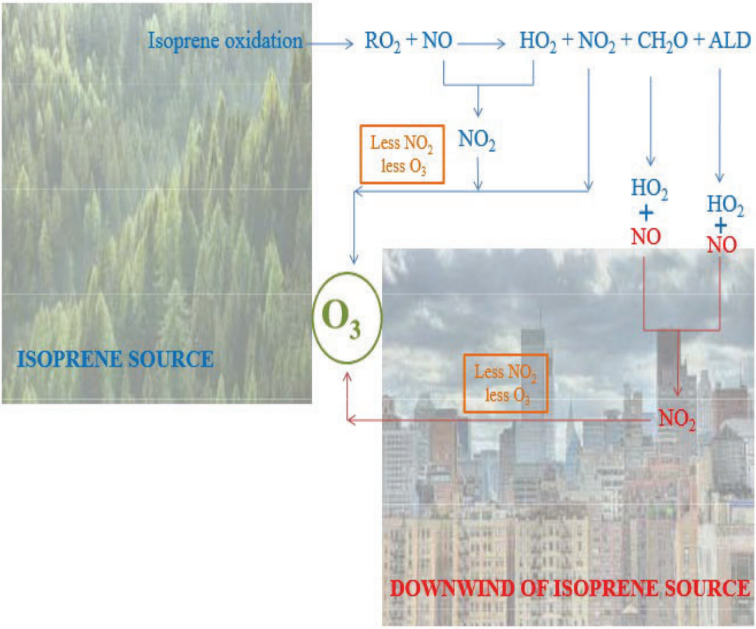

Fig. 14.2. Interrelationship between isoprene emissions and O_3 production (Modified from, Geng et al. 2011)

Isoprene is considered to be the most important VOC in terms of the amount emitted and its high reactivity (Fitzky et al. 2019). Isoprene is about 22 times more reactive than the important anthropogenic VOC, benzene (Wagner and Kuttler 2014). Furthermore, CO’s and methane's reaction constants are about 410 and 14,000 times lower than that of isoprene (Guenther et al. 2012). As such, isoprene can very well compete with other O_3 precursors, thus emerging as an important entity in O_3 formation (Wang et al. 2017).

Dunker et al. (2016) studied the role of isoprene chemistry in O_3 formation in California using three simulated chemical mechanisms; the Statewide Air Pollution Research Centre 2007 mechanism with updated toxics and isoprene chemistry (S07TIC); the Carbon Bond6, revision 2 (B6r2) mechanisms; and a modified CB6r2 mechanism with increased OH production from isoprene oxidation at low NOx concentration (CB6r2OH). The results showed that O_3 had both positive and negative sensitivities to isoprene emission depending upon the extent to which isoprene nitrate recycled to NOx (Dunker et al. 2016). The O_3 sensitivity to isoprene emissions was positive at most locations but was negative at locations in Northern California where NOx emission was small.

There existed an insubstantial balance between O_3 formation and O_3 destruction in the low NOx environment, and the isoprene nitrate formed via the isoprene chemistry played an important role (Paulot et al. 2012). In a study done by Dunker et al. (2016), it was observed that the second-generation isoprene nitrates produced in CB6r2 isoprene chemistry do not recycle to NOx and O_3 formation, while in S07TIC, all the isoprene nitrates produced recycle to NOx. Therefore, the behavior of isoprene nitrates produced through the isoprene chemistry is responsible for the differential sensitivities of CB6r2 and S07TIC, thus giving different estimates of O_3 prediction (Dunker et al. 2016). Mao et al. (2013) have also observed a positive sensitivity of O_3 to isoprene emission throughout the US using their chemical mechanism GEOS-Chem version 9-01-03, but a negative sensitivity was observed in Southeastern and Western US using the GEOS-Chem global model of Zhang et al. (2011).

14.4 Factors Regulating Biogenic Isoprene Emissions

Biogenic isoprene emissions depend upon many factors such as vegetation cover, plant functional types, and meteorological conditions like temperature and radiations (Hollaway et al. 2017; Lu et al. 2019a). Guenther et al. (2006) have shown exponential enhancement of biogenic isoprene emissions with rising temperatures using the chemical model MEGAN (Model of Emissions of Gases and Aerosols from Nature). The increased isoprene emission at high temperatures is identified to be the main driver in governing the O_3 temperature correlation over urban areas where NOx emissions are high (Pusede et al. 2015; Lu et al. 2019b). Doherty et al. (2013) used three coupled climate change chemistry models (CCMs). They observed that a 3ºC temperature enhancement would increase the biogenic isoprene emissions by 6–31% and surface O_3 by >2 ppbv in northern mid-latitudes. Several workers have stated that the O_3 response to temperature depends on the amount of NOx recycling from isoprene nitrates (Ito et al. 2009; Fiore et al. 2012). The decline in O_3 concentrations at a fairly high temperature can also be explained by the temperature dependent variations in isoprene emissions (Lu et al. 2019a). The rate of isoprene emission increases with temperature until an optimum of about 40ºC (Niinemets et al. 1999). Steiner et al. (2010) observed that a decline in surface O_3 at high temperatures (38.85ºC) could be attributed to the suppression of biogenic isoprene emission from that area's vegetation.

Hollaway et al. (2017) studied the sensitivity of nineteenth-century tropospheric O_3 to vegetation distribution and atmospheric chemistry vegetation interaction processes using an Earth-System model-HadGEM2-ES. It has been observed that changes in vegetation cover during the mid-nineteenth century led to an increase in global isoprene emission by 19%, which resulted in a rise in surface O_3 over the continents up to 2 ppbv and of 2–6 ppbv in the tropical upper troposphere (Hollaway et al. 2017). Isoprene emission is also sensitive to vegetation type and foliage density represented by indices like the leaf area index (Niinemets et al. 2010a, 2010b). Fu and Tai (2015) demonstrated that in high NOx-VOC limited regions over many parts of China, O_3 concentration decreased despite increased isoprene emission. It was also shown that the higher summertime leaf area index enhanced the dry deposition of O_3, resulting in a lesser increase in O_3 concentration in the troposphere. Contrary to this, in NOx limited areas of western China, isoprene emission dominated, and O_3 levels were shown to increase despite high deposition (Fu and Tai 2015).

Further, the type of vegetation and the plant functional types (cropland or forestland) also play a significant role in determining the dominance of isoprene emission or dry deposition, thus governing the surface O_3 pattern (Wu et al. 2012; Fu and Tai 2015). The conversion of isoprene-emitting forest to lowemitting cropland to cope with worldwide food demand decreased isoprene concentration by 15% globally during the last century (Lathiere et al. 2010). However, the prominence of the cultivation of bioenergy crops like giant reed and short-rotation forests like poplar will likely increase isoprene load, particularly at the regional scale (Hardacre et al. 2013; Sharkey and Monson 2014). Studies have shown that in South East Asia, 27 Mha of land are utilized for the cultivation of oil palm, which emitted three times more isoprene than the native crops (Fowler et al. 2011) and increased surface O_3 by 11% (Ashworth et al. 2012). Similarly, the expansion of short rotation forests in the temperate northern hemisphere was predicted to trigger the increase in isoprene burden for boreal Eurasia, North America, and China, where O_2/O_3 mixing ratios are expected to increase to 2.26 ppb (Ashworth et al. 2012; Hard acre et al. 2013; Zenone et al. 2016). Hollaway et al. (2017) studied the isoprene emission, using the model VEG-2000 and reported a reduction of 109 $TgCyr^{-1}$ in isoprene emissions as compared to PI-CTRL (control simulation) of 580 $TgCyr^{-1}$, upon the replacement of high isoprene-emitting broad-leaved trees with grasses and crops from the mid-nineteenth century to the present day. This reduction in isoprene emissions results in an increased O_3 concentration because the reduced isoprene emission caused a decrease in PAN formation, leading to a higher NOx concentration, which enhanced O_3 formation in the atmosphere.

Surface O_3 concentration in the VEG-2000 simulation is upto 2 ppbv greater over parts of North America, South East Asia, South America, and Africa compared to the mid-nineteenth-century control scenario, in the regions of the most significant loss of tree cover and replacement with a grass type of vegetation (Hollaway et al. 2017). Contrary to this, O_3 concentration under the VEG-2000 scenario is lower than that of PI-CTRL simulations by 1.5 ppbv in Northern Africa, Central Asia, and Australia, which are the areas marked by insignificant isoprene emission sources, i.e., isoprene doesn't play an important role in O_3 formation (Hollaway et al. 2017). It is important to note that a decrease in isoprene results in a lower O_3 concentration over marine regions, which can be attributed to a decrease in PAN formation and a subsequent reduction in the transport of NOx to these regions, reducing O_3 formation (Hollaway et al. 2017).

Isoprene emission and its interaction with NOx also act as a deciding factor in the surface O_3 pattern. In this context, the formation of PAN due to isoprene oxidation also influences the O_3-isoprene chemistry in the troposphere. PAN is produced by photochemical oxidation reactions with abundant NOx and VOCs. PAN formation serves as a sink for NOx and peroxy radicals, thereby lowering the O_3 production near the source region. PAN, so formed, is transported across the cold troposphere and eventually decomposed due to air heating, releasing NOx, and consequently enhances O_3 production in remote areas (Fischer et al. 2014; Xu et al. 2015; Han et al. 2017). Doherty et al. (2013) observed that PAN chemistry changes would lead to an enhancement of 4.2 ppbv O_3 over land and a reduction of 1 ppbv over oceans.

Because of the present climate change scenario, which predicts a remarkable increase in atmospheric CO_2 concentration shortly, a few studies have investigated the effect of elevated atmospheric CO_2 on biogenic isoprene emission in several plants (Possell and Hewitt 2011; Tai et al. 2013; Fu and Liao 2016). It has been demonstrated that atmospheric CO_2 concentration might promote or limit isoprene emissions from vegetation. Increased atmospheric CO_2 is accompanied by a phenomenon of "CO_2 fertilization," which stimulates the growth of vegetation cover, thus indirectly enhancing isoprene emission (Piao et al. 2011). However, at the leaf level, isoprene emission shares an inverse relation with rising CO_2 concentration (Fu and Liao 2016). The biochemical basis for the CO_2 inhibition of isoprene emission is not fully understood but may involve competition for substrates between terpenoid biosynthesis and cytosolic carbon metabolism (Rosenstiel et al. 2003). It is now well accepted that assimilated CO_2 from photosynthesis is channeled into isoprene biosynthesis (Monson et al. 2012). The 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway in the chloroplast of leaves (Lichtenthaler et al. 1997) provides a proper understanding of the connection between photosynthesis and isoprene biosynthesis. This pathway is dependent on photosynthesis for carbon substrates and NADPH reductant and ATP (Lichtenthaler et al. 1997). In the model produced by Martin et al. (2000), the isoprene emission is driven by ATP's competitive partitioning between photosynthesis and isoprene biosynthesis. In this model, as the internal CO_2 concentration increases, negative feedback is imposed on isoprene biosynthesis due to the limited turnover of sugar-phosphates and associated limitations by inorganic phosphate on ATP production (Monson et al. 2012). However, more assessment needs to be done to analyze the energetic and reductant requirements of isoprene biosynthesis.

Fu and Liao (2016) studied the effect of CO_2 inhibition on isoprene emission in China over the period 2006-2011 using the global chemical transport model (GEOS-Chem). They observed an annual reduction of 5.6% in isoprene emissions due to CO_2 inhibition. The annual isoprene emission over China averaged 12.62 $TgCyr^{-1}$ without including the CO_2 inhibition factorduring the period 2006-2011. However, when the CO_2 inhibition scheme was included, the average annual isoprene emission decreased by 2.7–7.4% during the same period (Fu and Liao 2016). Arneth et al. (2007) also reported a reduction of ~7% in annual isoprene emission over China, using the CO_2 inhibition effect in the dynamic vegetation model framework, LPJ-GUESS. Wilkinson et al. (2009) had also demonstrated that isoprene emission from the leaves of *Eucalyptus globules* showed a reduction in a continuous gradient when CO_2 concentration varied from 240 to 520 ppmv. *Populus deltoides* and *P. tremuloides* showed 30–40% reductions in isoprene emission rate when grown in environmentally controlled chambers at 800 ppmv CO_2 compared to 400 ppmv (Wilkinson et al. 2009).

The inhibitory effect of CO_2 on isoprene emission becomes significant when the studies related to the effect of climate change on isoprene emission are taken into account. Fu and Liao (2016), working with the MEGAN module embedded with GEOS-Chem, reported that meteorological changes between the late 1980s and mid-2000s led to increased isoprene emission by 17%.This value can be modified if the CO_2 inhibitory effect is incorporated in the model (Fu and Liao 2016). Heald et al. (2009) investigated the probable role of atmospheric CO_2 on isoprene emissions using a global coupled land-atmosphere model, CAM–CLM (Community Atmospheric Model–Community Land Model) for conditions inthe years 2000 (365 ppm CO_2) and 2100 (717 ppm CO_2) and reported that CO_2 effects would completely nullify the large increments in isoprene emission predicted due to warmer climates. Projected global isoprene emissions in 2100 dropped from 696 to 479 $TgCyr^{-1}$ when CO_2 inhibitory effects were included, maintaining future isoprene sources at levels similar to the present day (Heald et al. 2009).

14.5 Biogenic Isoprene Emission, Mechanisms Involved

As discussed earlier, isoprene emission is a trait adopted by a few specific plants to cope with the oxidative stress by quenching the ROS/reactive nitrogen species (RNS) (Jardine et al. 2011). Chemically, isopreneis a diene with conjugated double bonds, making it a highly reactive molecule and explaining its ability to combat oxidative stress (Loreto and Fineschi 2015). Although the concept of isoprene emission under stress was established in the 1960s (Rasmussen and Went, 1965), the “how and why” of isoprene emission by specific plants remains enigmatic (Dani et al. 2014; Velikova et al. 2016). The most recent emerging view regarding isoprene emission states that isoprene acts as an important agent in cellular protection from ROS generated during stress events that impair optimum coupling between light and dark reactions within the chloroplasts (Harrison et al. 2013). The main driver for isoprene emissions during stress conditions is most likely to be photosynthetic inhibition and increased generation of ROS because of the oversupply of reducing equivalents (Selmar and Kleinwachter 2013). The isoprene formed serves as an efficient scavenger of ROS and the excessive reducing power under stressful conditions (Agati et al. 2012; Harrison et al. 2013; Esteban et al. 2015). Behnke et al. (2007) reported a reduction in the photosynthetic electron transport rate (ETR) in *Populus* x *canescens* lines when isoprene biosynthesis was suppressed during heat stress. It was also observed that these plants did not recover photosynthesis at the rate of isoprene emitting lines once the stress was removed (Behnke et al. 2007). Tattini et al. (2014) reported that isoprene emission provided resistance to severe drought even when artificially induced in transgenic tobacco lines (*Nicotiana tabacum* cv Samsun). It was further observed that photosynthetic recovery was incomplete after removing stress as the plants were investing a greater proportion of their carbon budget into isoprene production even after the removal of stress (Tattini et al. 2014). These studies indicate that isoprene emission protects the photosynthetic apparatus from oxidative stress, not only by quenching the enhanced ROS production but also through improved stabilization of photosynthetic membranes (Ryan et al. 2014), possibly enhancing the stability of PS II light harvesting complexes (Velikova et al. 2016).

Pollastri et al. (2014) studied the non-photochemical quenching (NPQ) of chlorophyll fluorescence of leaves of the isoprene emitter *P. nigra* and transgenic isoprene emitting lines of *Nicotiana* and *Arabidopsis*. It was reported that the NPQ of fluorescence was reduced in species in which isoprene emission occurred naturally or when they are genetically programmed to produce isoprene with respect to when isoprene is not produced (Pollastri et al. 2014). This work confirms the hypothesis that isoprene emission reduces pressure on the photosynthetic membranes by removing the thermal energy at the thylakoid level at high temperatures (Pollastri et al. 2014). As the isoprene emitters enhance the PS II efficiency and reduce the need for heat dissipation in photosynthetic membranes, it is postulated that isoprene emitters will be more suitably adapted in the warmer climates of the near future (Pollastri et al. 2014).

Besides, isoprene stabilizes the thylakoid membrane structure (Velikova et al. 2014, 2015, 2016). Studies have shown that isoprene's protective function on the membrane may not merely depend upon the hydrophobic interaction between the isoprene and lipid acyl chains of thylakoid membranes. The isoprene concentration within the cell is too low to effectively modulate their lipid phase (Harvey et al. 2015). The suppression of isoprene biosynthesis caused considerable metabolic changes, alteration of lipid biosynthesis being one of them (Way et al. 2013; Kaling et al. 2015). The thylakoid lipid bilayer of the chloroplast is characterized by a high proportion of galactolipids consisting of monogalactosyldiacylglycerol (MGDG) (~50%) and digalactosyldiacylglycerol (DGDG) (~30%) molecules (Joyard et al. 2010). Sulphoquinovosyldiacylglyceral (~5–12%) and phosphatidylglycerol (~5–12%) are other important galactolipids of the thylakoid membrane (Kirchhoff et al. 2002). Galactolipids contain a large proportion of polyunsaturated fatty acids, which provide more fluidity to the thylakoid membrane than other biological membranes, a feature essential for photosynthetic processes (Gounaris and Barber 1983).

The lipid composition of thylakoid membranes and the chloroplast ultrastructure of isoprene emitting (IE) and non-emitting (NE) poplars (*Populus* x *canaescens*) were studied by Velikova et al. (2015). A significant reduction in the content of galactolipids (MGDG and DGDG) was observed along with the down-regulation of unsaturated linolenic acid in the NE chloroplasts as compared to the IE ones (Velikova et al. 2015). The low linolenic content in the lipid fraction indicates reduced fluidity of the thylakoid membranes, which affects the efficiency of PS II in NE compared to IE poplar lines (Velikova et al. 2015). Reductions in MGDG and DGDG resulted in a significant decline in the grana stacks per chloroplast in NE compared to IE poplar lines, which was responsible for a significant decrease in PS I and PS II proteins (Velikova et al. 2015). Velikova et al. (2011) have demonstrated modifications in the thylakoid lipid bilayer of transgenic *Arabidopsis* modified for isoprene emission and suggested that isoprene enhances the stability of PS II light-harvesting complex II (LHC II) through modifications of pigment-protein complex organization in thylakoid membranes (Velikova et al. 2011).

The role of isoprene in providing stability to the photosynthetic apparatus has been studied using transgenic approaches, either by inhibiting the natural isoprene emission in *Populus* x *canescens* (Behnke et al. 2007), or by the introduction of this trait in non-emitters like *Arabidopsis thaliana* (Fini et al. 2017; Moritz et al. 2017) and *Nicotiana tabacum* (Sainger et al. 2017). It has been proved that isoprene improves thylakoid membranes' integrity and functionality under optimum conditions in transgenic *Arabidopsis* emitting *isoprene* (Jud et al. 2015; Harvey et al. 2015). Brunetti et al. (2018) have shown significant metabolome-wide rearrangements of cellular metabolism between isoprene-emitting and non-emitting lines of poplar under stress conditions. Velikova et al. (2014) indicated that suppression of isoprene production in lines of transgenic grey poplar (*Populus* x *canescens* syn. *P. tremula* x *P. alba*) caused changes in the chloroplast protein pattern, which was responsible for changes in chloroplast functionality. Down regulation of isoprene synthase leads to decreased levels of chloroplast proteins involved in photosynthesis and increased levels of histones, ribosomal proteins and proteins related to other metabolic activities (Velikova et al. 2014).

14.6 Effect of Tropospheric Ozone on Isoprene Emissions in Plants

It is very important to decipher the interconnection between O_3 and isoprene because the present global climate change scenario is speculated to cause a circumstantial increase in both gases (Fares et al. 2006). Scientists, based on modular studies, have predicted an increase in tropospheric O_3 concentration by 50% by the end of this century because of the rise in the emission of the O_3 precursors (NO_x and VOCs) along with greenhouse gases, which will eventually result in global warming (Fowler et al. 1999). Global warming is also expected to increase isoprene biosynthesis because of the firm inter-dependence between isoprene and heat stress (Loreto and Sharkey 1990). This may result in a dual impact, adding extra O_3 at a surface level while negating the effect of O_3 on plants (Fares et al. 2006). However, the quantifiable impacts of climate change on BVOCs are still very challenging to predict due to complex interdependence mechanisms (Sharkey and Monson 2017).

According to Fares et al. (2010), tropospheric O_3 has a strong impact on isoprene emission. It was observed that the first leaf (A_1) of *P.alba*had significant isoprene emission under ambient O_3 stress. Isoprene emission was lower in leaves growing at more distance than the O_3-fumigated leaves, and it was also found that the isoprene emission from the second leaf (A_2) was surplus but insignificant (Fares et al. 2010). Wiberley et al. (2005) concluded that the low isoprene emission from young leaves is regulated at the transcriptional level because of low isoprene synthase mRNA and protein. Expression analysis of isoprene synthase mRNA ($PAL^{sp}s$ mRNA) in *P. alba* leaves suggested that the $PAL^{sp}s$ mRNA was abundant in leaves close to O_3 fumigation, and the mRNA content was significant and decreased gradually in the farther leaves (Fares et al. 2006). A few laboratory studies have shown that O_3 exposures were able to trigger isoprene emission in common reed (*Phragmites australis* L., 300 ppb for 3 hours) (Velikova et al. 2005a), poplar (*P. alba* L., 150 ppb, 11hr/day for 30 days) (Fares et al., 2006) and downy oak (*Q. pubescens*, 190 ppb, 8 hr/day for 3 days) (Pinelli and Tricoli 2008). This stimulation, however, depended on several factors, such as the level of leaf damage and photo-inhibition induced by the pollutant (Yuan et al. 2016).

Several other studies have contradicted the above results and indicated that O_3 exposure (80 ppb for 2 weeks) resulted in an inhibition of isoprene emissionalong with other volatiles from poplar (*P. nigra* L.) (Fares et al. 2010). Similar findings were also reported by Hartikainen et al. (2012), where elevated O_3 episodes [32.6 ppb and 30.6ppb as a daily average (14 hr/day) from June to August in 2007 and 2008, respectively] showed a significant reduction in the emission of isoprene from leaves of silver birch (*Betula pendula* Roth). Elevated O_3 (91 ppb on average) significantly reduced isoprene emission by 21%, while it did not vary considerably in the case of other VOCs (Feng et al. 2019). Chronic O_3 exposure (69 ppb on average) showed a considerable reduction in isoprene emission by 25%, whereas acute O_3 exposure (about 250 ppb for a few hours or days) showed insignificant emission (Feng et al. 2019). Table 14.1 gives an account of changes in isoprene emissions in selected plant species under elevated O_3.

Table 14.1. Changes in isoprene emissions in different plant species under elevated ozone

Plant Species	**Ozone Concentration (ppb)**	**Change in isoprene emission (%)**	**References**
***Betula pendula* Roth**	300	-60%	Loreto et al. (2001)
***Populus alba*L.**	150	-82.8%	Fares et al.(2006)
***Quercus serrata* Thunb. ex Murray**	120	-60.52%	Tani et al. (2017)
***Q. mongolica* var. crispala Blume**	120	-84.37%	Tani et al. (2017)
Hybrid poplar clone 546 (*Populus deltoids* cv. 55/56 x *P. deltoids* cv. Imperial)	80.3	-40.4%	Yuan et al. (2016)
***Populus tremuloides* Michx. (clone 271-O_3 tolerant)**	65	-14.2%	Calfapietra et al. (2008)
***Populus tremuloides* Michx. (clone 42E-O_3 sensitive)**	65	-20.22%	Calfapietra et al. (2008)
***Quercus pubescens* L.**	300 ppb	-29.73%	Velikova et al. (2005)
	300 ppb (72 hrs)	-4.83%	Velikova et al. (2005)
Meta-analysis (from 1980–2017)	91	-21.0%	Feng et al. (2019)
***Populus alba* L.** **A1 – First leaf extending above the cuvette**	150	+48.71%	Fares et al. (2006)
A2 – Second leaf	150	+12%	Fares et al. (2006)
A3 – Third leaf	150	+9.09%	Fares et al. (2006)

(-), decrease; (+), increase

Isoprene emission has been reported for short-term O_3 stimulation (Carriero et al. 2016; Loreto and Pinelli 2004). Isoprene's function as a defender of photosynthetic machinery is well known during the rapid growth of fast-growing plants (Behnke et al. 2007) and during oxidative stress (Fanciullino et al.2014; Loreto and Velikova 2001). However, more recent experiments suggest that both isoprene and monoterpenes act indirectly during the stress period by provoking antioxidant responses and systemic acquired resistance (Riedmeier et al. 2017; Harvey and Sharkey 2016). A meta-analysis done by Feng et al. (2019) suggests that chronic exposure to O_3 might lead to the disproportionate allocation of photosynthates for isoprene biosynthesis, thus resulting in a reduced effect on isoprene. A dose-response study postulated by Calfapietra et al. (2009) suggested that exposure to high O_3 episodes might lead to a negative impact on isoprene emission. In contrast, moderate O_3 exposures result in significant emission of isoprene, with better protection for the photosynthetic apparatus (Feng et al. 2019). Agathokleous et al. (2018) also observed a similar response of isoprene emission under O_3 stress. Similar responses were also reported by Kanagendran et al. (2018) in which O_3-induced hormesis in the emission rates of several VOCs was disclosed in tobacco plants (*N. tabacum* L. “Wisconsin”), which were exposed to 0, 400, 600, 800, and 1000 ppb for 30 minutes. Then VOC emission rates were measured at 0.5, 3, 10, 24, and 48 h.

In an experiment conducted by Yuan et al. (2016) on a hybrid poplar clone 546 (*P. deltoides* cv. 55/56 x *P. deltoides* cv. Imperial), it was found that isoprene under O_3 stress was affected by several other factors like leaf position, sampling time, drought conditions, etc. Elevated O_3 (80.3 ppb) exposure significantly reduced the isoprene emission by 40.4%, with the upper leaves emitting 1.4 times more isoprene than the middle leaves. Further, the emissions were significantly higher in July than in September. Due to identical effects of elevated O_3 in WW (Well-watered) (-45.3%) and MD (Mild drought) (-36.7%), the interaction between O_3 and drought was insignificant. Still, the significance level reversed when the leaf position was taken into account (significant O_3 x drought x leaf position). It was observed that high O_3 conditions induced more negative effects on the middle leaves (-61.1%) than on the upper leaves (-20.9%). When the measurements were done at the whole plant level, it was found that elevated O_3 episodes induced a significant decline in isoprene emission only in WW plants, but the reduction was insignificant for MD poplar plants (Yuan et al. 2016).

Elevated O_3 results in an uncoupling of the inter-dependence between photosynthesis rate and stomatal conductance, thus showing increased stomatal aperture even though the rate of photosynthesis shows a declined response. This type of behavior can be explained through a well-known phenomenon called stomatal sluggishness (Paoletti 2005; Paoletti and Grulke 2010). Such a decline in photosynthesis rate results in a shortage of photosynthates available for isoprene biosynthesis (Lichtenthaler 1997). To justify the results and the above statement, Yuan et al. (2016) have postulated a theory that might explain the reason behind such behavior. Elevated O_3 is well-known to damage the leaf membranes, thus declining the photosynthesis rate (-20%), which leads to an ephemeral rise in internal CO_2 concentration (+11%). This modification leads to increased competition for phosphoenolpyruvate (PEP) with the methyl-erythritol phosphate pathway (MEP), through which isoprene is generated. All of these resulted in a reduced emission of isoprene by 40%. According to Banerjee and Sharkey (2014), the reduction in isoprene in stress periods can be attributed to the expenditure of methyl erythritol cyclodiphosphate (MEcDP) content in the leaves, a key intermediate in the MEP pathway. A model developed by Morfopoulus et al. (2014) suggests that isoprene biosynthesis depends on a balance between the supply of photosynthates and the demand for carbon fixation.Tiwari et al. (2016) proposed a conceptual model to establish the interrelationship between isoprenoid emissions and O_3 stress responses. Isoprene emission not only assists the induced anti-oxidative defense system of the plants under O_3 stress, but it is also known to provide additional stability to the membranes of thylakoids, which in turn helps in sustaining the normal photosynthetic process (Fig. 14.3).

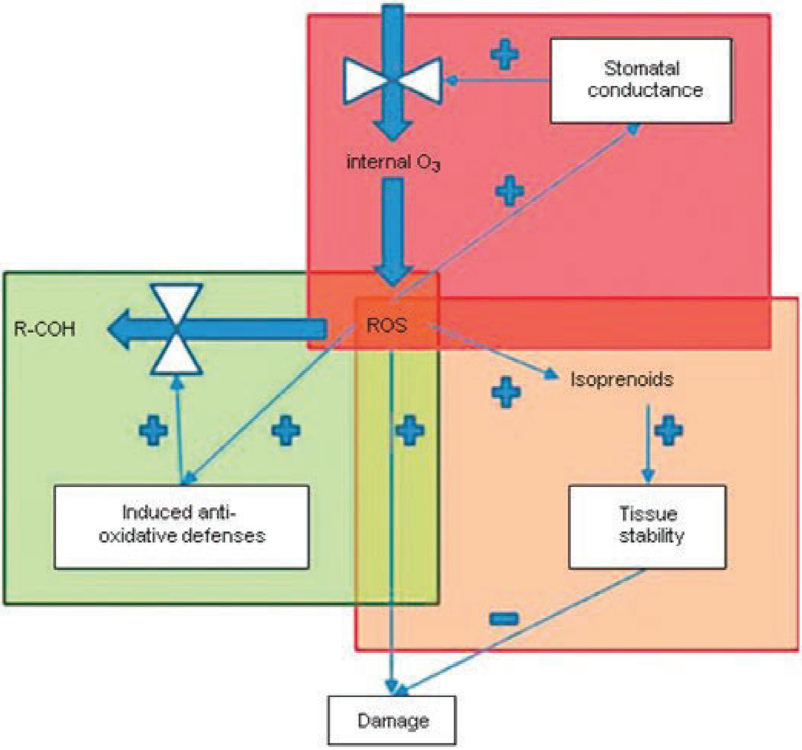

Fig. 14.3. Conceptual model explaining the role of isoprene in protecting plants against O_3 stress (Source, Tiwari et al. 2016)

Experiments done by Tani et al. (2017) suggest that the isoprene emission values started declining at around an AOT40 value of 10 ppm h. Yuan et al. (2016) also reported similar results of reduced rate of isoprene emission under elevated O_3 conditions at the end of the experiment at AOT40 values of 4.4 and 38.7 ppm h under filtered air and O_3-enriched air, respectively.Although the threshold level of AOT40 affects each physiological parameter differently, the emission of isoprene seems to be negatively affected at the highest degree compared to other parameters (Yamaguchi et al. 2011), suggesting that isoprene emission being the receptive parameter, can be used to understand the O_3 stress response. The lowered rate of photosynthesis and eventually, high internal CO_2

concentration might reduce the availability of phosphoenolpyruvate (PEP) and hence the isoprene biosynthesis (Yuan et al. 2016). A similar type of result was also reported by Monson et al. (2016) through the ACi curve, which showed that with an increase in Ci, the isoprene emission declined proportionately at ambient O_3 concentrationsin field-grown poplar clones. The reduced pool size of dimethylallyl pyrophosphate (DMAPP) was inversely proportional to the internal CO_2 concentration (Tani et al. 2017). Calfapietra et al. (2008) reported that the DMAPP content was significantly low in aspen plants under elevated O_3 conditions compared tothe control FACE experiment. In the FACE experiment on *P. tremuloides* L., Calfapietra et al. (2007) observed that isoprene's lower emission is due to the reduced gene expression and protein content of isoprene synthase under the elevated O_3 condition. Isoprene emission was found to depend upon concentrations and the duration of O_3 exposure. This statement was proved by Fares et al. (2006), who reported that short-term exposure to O_3 increased the expression level and mRNA copy number of isoprene synthase and eventually isoprene emission.

14.7 Conclusion

Although confined to limited plant species, biogenic isoprene emission largely affects the plant's metabolic functioning and plays a very significant role in atmospheric chemistry. It acts as a significant precursor of tropospheric O_3 and the oxides of nitrogen, which is the most essential factor in determining the concentration of O_3 in the troposphere. On the one hand, it increases O_3 production by increasing the concentration of NO_2 in urban areas. On the other hand, it protects the O_3 stressed plants by increasing the membrane stability. In view of the present-day climate change scenario, which predicts a warmer climate in the coming future, the biogenic isoprene emission is expected to increase. However, the increasing atmospheric CO_2 applies an inhibitory check on isoprene emission, the mechanism of which is still under investigation. Isoprene emission is an important characteristic of plants experiencing oxidative stress due to air pollutants such as O_3. Isoprene assists the plant's defense machinery in quenching the enhanced ROS production due to O_3 stress. The meteorological conditions during the coming times present favorable conditions for the formation of O_3 in the troposphere. Tropospheric O_3 and biogenic isoprene emissions share a direct relationship with one another, as an increase in one entity promotes the increase in the other. The warmer climate of the near future is expected to increase the biogenic isoprene emission as well as the O_3 concentration in the troposphere. However, examination of the molecular basis of isoprene emission, the response of isoprene emissions to changing environmental conditions, why isoprene emission is limited to a few specific plants, and the response and role of biogenic isoprene emission in the future atmospheric chemistry are a few of the important questions that need to be investigated in future.

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CHAPTER FIFTEEN

GLOBAL CROP YIELD LOSSES DUE TO TROPOSPHERIC OZONE: THE PRESENT AND FUTURE SCENARIO

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Abstract

Concentrations of ozone (O_3) have more than doubled since pre-industrial times. We reviewed the current O_3 levels across the world, its spatial variation, and its trend in different regions to provide a basic background of yield losses in major crops (wheat, rice, maize, and soybean) at the global, regional, and local scales. Global O_3 concentration is increasing at a rate of 0.5–2 ppbv decade^{-1}. Because of the high population, industrialization, and energy consumption in Asian countries, a gradual increase in O_3 precursors resulted in elevated tropospheric O_3 concentrations. Furthermore, current global maximum yield losses are calculated as 15.4% for wheat, 3.7% for rice, 2.4% for maize, and 8.5% for soybean using AOT40 metrics. However, a slightly different pattern has been found using stomatal O_3 uptake metrics, where global maximum crop yield losses of 7.1, 4.4, 6.1, and 12.4% for wheat, rice, maize, and soybean, respectively, were reported. Additionally, Asian wheat and rice cultivars are found to be more sensitive to O_3 compared to the USA and Europe. However, studies reported maximum productivity losses for maize and soybean in North America. The modern cultivars of crops are more sensitive due to their high stomatal density and conductance compared to old ones. Thus, the inadvertent selections by plant breeders are affecting the O_3-sensitivity of modern crop cultivars. Considering O_3 as a threat to agricultural crops, breeders need to develop highly resilient and O_3-tolerant varieties to combat agricultural yield losses. Adopting relatively tolerant crop varieties in highly polluted regions might help to reduce crop losses to some extent to reduce the threat to global food security.

Keywords: Ozone; Trend; Major crops; Yield losses; Crop sensitivity

15.1 Introduction

Ozone (O_3) in the upper atmosphere (stratosphere) is formed in the presence of ultraviolet-B radiation as a by-product of reactions associated with the formation of oxygen, which acts as a primary UV protection shield. Later, it was found that O_3 exists in the lower atmosphere (troposphere) and acts as a strong oxidant, which causes toxic effects on biological organisms (Sharma et al. 2012). The anthropogenically induced depletion in the stratospheric O_3 layer has now been under-recovery due to the Montreal Protocol (1987) (UNEP 2014; Solomon et al. 2016). On the other hand, the continuous increase in tropospheric O_3 (IPCC 2013; Cooper et al. 2014; Lin et al. 2014) and its harmful impacts on agriculture have attracted global concern related to food security (Teixeira et al. 2011; Mishra et al. 2013; Sinha et al. 2015).

Unlike other gaseous pollutants, the peculiarity of tropospheric O_3 is in its formation (secondary pollutant) involving some photochemical reactions and O_3 precursor gases such as nitrogen oxides (NOx), carbon monoxide (CO), and volatile organic compounds (VOCs) (The Royal Society 2008; Monks et al. 2015). The precursors of the troposphere can be natural and anthropogenic, and its budgeting varies with the meteorological conditions, geographical location, and proximity to the precursor sources (Khiem et al. 2010; Monks et al. 2015). Due to the global increase in O_3 precursor emissions, background concentrations of O_3 rise in the northern hemisphere and are projected to increase over this century unless the regulations on emissions of O_3 precursor gases are effectively implemented (Meehl et al. 2007; Fowler et al. 2009). Tropospheric O_3 is not only the third most important contributor to the human-induced greenhouse gases after CO_2 and CH_4 (IPCC 2013), but also an important gaseous air pollutant causing negative effects on net primary production, soil carbon sequestration and other ecosystem services (Sicard et al. 2017; Wang et al. 2019).

Extensive researches were conducted during the 1970s and 1980s explaining the photochemical mechanisms involved in tropospheric O_3 formation in the presence of primary pollutants such as NOx, CO, and hydrocarbons (Crutzen 1973; Chameides and Walker 1973). Earlier, the downward transport of stratospheric O_3 was assumed to be a vital contributor to tropospheric O_3 (Neu et al. 2014). Later, photochemical reactions were found to be mostly involved in increasing the tropospheric O_3, which is also influenced by the concentrations of precursors and climatic conditions (Lee et al. 2014). The O_3 pollution level is reported to be higher in North America, Central Europe, North Africa, and South and East Asia (Mills et al. 2018a).

At first, C.H. Schönbein (Switzerland), the discoverer of O_3, measured the concentration of O_3 in the atmosphere during 1840 by developing his semi-quantitative method. Despite several limitations, ~300 monitoring stations were created to measure the ambient O_3 concentrations in different parts of the USA and Europe during the 1850s. However, the method suggested by Schönbein failed to estimate the quantity of O_3. Henceforth, the data reported by the Paris Municipal Observatory in Park Montsouris (1876-1907) were considered as the first quantitative measurement of ambient O_3. The re-analysis of Montsouris data by Volz and Kley (1988) reported that average O_3 mixing ratios between 1876 and 1910 were 11±2ppbv. The past O_3 measurement data revealed that the peaks in seasonal O_3 were mostly observed in spring during the nineteenth century (Cooper et al. 2014). As per the Fifth Assessment Report of the IPCC, the magnitude of the observable O_3 increments corresponded with an increase in fossil fuel combustion throughout the globe (IPCC 2013). Additionally, the rise in NOx emission by a factor of 4.5 from 1955 to 1985 in Europe can be correlated with rising O_3 concentrations (Staehelin et al. 1994).

The concentration of O_3 varies with the rate of emission of O_3 precursors, existing meteorological conditions, and stratospheric-tropospheric exchange. Additionally, the diurnal, seasonal, and spatio-temporal variations in the O_3 concentrations are also documented (Sicard et al. 2016; Xu et al. 2016; Gaudel et al. 2018). Diurnal O_3 variations are accorded the intensity of solar radiation; for instance, the concentration of O_3 remains low at night time due to the unsuitable environment for photolysis reactions of NO_2 and photo-oxidation of VOCs, CO, and other O_3 precursors. It also coincides with the fastbreaking down of O_3 molecules by NO and no new replacement because of the stable air temperature profile at night (Mavrakis et al. 2010; Monks et al. 2015; Sicard et al. 2016). During day time, the increase in concentrations of O_3 is attributed to the photo-oxidation of O_3 precursors and the downward movement of O_3 by convective heating (Mavrakis et al. 2010; Derwent et al. 2015). However, lower O_3 concentration is more noticeable in urban areas at night than in rural areas (Sicard et al. 2016). This is because of the low emissions of NO in rural areas, which reduces the chances of O_3 titration by NO, leading to relatively high O_3 levels (Mavrakis et al. 2010). The peak episodes of O_3 occur when precursor gases of O_3 accumulate under sunny weather (intense solar radiation) and warm-dry conditions with stagnant air conditions (Oksanen 2009). In the monsoon season, the reduction in O_3 concentration could be the washout effects of rainfall, leading to lower O_3 precursors' availability. However, comparatively higher O_3 concentrations in winter might result from long-range transport of O_3 precursors; since their thermal decomposition reduces due to the lower temperature in winter, thus increasing their lifetime in the atmosphere (Gaur et al. 2014).

As the global ambient O_3 levels have been continuously increasing over several decades, the existing concentrations are a cause of concern due to the risk for crops and several other plant species in the areas where O_3 levels have exceeded the tolerance level. Moreover, seasonal maximum O_3 concentrations coincide with the growing season, and hence crops are often exposed to chronic O_3 stress, causing differential responses of physiological and biochemical traits (Oksanen 2009). Variations in plant responses to O_3 with particular reference to yield losses in crops, mostly for grain crops, have been reported at global, national, and local scales (Van Dingenen et al. 2009; Avnery et al. 2011a, 2011b; Mills et al. 2018c; Feng et al. 2020). Major crop producer countries like China, the USA, and India face considerable crop yield losses, as they bear more than half of all high O_3 risk areas globally (Teixeira et al. 2011).

If we identify the high O_3 risk regions globally, we may introduce O_3-tolerant crop varieties in O_3 polluted areas to reduce the magnitude of yield losses by O_3. The yield of crops is a crucial trait to determine O_3 sensitivity (Mills et al. 2007; Rai et al. 2016). For understanding the crop yield responses, there is a need to identify the areas with a high O_3 concentration. We have compiled the literature from 2009 to 2020 to provide the recent updates in crop yield losses due to elevated O_3 in different regions of the world. This review emphasized the global O_3 scenario and O_3-induced yield losses in four major cultivated crops (wheat, rice, maize, and soybean) under present and future O_3 pollution situations.

15.2 Status of O_3 occurrence in different regions of the world

The lifetime of O_3 is significantly shorter than that of other air pollutants, and the global average lifetime is around 23 days (Young et al. 2013). Tropospheric O_3 budgeting is dependent on the availability of sunlight and O_3 precursor emissions. The abundance of O_3 varies with time, seasonal, inter-annual, and decadal time-scales (Cooper et al. 2014). The role of O_3 precursors is not only confined to the formation of O_3 but also in the long-range intercontinental transport of O_3. The intercontinental transport of European and Asian O_3 precursor emissions significantly affected the ground-level O_3 concentration in western North America (Doherty 2015). Henceforth, O_3 is no longer considered to be a local/regional problem but has assumed a global concern (CLRTAP 2017). The increases in precursors of O_3 in East Asia are responsible for the rise of O_3 concentration in Asia and western North America during the spring season (Lin et al. 2012; Parrish et al. 2012). Cooper et al. (2012) reported a decline in NOx, CO, and VOCs by 49, 45, and 44%, respectively during the period 1990-2010, and a significant rise in O_3 concentration by 0.41 ± 0.027ppbv yr^{-1} from 1995 to 2011 over western North America. Verstraeten et al. (2015) studied the tropospheric O_3 column using satellite and global chemistry-climate model generated chemical transport data over the USA and China from 2005 to 2010. This comparison found an increase of 21% in O_3 precursor emission and 1.08% in NOx emission between the altitudes of 3 and 9 km over China. However, in the same study over the USA (2005-2010), no significant change in the tropospheric O_3 column was recorded along with a 21% decline in NOx emission. Ziemke et al. (2006) suggested that the northern hemisphere of the globe experiences its high O_3 concentration at mid-latitude during summer, while the southern hemisphere experiences maximum O_3 levels in spring, especially in tropical and sub-tropical regions.

Mills et al. (2018a) have presented the long-term trends of tropospheric O_3 global distribution patterns and their impact on vegetation. The highest O_3 values were reported in the mid-latitudes of the northern hemisphere (particularly southern USA, China, North India, Japan, Republic of Korea and the Mediterranean basin) during the period 2010-2014. However, the lowest concentrations of O_3 were reported in south-east USA, southern parts of South America, Canada, northern parts of Europe, Australia, and New Zealand.

Table 15.1. The estimated current tropospheric O_3 (Mean ±SD) levels in different regions of the world using three months of M12 and AOT40 metrics (2010–2014) for the wheat- and rice-growing period (adopted from Mills et al. 2018a)

Regions of the world	**Included countries for wheat- and rice-growing areas**	**Wheat-growing period**		**Rice-growing period**	
		M12 (ppb) (Mean± SD)	**AOT40 (ppm h) (Mean± SD)**	**M12 (ppb) (Mean± SD)**	**AOT40 (ppm h) (Mean± SD)**
North America	Canada, USA	41±4.9	6.01±2.82	39.3±9.4	7.1±5.9
West-East Europe and Turkey	Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Gibraltar, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Macedonia, Malta, Netherlands, Norway, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Somalia, Spain, Sweden, Switzerland, United Kingdom.	39±5.4	5.32±2.88	46.3±6.4	10.5±5.8
South Asia	India, Nepal	41.4±13.6	9.05±6.59	30.7±14.0	4.9±6.0
East Asia	China, Japan, Republic of Korea, Taiwan	45.8±4.2	10.37±3.1	36.9±5.9	7.5±3.7
Southeast Asia	Indonesia, Thailand	NA	NA	19.2±4.5	0.9±0.7

Pacific, Australia, and New Zealand	Australia, New Zealand	26.4±3.1	0.32±0.35	23.3±3.4	0.7±0.5
Middle East	Israel	41.2±8.2	6.62±5.94	NA	NA
Mexico, Central America, Caribbean	Barbados, Bermuda, Guadeloupe, Martinique, Mexico	39.2±9.7	7.03±8.39	40	7.0
South America	Argentina, Brazil, Chile	22.1±1.7	1.14±0.59	28.6±9.6	3.4±1.4
Russia, Belarus, Ukraine, Central Asia	Armenia, Russia	49.5±4.2	10.62±5.2	NA	NA
Global		**40.2±5.9**	**6.06±3.4**	**38.5±7.6**	**7.9±4.5**

M12, the twelve-hour mean O_3 during 08.00–19.59; AOT40, the accumulation of hourly mean O_3 values over 40 ppb during daylight hours; NA, not applicable

Table 15.1 lists the current O_3 situation in different regions of the world during wheat- and rice-growing periods. According to Mills et al. (2018a), the global three-month O_3 was 40.2 ppb for M12 and 6.06 ppm h for AOT40 during the wheat-growing period. However, global mean O_3 was 38.5 ppb for M12 and 7.9 ppm h for AOT40 during the three-month rice growing period (Table 15.1). The regional mean three-month M12 and AOT40 values were higher in East and Central Asia than in North America, South Asia, and Europe during the wheat-growing period (Table 15.1). Moreover, the lowest regional M12 and AOT40 values for wheat were reported in the southern hemisphere, particularly in Australia, New Zealand, and South America (Table 15.1). The highest mean AOT40 values were in Japan, the Czech Republic, the USA and Italy, and the lowest were in Canada, Germany, and France. The values of AOT40 above the critical level for crops suggest a potential risk to crop productivity in these countries (CLRTAP 2017).

However, M12 values were highest in northern Italy and South-west Spain and the lowest were in South-east Asia during the rice-growing period (Mills et al. 2018a). In North America, M12 values were reported in the range of 21–35 ppb in Florida, 41–50 ppb in the USA's eastern states, and more than 51 ppb at several California sites during the rice-growing period (Mills et al. 2018a). Globally, 13.3% of monitoring sites have AOT40 values above the value of 12.8 ppm h and are expected to cause around 5% yield losses (Mills et al. 2007).

15.2.1 Current trends of O_3 and future projections

Global spatial reports on surface O_3 are minimal; however, data available from remote locations suggest a much higher concentration of O_3 in the 21st century compared to the 1970s and 1980s. In the past two decades, some geographical areas (South and East Asia, South America) exhibited increasing pollution trends, while many other areas displayed decreasing trends of pollution (Gaudel et al. 2018; Fig. 15.1). There has been a decline in O_3 precursor emissions in North America and Europe, while a rise in Asia has been reported (Granier et al. 2011; Cooper et al. 2020). Ozone concentrations in North America showed a significant declining trend during the period 1995–2014, whereas no change in Europe and a significant increasing trend in East Asia are reported (Mills et al. 2018a; Fig. 15.1). A long-term analysis of O_3 trends between 1990 and2010 in rural areas of selected sites of western and eastern USA showed a reduction of 43% at those sites located in the eastern USA during spring; while at sites in the western USA, a significant increase of

50% was found (Cooper et al. 2012). The increase in O_3 concentration is attributed to the increases in population, industrialization, and energy consumption (Cooper et al. 2014) and meteorological changes and intercontinental transport of O_3 and its precursors (Parrish et al. 2012).

The satellite observation-based studies using the Total O_3 Mapping Spectrometer (TOMS) between 2005 and 2014 suggested an increase by 0.71% yr^{-1} in O_3 load across the 60°S to 60°N region of the globe (Cooper and Ziemke 2013). Similarly, global variations in the tropospheric O_3 column (TOC) estimated from SCIAMACHY limb-nadir matching (LNM) observations between 2003 and 2011 found a 0.1% yr^{-1} increase in O_3 values in the southern hemisphere between 50 and 30°S, and a decline of 2% yr^{-1} was observed in the northern hemisphere (30–50°N) (Ebojie et al. 2015). The positive changes in the TOC over South America was correlated with the rise in O_3 concentration over the southern hemisphere (Ebojie et al. 2015). Also, the TOC's negative changes can be attributed to the decline in O_3 values over North America and Europe (Ebojie et al. 2015).

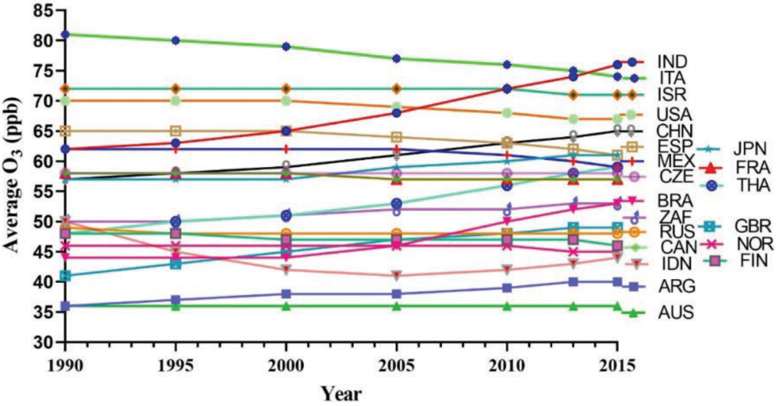

Fig. 15.1. Country-wise population-weighted annual average surface O_3 (ppb) concentrations in different regions of the world from 1990 to 2015. ARG, Argentina; AUS, Australia; BRA, Brazil; CAN, Canada; CHN, China; CZE, Czech Republic; ESP, Spain; FIN, Finland; FRA, France; GBR, United Kingdom; IDN, Indonesia; IND, India; ISR, Israel; ITA, Italy; JPN, Japan; MEX, Mexico; NOR, Norway; RUS, Russia; THA, Thailand; the USA, United States of America; ZAF, South Africa.Data obtainedfrom the State of Global Air(2017) (https,//ourworldindata.org/grapher/ozone-o3-concentration-in-ppb?tab=chart&country)

Cooper et al. (2020) have analyzed surface O_3 trends from 27 globally distributed remote sites in the northern and southern hemispheres on 20 years of data since 1995. It was observed that some northern hemisphere sites showed a positive, and some a negative O_3 trend; however, five sites of the southern hemisphere out of seven displayed positive trends. The O_3 increased trend was reported in the range of 0.5–2 ppbv decade^{-1} (Cooper et al. 2020). During the given period, the background tropospheric O_3 concentration over the northern hemisphere has increased at the rate of 0.5–2% yr^{-1} (Vingarzan 2004) and is projected to increase under the current emission scenarios to 68 ppb by 2050 (Vingarzan 2004; Meehl et al. 2007). Moreover, Seinfeld and Pandis (2012) found that ground-level O_3 has increased about four-fold over the last century and is still expected to increase at a rate of 0.3 ppb yr^{-1} (Wilkinson et al. 2012). Summer mean O_3 levels in Europe are expected to reach 40–60 ppb by 2030 (The Royal Society 2008) and may exceed 75 ppb in many parts of Europe by 2100 (Sitch et al. 2007). Pommier et al. (2018) used the EMEP/MSC-W chemical transport model. They predicted that surface O_3 concentration in India's major parts would rise to 13% by 2050, mainly due to an increase in anthropogenic emissions.

15.2.2 Regional trends of O_3 pollution

Oltmans et al. (2013) found that the concentrations of surface O_3 in North America have increased in eastern and arctic Canada, but central and western Canada showed no significant changes. The USA's west coast has encountered a significantly higher concentration of surface O_3 since the mid-1980s (Parrish et al. 2009). The surface O_3 concentration is increasing over Mauna Loa (Hawaii) while it is reducing in Miami Tori Shima (Western North Pacific) (Oltmans et al. 2012). In an air monitoring study of surface O_3 concentration during the period 1980-2007 over several coastal sites of the US, the baseline O_3 levels flowing onshore from the North Pacific Ocean showed a significant rise in winter, spring, and summer seasons (Parrish et al. 2009). Similarly, Cooper et al. (2012) studied the rural and urban trends of O_3 between 1990 and 2010 from 12 to 41 sites in western and eastern USA, respectively, and found a reducing trend in 43% of sites in the east of the USA and 17% of sites in the west of USA during summertime. Various studies related to the photochemical O_3 formation processes explained that the reduction in emission of O_3 precursors caused a decrease in O_3 concentration in the eastern USA (Hogrefe et al. 2011; Butler et al. 2011). Though the frequency of 8 h means of O_3 shows a decreasing trend, various studies have confirmed an increase in baseline O_3 concentration (Lin et al. 2000). Several studies have suggested that Asian pollution plumes are responsible for increasing the baseline O_3 concentration in various regions, including the western USA (Verstraeten et al. 2015).

Over five decades (1950s–2000s), the European surface O_3 concentration has probably shown a two-fold increment, but after 2000 it showed a reduction over Western Europe (Parrish et al. 2012; Hartmann et al. 2013). Sicard et al. (2016) have analyzed the short-term trend of $O_3$from 332 background monitoring stations in France over the period 1999–2012 and found a decline of 0.12 ppb yr^{-1} in mean annual concentration at the rural stations, particularly during the warm season, which is associated with reductions of NOx and VOCs compared to the early 1990s. Contrary to this, the background O_3 level showed an increasing trend at urban sites mostly at a rate of +0.14 ppb yr^{-1}, during the cold period. This pattern is attributed to rising imported O_3 by long-range transport and low O_3 titration by NO because of the low emission of NOx (Sicard et al. 2016). A gradient between North and South was also observed showing a lower O_3 concentration in the North, while it was higher towards the Mediterranean region. This study further showed increments in O_3 levels in France, the Iberian Peninsula, Eastern Europe, mid-Europe, and northern Italy by 5.9, 5.8, 5.3, 5.0, and 2.4 ppb, respectively. A significant rise in O_3 levels over Eastern Europe, North Africa, and the Mediterranean region was reported, while a decline was recorded over the British Isles and Scandinavia (Lemaire et al. 2016). In a study over different locations of the EU-27 countries during the period 2002-2012 based on an 8 h mean O_3 concentration in Europe, there were significant reductions over 18% of the selected locations, while 2% of the stations were reported to show an increasing trend, mostly in the Iberian Peninsula sites (Guerreiro et al. 2014).

The O_3 concentrations over East and South Asia have drawn the attention of the global scientific community, as these regions experience higher O_3 concentrations due to the increase in O_3precursor gases. In a study, Hakim et al. (2019) evaluated O_3 levels from the HTAPII and CCMI models. They observed the area-weighted O_3 range to be 37.26–56.11 ppb over the Indian subcontinent with higher values in the northern part. Wang et al. (2005) have closely analyzed the surface O_3 data at sixteen different locations in East Asia from 2000 to 2004 under the Acid Deposition Monitoring Network in East Asia (EANET) programme. In their report, it was observed that the concentrations of O_3 over mid-latitudes were higher compared to the tropical sites. For instance, the sites at mid-latitude like Russia (Mondy), Korea (Kanghwa), and Japan

(Happo) had 44 ppb (three-yearly), 41 ppb (three-yearly), and 55 ppb (five-yearly) annual average mean O_3 concentration, respectively. In comparison, the Thailand sites (tropical sites) showed a 20-ppb two-year average O_3 concentration. In a recent study of tropospheric O_3 over China during the period 2005-2010, using the TM5 global chemistry model, a 7% increase in O_3 concentration was calculated, which is correlated with the rise in O_3 precursor emissions and the downward transport of stratospheric O_3 (Verstraeten et al. 2015).

According to the IPCC (2013), the increase in O_3 concentration is very fast in south Asia and predicted to increase in the near future compared to other regions. Industrialization and urbanization act as major sources of O_3 precursors in developing countries like India. The prevailing tropical meteorological conditions of the Indian peninsula also offer a favorable environment for O_3 formation (Tiwari et al. 2008). Using the regional episodic chemical transport model (HANK), Mittal et al. (2007) predicted 8 h daily O_3 levels ranging from 33–40 ppb during February-April, 2000 in Varanasi. A similar range of O_3 concentrations was also observed in urban-rural sites of Maharashtra (Debaje and Kakade 2009). During the period 2002-2012, the continuous monitoring data recorded at a suburban site in Varanasi showed a significant increase in surface O_3 concentration (Tiwari and Agrawal 2018). The study also revealed the seasonal variations in O_3 concentrations throughout the study. During 2011-2012, the summertime O_3 concentration at the same site varied between 55.21 and 62.2 ppb (Singh et al. 2014; Sarkar et al. 2015), and between 32.22 and 53.22 ppb in winter (Mishra et al. 2013).

15.3 Global crop yield losses caused by O_3

The serendipity of high O_3 concentration during the major crop growing period accelerates the global crop yield loss. Several model-based studies have developed statistical relationships between prevailing O_3 concentration and crop yield to estimate both present and future crop yields on national and global levels (Van Dingenen et al. 2009; Avnery et al. 2011a, 2011b; Lal et al. 2017; Mills et al. 2018c; Fischer 2019; Schauberger et al. 2019). Other studies depend upon statistical models using historical yield data of the state or county with past air pollution and climate data to estimate variations in yield patterns associated with O_3 (Burney and Ramanathan 2014; McGrath et al. 2015). The results of both approaches support the view that current O_3 levels are sufficiently high to reduce the crop yield (**Table 15.2**).

Table 15.2 Estimated global annual relative crop (wheat, rice, maize, and soybean) yield losses due to present and near future O_3 pollution

Crop	Ainsworth (2017), based on AOT40 for the year 2000 (combining two studies; Avnery et al. 2011a; Van Dingenen et al. 2009)	Mills et al. (2018c), based on stomatal uptake of ozone (mean of 2010–2012)	Avnery et al. (2011b), based on AOT40 predictions for the year 2030	
			A2	**B1**
Wheat	12.3–15.4%	7.1%	25.8	17.2
Rice	3.7%*	4.4%	na	na
Maize	2.2–2.4%	6.1%	4.4	2.5
Soybean	5.4–8.5%	12.4%	19	9.5

na, not available; *(Van Dingenen et al. 2009); AOT40, cumulative exposure to O_3 concentrations above 40 ppb; A2 and B1 are pessimistic and optimistic (upper and lower boundary) future projections

According to Van Dingenen et al. (2009) and Avnery et al. (2011a), the estimated global yield losses were 12.3–15.4% for wheat, 5.4–8.5% for soybean, and 2.2–2.4% for maize for the year 2000 using AOT40 metrics. Furthermore, Van Dingenen et al. (2009) have estimated a 3.7% global yield loss in rice at the same O_3 metrics (Table 15.2). Mills et al. (2018c) have estimated global annual yield reductions of 12.4, 7.1, 6.1, and 4.4% for soybean, wheat, maize, and rice, respectively, at the global O_3 level during the period 2010–2012 (Table 15.2).

Avnery et al. (2011b) predicted O_3-induced yield losses in wheat, maize, and soybean crops by 2030 compared to the year 2000 based upon MOZRAT-2 and according to the two trajectories of O_3 pollution, which represent the upper and lower boundary future projections, A2 (pessimistic emission scenario) and B1 (optimistic emission scenario). Globally, it was estimated that under the A2 scenario, O_3-induced relative yield losses will increase by 25.8% for wheat, 19% for soybean and, 4.4% for maize in 2030. However, under the B1 scenario, the increase in yield losses would be 17.2% for wheat, 9.5% for soybean, and 2.5% for maize crop (Table 15.2).

15.3.1 Ozone-induced wheat yield losses in different countries

The potential risks of O_3 to agricultural production have been estimated through small-scale field experiments and model-based studies. The results of exposure-based field studies conducted to estimate yield losses in wheat due to surface O_3 in South and East Asia are summarized in Table 15.3.Feng and Kobayashi (2009) have reported a 10% yield loss in wheat under the current O_3 (31–50 ppb) situation compared to O_3 free air from the meta-analysis on 81 examples of peer-reviewed literature between 1980 and 2007. Similarly, Tomer et al. (2015) compared two Indian wheat species namely, *T. aestivum* (PBW343) and *T. durum* (HD2936), under ambient (34.9 ppb 7 h mean) and elevated (ambient+ 30ppb) O_3 and observed the reductions in grain yield ranging between 11 and 19% during the period 2008-2010 (Table 15.3). Feng et al. (2015) evaluated the yield loss in wheat to be 6.4–14.9% and 14.8–23% under current and future O_3 levels, respectively, in China. A field study conducted in India's IGP region with 14 wheat cultivars showed 10–31.3% reductions in the grain yield under future O_3 levels (ambient+ 30 ppb) during the year 2014-2015 (Singh et al. 2018; Table 15.3). Grain yield losses of 10.2, 18.0, 22.1, and 23.8% were reported in Indian wheat cultivars, HD 2967, Sonalika, HUW234, and HD3118 under a future O_3 scenario (ambient+ 20 ppb) during the period 2015-2017 (Pandey et al. 2018; Yadav et al. 2020; Table 15.3).

Table 15.3. Wheat yield losses due to O_3 pollution in different regions of the world

Reference	Type of study	Year(s) considered and location (country)	Cultivars	Yield loss
Tang et al. (2013)	Estimations using the chemical transport model; based on the AOT40 index in 2020	2000–2020; India China	na	Increased yield loss of 13.6–30% 14.5–24.3%
Debaje (2014)	RYL estimated using AOT40 indices for the period 2002–2007	2002–2007; India	na	21.79%
Ghude et al. (2014)	O_3 estimated using the WRF-Chemical regional model	2005; India	na	5%
Tomer et al. (2015)	In OTCs; Ambient (34.9) Ambient (34.9) +30 ppb (7 h mean)	2008–2009 2009–2010; IARI, New Delhi, India	PBW343 HD2936	17–19% 11–16%
Singh et al. (2015)	In OTC; Ambient (58.2) (12 h mean)		LOK-1	16.2%
Sinha et al. (2015)	O_3 estimated using M7 metrics	2011–2013; Punjab Haryana	na	25% 11%
Lal et al. (2017)	RYL estimated using AOT40 indices	2012–2013; India	na	15%
Ainsworth (2017)	RYL estimated using AOT40 indices	2000; North America European Union China/East Asia India/South Asia	na	4.1–11.0% 4.1–12.1% 16.3–19.0% 26.7–27.6%

Mills et al. (2018b)	EMEP MSC-W, chemical transport model, v. 4.8; RYL estimated using POD_3	2010–2012;		
		Northern Hemisphere		9.9%
		Southern Hemisphere		6.2%
		India		12.2%
		Pakistan		9.5%
		Egypt		10.9%
		Ukraine		12.9%
		China		11.7%
		Turkey		8.2%
		Kazakhstan		9.1%
		Argentina		6.0%
		United States		10.1%
		Russia		10.8%
Singh et al. (2018)	In OTC; Ambient (47.2) +30 ppb (8 h mean)	2014–2015; BHU, Varanasi, India	HD2967	23.4%
			HD3059	22.9%
			HD2987	31.3%
			Kharchiya65	10.0%
			PBW502	22.3%
			PBW550	26.1%
			DBW50	29.1%
			DBW77	27.6%
			NIAW34	23.6%
			Kundan	11.8%
			HUW12	16.7%
			HUW55	10.7%
			HUW213	20.4%
			HUW251	20.3%

Pandey et al. (2018)	In OTC; Ambient (52.4) +20 ppb (8 h mean)	2016–2017; BHU, Varanasi, India	HD2967 Sonalika	10.2% 18.0%
Yadav et al. (2019)	In FAOCE; Ambient (30.7 ppb) + 40 ppb (7 h mean)	2016–2017, 2017–2018; IARI, New Delhi, India	HD-2967 C-306	17.3% 18.1%
Schauberger et al. (2019)	Ensemble of chemical transport models; RYL estimated using POD-based crop model	2008–2010 Argentina/Australia Canada/USA France/Egypt Iran/Turkey India/China/Pakistan	Western wheat Asian wheat	1% 3–4% 23–27% 4–6% 33–39%
Sharma et al. (2019)	Improved WRF-Chemical regional model; RYL estimated using AOT40 indices	2014–2015 India	na	21%
Yadav et al. (2020)	In OTC; Ambient (51.7) +20 ppb (8 h mean)	2015–2016; BHU, Varanasi, India	HUW234 HD3118	22.1% 23.8%

na, not available; OTCs, open-top chambers; POD, Phytotoxic Ozone Dose; EMEP MSC-W, European Monitoring and Evaluation Programme, Meteorological Synthesizing Centre-West.

The modeling studies conducted in many parts of the world evaluated the yield of major crops based on past data and predicted future yield losses. The relative yield losses in wheat between 2000 and 2020 were estimated to be in the range of 13.6–30% and 14.5–24.3% for India and China, respectively, based on exposure to AOT40 metrics (Tang et al. 2013; Table15.3). Debaje (2014) estimated a 21.8% yield loss in India using AOT40 metrics for the period 2002–2007. Using a regional chemistry transport model, Ghude et al. (2014) have predicted that yield losses in India's many states were based on the standard AOT40 for the 2004–2005 wheat season. Plausible yield losses due to O_3 were low in Haryana (2.5%), Uttar Pradesh and Bihar (3.5%), Punjab (4%), and West Bengal (6%). However, higher wheat yield losses were estimated in Madhya Pradesh (8%) and Maharashtra (17%), with a national average of yield loss of 5% during the year 2004-2005. Sinha et al. (2015) calculated crop yield losses in the northwest IGP using M7 metrics during a two-year experiment (2011–2013) and estimated 25% yield losses in Punjab and 11% in Haryana (states of India) due to present tropospheric O_3. Lal et al. (2017) measured tropospheric O_3 levels at 6 sites across the IGP region, India, during 2011–2012 using the standard AOT40 metrics and have estimated a 15% annual yield loss in wheat. Similarly, Sharma et al. (2019) estimated a 21% reduction in Indian wheat production using AOT40 metrics for the year 2014-2015.

Ainsworth (2017) calculated relative yield losses of wheat varying from4.1–11.0%, 4.1–12.1%, 16.3–19.0%, and 26.7–27.6% in North America, the European Union, East Asia, and South Asia, respectively, by combining two studies of Van Dingenen et al. (2009) and Avnery et al. (2011a) based on AOT40 metrics (Table 15.3). Mills et al. (2018b)modeled the global wheat production data and estimated a reduction in yield based on phytotoxic doses for major wheat-producing countries such as India, Pakistan, Egypt, Ukraine, China, Turkey, Kazakhstan, Argentina, the United States and Russia during the period 2010-2012 from the year 2000 (Table 15.3). Schauberger et al. (2019) compared Western and Asian wheat production losses using the POD-based crop model and predicted yield reductions in different world regions. The O_3 damage was predicted to be highest for the Asian wheat (relatively more sensitive to O_3) in India, China, and Pakistan (33–39%), followed by western wheat in France/Egypt (23–27%) than in the USA and other western countries.

15.3.2 O_3-induced rice yield losses in different countries

Rice is a moderately sensitive crop to O_3, and the highest negative impact on yield was in the warm temperate moist climates of Europe and warm temperate dry climates of North America. Rice is properly irrigated in both climates; therefore, stomatal uptake is not limited by soil moisture (Mills et al. 2018a). Van Dingenen et al. (2009) evaluated the global relative yield loss in rice for the year 2030 based on the optimistic "current legislation scenario (CLE)." Using AOT40 metrics, RYL for the year 2000 was estimated as 4.7, 3.2, 3.9, and 8.3% for Europe, North America, China, and India, respectively (Table 15.4). Rai et al. (2010) found reductions of 11.2 and 7.4% in the yield of rice cultivars NDR97 and Saurabh950, respectively, under ambient O_3 concentration (Table 15.4). Likewise, Sarkar et al. (2015) have analyzed the yield of two Indian cultivars of rice under ambient, ambient+ 10 ppb and ambient+ 20 ppb O_3 and found 11, 22.6 and 36.6% yield losses in Malviya 36 and 13, 28.6, and 43.3% in Shivani, respectively (Table 15.4). Danh et al. (2016) have estimated 0.4–5.9% production losses in rice of the eastern region of southern Vietnam during 2009–2010 using AOT40 metrics. Using the same metrics, an O_3-induced loss of yield for the rice crop in India has been estimated as 1.66% under the current O_3 level during the period 2002–2007 (Debaje 2014), 6.3% for the year 2011–2012 (Lal et al. 2017), and 6% during 2014–2015 (Sharma et al. 2019; Table 15.4). Mills et al. (2018c) have reported 7.5–12.5% reductions in the yield of rice in major rice producer countries, i.e., South Asia (India, Bangladesh), East Asia (China), and South-East Asia (Indonesia), due to O_3 concentrations during the period 2010–2012 (Table 15.4).

Table 15.4. Rice yield losses due to O_3 pollution in different regions of the world

Reference	Type of study	Year(s) studied and Location (country)	Cultivar	Yield loss
Van Dingenen et al. (2009)	RYL estimated using AOT40 indices	2000; Europe North America China India	na	 4.7% 3.2% 3.9% 8.3%
Rai et al. (2010)	In OTCs; Ambient (35 ppb) O_3 (12 h mean)	BHU, Varanasi, India	NDR 97 Saurabh 950	11.2% 7.4%
Sarkar et al. (2015)	In OTCs; Ambient (49.36) Ambient (49.36) +10 ppb Ambient (49.36) +20 ppb (12 h mean)	BHU, Varanasi, India	Malviyadhan 36	11.1% 22.6% 36.6%
			Shivani	13% 28.6% 43.3%
Debaje (2014)	RYL estimated using AOT40 indices for the period 2002–2007	2002–2007; India	na	1.66%

Danh et al. (2016)	RYL estimated using AOT40 indices	2009–2010; Vietnam	na	0.4–5.9%
Lal et al. (2017)	RYL estimated using AOT40 indices	2012–2013; India	na	6.3%
Mills et al. (2018c)	RYL based on stomatal uptake of O_3	2010–2012 India, Bangladesh, China, and Indonesia	na	7.5–12.5%
Sharma et al. (2019)	Improved WRF-Chemical regional model; RYL estimated using AOT40 indices	2014–2015 India	na	6%

na, not available; OTCs, open-top chambers

15.3.3 O_3-induced yield losses in maize in different countries

During wet years, the historical yield of maize showed no effects of O_3 in the USA (McGrath et al. 2015). However, the present background O_3 concentration showed 10–20% yield losses in maize during dry years. McGrath et al. (2015) provided evidence that the maize yield has been less severely affected due to background O_3 in the USA since 2000, which coincided with the EPA's enactment for regulation on O_3 precursor emissions. The declining trend of O_3 precursors in the USA encourages other countries to also take the necessary action in reducing emissions of O_3 precursors. Ainsworth (2017) reported maize yield losses in North America, the European Union, China/East Asia, and India/South Asia from2.0–2.2, 3.1–3.5, 3.8–4.7, and 2.0–3.4%, respectively, at the existing O_3 scenario in 2000 (Table 15.5). Mills et al. (2018c) estimated 7.5–15% and 12.5–15% yield reductions due to O_3 in maize for the USA and China during the period 2010–2012 (Table 15.5). An exposure-based field study in the IGP region of India reported a significant decline in grain yield by 8 and 11% and 10 and 15% in normal maize cultivar DHM117 and quality protein maize cultivar HQPM-1 under ambient+ 15 ppb and ambient+ 30 ppb O_3 exposures (Singh et al. 2019; Table 15.5). Using AOT40 metrics, Feng et al. (2020) estimated an 8.2–13.4% yield loss in maize over the north China plain region during the period 2014–2017 (Table 15.5).

Table 15.5. Maize yield losses due to O_3 pollution in different regions of the world

Reference	Type of study	Year(s) studied and Location (country)	Cultivar	Yield loss
Ainsworth (2017)	RYL estimated using AOT40 indices	2000; North America European Union China/East Asia India/South Asia	na	 2.0–2.2% 3.1–3.5% 3.8–4.7% 2.0–3.4%
Mills et al. (2018c)	RYL based on stomatal uptake of O_3	2010–2012 USA China	na	 7.5–15% 12.5–15%
Singh et al. (2019)	In OTCs; Ambient (56.1) +15 ppb Ambient +30 ppb (10 h mean)	2012–2013; BHU, Varanasi, India	HQPM1	7.8% 11.1%
			DHM117	10.2% 14.8%
Feng et al. (2020)	Used observational O_3 data (AOT40 indices) in combination with geostatistic methods to estimate yield losses	2014–2017 North China Plain	na	8.2–13.4%

na, not available; OTCs, open-top chambers

15.3.4 O_3-induced yield losses in soybean in different countries

Soybean is not a widely grown crop; therefore, related studies are very limited. Modeling studies showed around 10% of soybean production losses because of the current O_3 levels in the USA during 2005 (Tong et al. 2007). However, yield loss of soybean in the USA is now reduced from 2000 due to declining ambient O_3 levels (McGrath et al. 2015). Singh et al. (2010) exposed two Indian cultivars of soybean at constant O_3 concentrations of 70 and 100 ppb and recorded 12–33.6% yield losses, suggesting a high sensitivity of Indian soybean to O_3 (Table 15.6). Osborne et al. (2016) compared the sensitivity of soybean cultivars based on studies conducted in India, China, and the USA and found Indian soybean cultivars to be most sensitive to O_3. Ainsworth (2017) displayed 7.1–12, 20.5–23.9, 11.4–20.9, and 3.1–4.7% yield losses in soybean in North America, the European Union, China/East Asia, and India/South Asia, respectively, using AOT40 metrics for the year 2000 (Table 15.6). Mills et al. (2018c) estimated yield losses of 12.5–15%, 7.5–10%, and >20% for the period 2010–2012 based on the stomatal uptake of O_3 in soybean in Brazil, Argentina, and the USA under ambient O_3 concentration (Table 15.6). However, yield losses of 10, 2–4, and 6–7% for the USA, Brazil/Argentina, and India/China, respectively, for the years 2008–2010 were reported using the POD-based crop model (Schauberger et al. 2019; Table 15.6).

Table 15.6. Soybean yield losses due to O_3 pollution in different regions of the world

Reference	Type of study	Year(s) studied and Location (country)	Cultivar	Yield loss
Singh et al. (2010)	In OTCs; Filtered (8.5) +70 ppb Filtered (8.5) +100 ppb (4 h mean)	BHU, Varanasi, India	PK 472 Bragg	20% 33.6% 12% 30%
Osborne et al. (2016)	RYL estimated using 7-h mean O_3 concentration	Studied literature from 1982 to 2014 USA India	na	13.3% 37.9%
Ainsworth (2017)	RYL estimated using AOT40 indices	2000; North America European Union China/East Asia India/South Asia	na	7.1–12% 20.5–23.9% 11.4–20.9% 3.1–4.7%
Mills et al. (2018c)	RYL based on stomatal uptake of O_3	2010–2012 Brazil Argentina USA	na	12.5%–15% 7.5%–10% >20%
Schauberger et al. (2019)	RYL estimated using POD-based crop model	2008–2010 USA Brazil/Argentina India/China	na	10% 2–4% 6–7%

na, not available; OTCs, open-top chambers

15.4 Sensitivity of crops to O_3

For crop plants, the yield is an important attribute to determine O_3 sensitivity (Rai et al. 2016); however, different experimental approaches modify crops' responses in relation to estimated yield loss (Emberson et al. 2009). The studies have shown considerable variability in the yield responses of crops and cultivars under ambient and elevated levels of O_3. Modern crop cultivars are found to be more sensitive than older ones (Pleijel et al. 2006; Biswas et al. 2009; Singh et al. 2010, 2018; Yadav et al. 2020). Theoretically, the increased sensitivity to O_3 over the recent decades is related to selective breeding for higher stomatal conductance (Roche 2015; Yadav et al. 2020) that unintentionally increased the uptake of O_3 into the foliage (Biswas et al. 2009; Osborne et al. 2016). Considerable variations in production losses in different regions correlated well with the O_3 richness of a particular area. Studies have further indicated that Asian wheat and rice cultivars are equally or more sensitive to O_3 than US cultivars (Aunan et al. 2000; Ghude et al. 2014; Schauberger et al. 2019).

Mills et al. (2007) categorized wheat and soybean asmost O_3-sensitive crops and rice and maize as moderately sensitive crops. Van Dingenen et al. (2009) supported this outcome and concluded that locally, wheat and soybean exceed a 30% relative yield loss due to background O_3 pollution. On a global scale, yield loss in wheat ranges between 7 and 12% at AOT40 metrics and soybean yield loss ranges between 5 and 16% at M12 metrics. Moreover, yield losses in rice and maize are 3–5% on a global scale. McGrath et al. (2015) found maize to be more vulnerable than soybean over 31 years of study in the USA. The average yield loss was 10% in maize and 5% in soybean due to background O_3 concentration. Contrary to this, globally and also for the European Union and South and East Asia, soybean was ranked the most O_3 sensitive amongst the agricultural crops (Mills et al. 2007, 2018c), suggesting that the sensitivity of the crop could vary in the different locations because of different edaphic and climatic factors and O_3 concentrations. Based on Asian studies for the yield losses of crops, the ranking for crop sensitivity to O_3 is in the order of legume/soybean>wheat>maize >rice (Mills et al. 2007, 2018c; Emberson et al. 2009).

15.5 Conclusion

The review concludes that mid-latitude countries of the northern hemisphere have experienced the highest O_3 concentrations in the world.

Developed countries like the USA have shown a declining trend of O_3 concentration since 2000 after the EPA's enactment for the regulation on O_3 precursors' emission. However, O_3 levels have been almost constant in the European Union since 1995, and a significant increasing trend of O_3 has been recorded in East and South Asia. Among the significant cultivated crops globally, wheat and soybean are highly sensitive crops, while maize and rice exhibit moderate sensitivity to surface O_3. Regarding relative yield loss, East and South Asia showed extreme vulnerability compared to other regions of the world due to the persistently high emissions of O_3 precursors, leading to increased surface O_3 concentrations. Regionally, wheat and rice are more sensitive crops in India, China, Bangladesh, and Pakistan. For soybean, the highest production losses were observed in North and South America. However, maize is relatively sensitive in the USA, China, and the European Union. Conclusively, surface O_3 plays a vital role in causing global crop yield losses, which qualifies O_3 as a hidden threat to global food security.

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CHAPTER SIXTEEN

IMPACT OF OZONE ON CROP YIELD AND QUALITY: A CASE STUDY WITH INDIAN CROP PLANTS

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Abstract

Plants face a wide range of environmental challenges during their developmental stages, which affect many physiological and biochemical processes, and ultimately their growth, thereby leading to reduced yield. Tropospheric ozone (O_3) is considered to be the most severe air pollutant, severely affecting the growth, yield, and produce quality in several major crops such as rice, wheat, and mustard due to its high concentrations and prevalence in agriculturally important regions of the world. The most increased risks of increasing O_3 concentrations are estimated for densely-populated areas of developing countries. Recent studies have shown very high O_3 concentrations in India, particularly in the Indo-Gangetic Plains (IGP) region, one of the most fertile agricultural land areas facing high pollution and population loads. Modelling-based assessments suggest considerable yield losses for the major Indian crop plants, but they rely mainly on European and North American dose-response relationships. Variation is high in such predictions. Ozone tolerance and the sensitivity mechanisms can be studied in laboratory and chamber conditions for each plant species and cultivar. Still, for reliable crop yield and quality assessments and the large-scale screening of genetic differences, long-term field experiments are required. EDU (ethylene diurea) treatments, acting as a chemical protectant against O_3, have been widely used to evaluate O_3 impacts in the field, particularly in the remote areas suffering from limited infrastructures for O_3 exposure facilities.

This chapter presents key findings from experimental studies performed with rice, wheat, and mustard cultivated in the high O_3 IGP region. We highlight the severity of O_3-induced damage on production and grain quality in these Indian crop species and show the genetic differences between the cultivars and varieties. The breeding and selection for O_3-tolerant crops or cultivars can be an important food security strategy in these areas. We will address the trade-off phenomenon in crop plants growing under high O_3 conditions, manifested as shifts in resource allocation between growth and defense, ultimately affecting both the production and the yield quality.

Keywords: India; Ozone; Mustard; Rice; Wheat; Yield

16.1 Introduction

Crop yield is adversely affected by various environmental challenges during the developmental stages such asdrought, air pollution, high temperature, and extreme weather events. These challenges can adversely affect the crop yield and crop nutritional quality, thereby further aggravating the global food security and nutritional risk (Myers et al. 2017). Tropospheric ozone (O_3) has been considered as the most important phytotoxic air pollutant and significant greenhouse gas (Ainsworth et al. 2012; Bytnerowiczet al. 2007; Wilkinson et al. 2012). O_3 is formed by the reaction between natural or anthropogenic O_3 precursors, such as volatile organic carbons (VOCs), carbon monoxide (CO), and nitrogen oxides (NOx) in the presence of sunlight (Finlayson-Pitts and Pitts 1993). Mills et al. (2018) ranked crops based on O_3 sensitivity in the order soybean>wheat>maize>rice. The annual O_3-induced global yield losses are12.4%, 7.1%, 4.4%, and 6.1% for soybean, wheat, maize, and rice, respectively, resulting in an economic loss ranging from 14 to 26 billion US$ on the global scale (Avnery et al. 2013; Mills et al. 2018). O_3 concentrations have begun to decrease in some parts of the world such as the Eastern United States and parts of Europe, due to a substantial reduction in precursor emissions (Klingberg et al. 2014); on the contrary, a further increase in the average O_3 concentration has been predicted for many regions of the world, especially in India, China, South Korea, and many African countries owing to unmitigated emissions of O_3 precursors from industrial and human activities (Hansen et al. 2019; Hayes et al. 2020; Wild et al. 2012). In India, the Indo-Gangetic plains (IGP) region, the most fertile part of the country, is also the most vulnerable hot-spot for O_3-induced negative impacts on crop productivity and alteration of nutritional quality. David and Ravishankara (2019) reported that a substantially high O_3 concentration (ranging from 6–7 Dobson units) prevails in the IGP region and central India. Eventually, these areas will be the major contributors for O_3 precursors over eastern India, Bangladesh, and southern Indian regions. The variation in daily average O_3 concentration during the experimental period of rice, wheat, and mustard (from August 2011 to April 2013) indicates that plants in the IGP region (at Lucknow, India) are frequently exposed to harmful O_3 concentrations (above 40 ppb) (Fig.16.1).

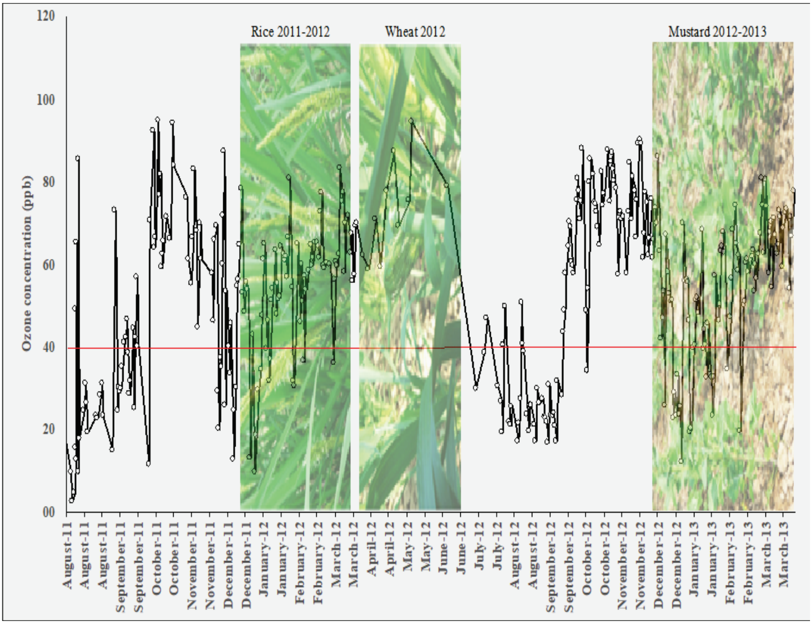

Fig.16.1. Daily O_3 concentration (ppb, 8 hours daily average), during the growing season of rice (5 August 2011 to 22 November 2011), wheat (6 December 2011 to 29 March 2012), and mustard (27 November to 1 April 2013). The redline in the figure indicates the threshold limit (>40 ppb) for the crops above, for which there has been estimated a 5% yield reduction (Mills et al. 2007)

The O_3 concentrations are high, especially during the flowering phase of these plants. A high O_3 concentration in this region may be attributed to high population density, increased emission of O_3 precursors (such as NOx and hydrocarbon generated by heavy traffic loads), and favorable tropical meteorology (hot, sunny weather) (Table 16.1; Dolker and Agrawal 2019; Han et al. 2020). Moreover, the increasing trend in O_3 concentration has been correlated with the increase in the demand for energy production and fossil fuel consumption, which can ultimately lead to a higher emission for O_3 precursors (VOCs and NOx) (Oksanen et al. 2013; Feng et al. 2018). A recent study estimated that about half of the total crop losses for wheat and rice in India due to O_3 occur in the IGP region where most of these crops are produced (Sharma et al. 2019).

Table 16.1 Ambient O_3 concentrations recorded at different experimental sites in India

Crop	**Month/year**	**Experimental site**	**O_3 concentration (ppb h)/AOT40/M7**	**Reference**
Wheat (*Triticum aestivum* L.)	1st year December 2016–April 2017 and 2nd year December 2017–April 2018	New Delhi	31232 ppb h $^{-1}$(AOT40)	Yadav et al. (2019)
Castor (*Ricinus communis*	September 2016–January 2017	Gujarat	14 ppb day^{-1} (monthly mean)	Rathore and Chaudhary (2019)
Wheat (*T. aestivum* L.)	December 2011–March 2012	Lucknow	52.8 ppb (seasonal mean), 86600 ppb h $^{-1}$(AOT40)	Pandey et al. (2019)
Wheat (*T. aestivum* L.)	December 2014–March 2015	Varanasi	47.2 ppb (seasonal mean) 8000 ppb h^{-1}(AOT40)	Singh et al. (2018)
Wheat (*T. aestivum* L.)	November 2016–March 2017	Varanasi	52.4 (seasonal mean), 11900 ppb h $^{-1}$ (AOT40)	Pandey et al. (2018)
Indian clover (*Trifolium alexandrium* L.)	January 2012–March 2012	Varanasi (Site 1) and Sultanpur (Site 2)	52.76 (monthly mean) 67800 ppb h $^{-1}$(AOT40) (Site 1) and 60.16 ppb (monthly mean), 73900 ppb h $^{-1}$(AOT40) (Site 2)	Singh et al. (2018)
Wheat (*T. aestivum* L.)	1styear December 2010–April 2011, 2nd year December 2011–April 2012	New Delhi	38 ppb (2010–2011) and 29 ppb (2011–2012) the (seasonal mean)	Daripa et al. (2016)

Rice (*Oryza sativa* L.)	August–November 2011	Lucknow	46 (seasonal mean), 5600 ppb h $^{-1}$ (AOT40)	Pandey et al. (2015)
Mung bean, (*Vigna radiata* L.)	1styear April–June (2010) and 2ndyear April–June (2011)	Varanasi	1st year 64.0 ppb (monthly mean) 11577.4 ppb h $^{-1}$(AOT 40), 2nd year 63.4 ppb (monthly mean), 11419 ppb h $^{-1}$(AOT 40)	Chaudhary and Agrawal (2015)
Mung bean, (*V. radiata* L.)	April–June, 2011	Varanasi	63.4 ppb (monthly mean), 11419 ppb h $^{-1}$(AOT40)	Mishra and Agrawal (2015)
Wheat (*T. aestivum* L.)	December 2010–March 2011	Varanasi	58.2 ppb (M 12) and 11600 ppb h $^{-1}$ (AOT 40)	Singh et al. (2015)
Wheat (*T. aestivum* L.)	1styear December–April (2008–2009), 2ndyear December–April (2009–2010)	New Delhi	30.9 ± 1.4 (2008–2009) and 34.9 ± 1.9 ppb (2009–2010) (seasonal mean)	Tomer et al. (2015)
Mustard (*Brassica rapa* syn. *B. campestris*)	November 2012–April 2013	Lucknow	54.8 ppb (monthly mean)16000 ppb h $^{-1}$(AOT40)	Pandey et al. (2014)
Maize (*Zea mays* L.)	December 2011–April 2012	Varanasi	55.6 ppb (seasonal mean), 84900 ppb h $^{-1}$(AOT40)	Singh et al. (2014)

16.2 Ozone entry in the plant cell

Ozone enters plant leaves during the process of gas exchange through the stomatal pores. It reacts with the apoplast constituents and alters the chemical compositions inside the plant cell by producing reactive oxygen species (ROS), such as hydrogen peroxide and hydroxyl radicals, thereby causing oxidative stress (Apel and Hirt 2004). ROS may lead to programmed cell death and accelerated senescence and may adversely affect the photosynthesis, stomatal responsiveness, root shoot biomass, yield, and product quality (Broberg et al. 2015; Broberg et al. 2017; CLRTAP 2015).

The regulatory response under oxidative stress may vary depending on the plant species by the up-regulation of antioxidative defense systems; comprising both non-enzymatic (ascorbate, glutathione, tocopherol, salicylate) and enzymatic (superoxide dismutase, catalase, ascorbate per oxidase), and by other strategies such as plant hormone signaling (Foyer and Noctor 2003). All these strategies are energy (carbon) demanding, resulting in a net decrease in resources available for growth and biomass (Ainsworth et al. 2012). A plant's stress response may also vary on the O_3 exposure regime, crop, cultivar, developmental phase of the plant, and other edaphic factors (Dolker et al. 2019; Fumagalli et al. 2019; Pandey et al. 2014; Yadav et al. 2019).

16.3 Different methods used in ozone studies

Many experimental approaches have been used to study the adverse effects of high O_3 concentrations on vegetation. (1) In chamber studies, plants can be grown in highly controlled conditions with the possibility of manipulating environmental variables as per the experiments' requirement, e.g., light, temperature, humidity, etc. Chamber-based studies have a good chance of reproducibility but are less realistic in responses than field experiments due to the enclosed system's environmental artifacts. (2) In Open-Top Chambers (OTC), plants can be exposed to a range of high O_3 or high CO_2 concentrations above the ambient concentrations to predict future predictions. The OTC technique's limitations are that plants inside the chambers may experience different growth conditions in terms of temperature, humidity, shade, and constant wind movement compared to the outside plants (Kimball et al. 1997). (3) Free air canopy exposure (FACE) is more realistic to the ambient conditions' exposure approach. The plants are exposed to high O_3 or high CO_2 in real field conditions, thereby removing the limitation from the previous approaches to study the effect of the high O_3. Although this approach is most recent and realistic, due to the high level of technology involved and expensive running costs, this approach has not yet been the most suitable/successful method in the remote rural areas, where the majority of land cover is used for agricultural practice and which are already experiencing high O_3 concentration and suffer from electricity limitations (Singh and Agrawal 2017; Oksanen et al. 2013). (4) Another approach which has been widely practiced is by applications of synthetic chemical protectants, for example, ascorbic acid, quercetin, and N-[2-(2-oxo-1-imidazolidinyl) ethyl]-N′-phenylurea (EDU). Of these, EDU has been extensively used as a research tool to study the response to ambient O_3 and assess the crop production losses and leaf-injury-based O_3-sensitivity screening in various plants, including crops and their cultivars (Paoletti et al. 2009; Manning et al. 2011) especially in the high O_3 rural areas lacking electricity and fumigation infrastructures (Oksanen et al. 2013). EDU was first used by Carnahan et al. (1978) to protect snap bean plants against O_3-induced damage. EDU is a synthetic chemical that has been widely used experimentally because EDU doesn’t interfere with the cell metabolism directly; EDU protects plants specifically against O_3-induced damages and does not affect other pollutants, e.g., SO_2 or peroxyacyl nitrates (Tiwari 2017); EDU has no growth-enhancing effects in non-O_3 conditions (Szantoi et al. 2007; Foster et al. 1983). The protective mechanism of EDU against high O_3 remains largely elusive, but there is enough scientific evidence that supports the use of EDU in field studies. Based on the transcriptome data of snapbean (*Phaseolus vulgaris*), Paoletti et al. (2014) indicated that EDU could halt O_3-induced ROS production. Ashrafuzzaman et al. (2018) suggested that EDU does not interfere with other abiotic stresses, such as iron toxicity, zinc deficiency, and salinity stress under O_3 stress in rice. In another comparative study between two agrochemicals, EDU and cytokinin, EDU was found to be more effective in ameliorating the toxic effect of O_3 in snap bean (Zhang et al. 2018).

16.4 Different metrics used to measure the ozone-critical level

To protect vegetation against O_3-induced damages, researchers have produced several metrics to establish limits to O_3 exposure. These metrics are based on field studies to develop a crop-specific O_3response in yield under a given concentration of O_3. Most of the studies have been carried out in North America and Europe and, to a lesser extent, in South and East Asia (Emberson et al. 2009; Marco and Sicard 2019). Currently, the

European standard uses the O_3 exposure index AOT40, i.e., the cumulative exposure to the O_3 hourly concentration exceeding 40 ppb over the daylight hours of the growing season over 3 months developed by Mauzerall and Wang (2001). Different metrics used to estimate the O_3 exposure of plants are presented in Table 16.2.

Table 16.2 Different metrics used to estimate the O_3 exposure in plants

Metrics	Definition	Unit
AOT40	Accumulated O_3 above a Threshold of 40 ppbv $\text{AOT40} = \sum_{i=1}^{n}[C-40]_i$ $for\ C \geq 40$ ppbv where C is the mean O_3 concentration per hour in parts per billion (ppb), "i" is the index, and n is the number of hours with C >40 ppb	ppm h
M7	M7 is defined as the mean daytime ozone concentration for 7 h (3-month period) $\text{M7} = \frac{1}{n}\sum_{i=1}^{n}[C]_i$ for 09:00–15:59 h C where n is the number of hours in the growing season. C is the hourly O_3 concentration, and i is the hour index	ppbv
M12	M12 is defined as the mean daytime O_3 concentration for 12 h (3-month period) $\text{M12} = \frac{1}{n}\sum_{i=1}^{n}[C]_i$ for 08:00–19:59 h C where n is the number of hours in the growing season. C is the hourly O_3 concentration, and i is the hour index	ppbv
W126	Sum of the hourly O_3 concentration (weighted by a sigmoidal function) $\text{W126} = \sum_{i=1}^{n}\left[\frac{C}{1+4403\ exp\ (-0.126 \times C)}\right]_i$ for C ≥0 ppmv	ppm h

	where n is the number of hours in the growing season. C is the hourly O_3 concentration and i is the hour index	
SUM06	Accumulated O_3 above a Threshold of 60 ppbv $\mathrm{SUM06} = \frac{1}{n}\sum_{i=1}^{n}[C]_i$ for $C \geq 60$ ppbv where n is the number of hours in the growing season. C is the hourly O_3 concentration, and i is the hour index	ppbv
POD	The accumulated stomatal flux of O_3 (POD_Y, the Phytotoxic O_3 Dose above a threshold flux of Y nmol m^{-2} PLA s^{-1}) $\mathrm{POD_y} = \int max\ (F_{st} - y, 0)dt$ y denotes a threshold of stomatal O3 flux where PLA is the projected leaf area	$nmol/m^2/s$

AOT40 has a limitation in that it measures the atmospheric O_3 concentration, but the effects on vegetation are dependent on the overall O_3 uptake through the stomata into the plants (Agathokleous et al. 2018). W126 is a non-threshold index where each hourly O_3 concentration value is assigned a weight in the range between zero and one (Hollaway et al. 2012). A M7 and M12 measure for over 3 months of the growing season and give equal emphasis to all O_3 concentrations (Tong et al. 2009) and SUM06 is measured over 3 months for O_3 concentrations above 60 ppbv (Van Dingenen et al. 2009). The impact of high O_3 on vegetation depends on ambient O_3 concentrations and the O_3 uptake through the stomata, which in turn depends on other environmental variables such as air humidity, soil moisture, solar radiation, temperature and plant sensitivity. The Phytotoxic Ozone Dose (PODY) is defined as the accumulated O_3 flux entering the leaves via the stomata over a Y (PODY) detoxification threshold. This index considers multiple climate factors, vegetation characteristics, and local and phenological inputs on O_3 uptake or flux (Emberson et al. 2000) (Table 16.2). There are still some uncertainties associated even with the most biologically sensitive index, PODY; for example, duration of the growing season, selection of the threshold for the Y value, and selection of the parameters which would define the damage to vegetation in the most accurate manner, in terms of yield loss, growth, visible injuries and defoliation. There exists a debate on which of these O_3 exposure indexes could provide the best fit to estimate the yield losses. To date, no single O_3-exposure metric has been considered to be the best for all the crops across the globe (Marco and Sicard 2019). Therefore, there is an absolute need to perform large-scale screening experiments involving many crop cultivars in the ambient field conditions. These can be executed using more robust and recent approaches, such as O_3-FACE, to develop O_3-exposure metrics by involving the environmental variables that can affect the stomatal responses and stomatal O_3 flux at each experimental site (Feng et al. 2018).

16.5 Need to develop metrics based on Indian crops/cultivars

A modeling based study indicated that Asian wheat and rice cultivars are more sensitive to O_3-induced yield losses than European and North American wheat cultivars (Emberson et al. 2009). On the contrary, Feng et al. (2018) used the AOT40 index to compare yield losses between European and Asian wheat cultivars (from experiments executed both in China and India) to assess the yield response to ambient O_3, and they did not find a significant difference between these cultivars in terms of yield losses. Moreover, the O_3-FACE based studies were more reliable than the studies using OTC to estimate the O_3-induced losses (Feng et al. 2018). Similarly, Fischer (2019), in a critical review on O_3 pollution and its impact on wheat yield in India, suggested that when studying O_3-induced losses, other parameters which can significantly affect the O_3 impact on real field conditions, such as the irrigation pattern, the time duration for the experiment and aerosol study, should be included. There exists an urgent need to perform more field experiments in the native environment of crops and cultivars to enhance the limited information with Asian/Indian vegetation and to formulate standards to protect the native vegetation against O_3-induced damages under O_3-FACE approaches to develop O_3 risk modelling and promote resistance breeding (Emberson et al. 2018).

16.6 Case studies for three important crop plants grown widely in the IGP areas of India

16.6.1 Case study1: Wheat (*Triticum aestivum*)

Wheat is a staple food crop grown in the largest area of the Earth (2220.1 M ha). India stands as the second-largest producer (95 M t) of wheat globally (Fischer 2019). The IGP region accounts for about 63% of the total wheat production of India. Wheat has been classified as an O_3-sensitive crop due to adverse effects on growth, yield production, and grain quality under high O_3 conditions (Mills et al. 2007; Singh and Agrawal 2009; Singh and Agrawal 2010; Singh et al. 2010; Singh et al. 2018; Pandey et al. 2019; Yadav et al. 2019). Wheat has been relatively more extensively studied than other crop plants in the IGP region of India. Ozone experiments with wheat suggest genetic variability in O_3 sensitivity, impaired growth, gas exchange, grain yield losses, and activated antioxidant defense (Fatima et al. 2018, 2019; Singh and Agrawal 2014, Mishra et al. 2013). Despite this, studies made under ambient field conditions involving locally relevant wheat cultivars have been scarce in the IGP region where the crop losses are significant (Sharma et al. 2019). Pandey et al. (2019) conducted an extensive experimental set-up involving eleven locally important wheat cultivars to study their productivity and performance in the ambient field conditions experiencing high O_3 concentration at two experimental (urban and semi-urban) sites in Lucknow, India. EDU was used as a plant protectant against O_3-induced damages at a concentration of 300 ppm (determined based on earlier studies). Plants were measured for different growth, biomass, gas exchange, biochemical (antioxidants), and yield attributes. During the vegetative phase (December-January), the O_3 concentration did not exceed the threshold limit prescribed for wheat (3ppm h). Still, it was higher during the most sensitive phase of wheat development, i.e., grain filling (February to March), being more than double the threshold limit for wheat (8.66 ppm h at the end of the experimental period) (Fig. 16.1).

Out of the eleven cultivars tested, three cultivars (i.e., Kundan, WR544, and PBW550) were found to be the most suitable in the present high O_3 environment. The study also suggested that both high antioxidant defense capacity and net assimilation rate play an important role in O_3 tolerance in these cultivars, supported by significant correlations between the grain yield and the photosynthetic rate, especially at the flowering phase, as well as between the grain yield and biomass. In general, the yield attributes (harvest index, grain weight plant^{-1}, and weight of inflorescence) were higher in the EDU treated plants than in the non-EDU treated plants, particularly at the urban site. The significant EDU treatment x cultivar interactions for grain weight plant^{-1} and 1000 grain weight indicate the high variability of wheat cultivars in their response to O_3. Interestingly, the most sensitive cultivars in this study (PBW-502, WH-711, and DBW-17) had a higher stomatal conductance, suggesting a chance for higher O_3 uptake. This view is supported by the fact that the recently developed high yielding wheat cultivars are more sensitive to O_3-induced damages (Biswas et al. 2008; Wilkinson et al. 2012). If the breeders select cultivars based on their yield performance, they unintentionally select cultivars having higher stomatal conductance, which are more prone to higher O_3 uptake so bound to face more significant oxidative damage (Biswas et al. 2008; Wilkinson et al. 2012).

16.6.2 Case Study 2: Rice (*Oryza sativa*)

Rice is the most important staple crop in Asia, including India, providing 21% of the calorific need of the global population (Fitzgerald et al. 2009). In India, rice contributes over 43% to the nations' food grain production (Oksanen et al. 2013). Rice in India is grown widely in Uttar Pradesh, West Bengal, West Odhisa, Andhra Pradesh, and Tamil Nadu and these five states alone account for about half of the rice production of India. O_3-induced yield losses are highest in the north (especially in Uttar Pradesh), ranging from 10% to 15%. To date, a minimal number of experimental studies have been conducted using OTCs and only with a few rice cultivars. A high genetic variation in rice crop response to O_3 is reported by Mills et al. (2018). Therefore, utilizing the genetic variation in O_3 sensitivity among rice cultivars would be an important strategy in decreasing the productivity losses in the IGP region.

OTC-based experiments for rice indicate that elevated O_3 can adversely affect growth, photosynthesis, and reproductive structures (fertile florets and pollen viability), thereby decreasing grain yield in the range of 7–45% (Rai and Agrawal 2008; Rai et al. 2010; Sarkar and Agrawal 2010, 2012). Pandey et al. (2015) studied the response to high ambient O_3 with eighteen locally important rice cultivars of the IGP region at two locations (urban and semi-urban) in India using EDU as an O_3 protectant in the ambient field conditions. Many parameters were studied in response to O_3, measuring gas exchange, antioxidative defense, and yield attributes throughout the growing season, i.e., the vegetative phase, flowering phase, and harvest phase. A significant EDU x cultivar interaction among the cultivars was detected in these parameters. The importance of the antioxidant defense system and chlorophyll concentrations across all the cultivars suggests that these parameters can be utilized in large-scale screening experiments performed under ambient O_3 conditions for rice even slightly. Lipid peroxidation proved to be an important indicator to the O_3 response. Seven out of the eighteen cultivars tested (i.e., NDR 359, HKR 47, Pusa Basmati 1, TCS-555, Narendra Usar 3, Pusa Sugandha 5, and Varadhan) were found to be suitable in terms of grain yield under present environmental conditions and can be recommended especially in the areas suffering from high O_3 concentrations.

16.6.3 Case Study: 3 Mustard (*Brassica rapasyn B. campestris)*

Mustard is the third most important source of oilseed crops grown globally after soybean and palm oil (Maity et al. 2019). India is the second-largest cultivator of rapeseed-mustards after China, with an annual production of 79170000 t (FAO-STAT 2017; Kumar et al. 2020). Rapeseed-mustard has been used widely as vegetable oil, as a spice, preservative, and seed meal for cattle, manure, and traditional medicine. India is still importing about 40% of its annual edible oil needs due to increasing demand as a result of high population and rising incomes (Bohmiraj et al. 2010) and to meet the future demand, the production of mustard should be increased 2.5–3 times and the productivity doubled by the year 2030 (Maity et al. 2019). Tripathi et al. (2012) reported that two cultivars of *B. campestris* (Sanjukta and Varda) under high ambient O_3 showed an increased degree of unsaturation and level of PUFA, ω-6fatty acid, linolenic acid and eruic acid in the seed oil, indicating a deterioration in the seed quality. A higher dosage of fertilizers (1.5 times that recommended) has been suggested to protect grain yield and seed quality damages in *B. campestris* against O_3 (Singh et al. 2009; Singh et al. 2012). A field experiment was conducted with two locally-grown mustard cultivars (Kranti and Peelasona) in India to study the impact of ambient high O_3 concentration on the gas exchange (photosynthesis), biochemical (non-enzymatic and enzymatic antioxidative defense), and yield attributes, and oil content at different stages of development (the vegetative, flowering and harvest phases) in the IGP region of India. EDU at two levels, 200 and 400 ppm, was used as a tool to identify the O_3 sensitivity in these cultivars in terms of production losses and quality deterioration. Mills et al. (2007) classified mustard as a moderately O_3-sensitive crop. The higher level of EDU concentration used in the study (400 ppm) proved to be more effective against O_3-induced damages, particularly increasing the oil content of seeds in both cultivars. High O_3 concentration affected the general growth and biomass partitioning patterns differently in the mustard cultivars. Kranti continued to accumulate biomass until the harvest phase, whereas, in Peelasona, seed production was favored under EDU treatment (reducing the adverse effects of high O_3 conditions). Biomass accumulation or allocation patterns were not strongly correlated with seed yield in mustard cultivars grown under high O_3 conditions. Strong induction of the antioxidative defense in both cultivars in response to high O_3 conditions demonstrated an important role of the antioxidative defense to protect plants against O_3. Seed oil content showed a decrease of 4–5% under ambient O_3 concentration as compared to the EDU-treated plants, which further indicates that the adverse effect of high O_3 concentrations not only impairs the growth and biomass of mustard in this region but also affects seed-oil production.

16.7 Conclusions

The present chapter highlights the importance of experiments with Indian crop plants under the high O_3 condition, which is severely affecting crop production in an agriculturally important region of India. Based on field experiments with three important crops (rice, wheat, and mustard) and selecting locally important cultivars, it can be concluded that (1) prevailing ambient levels of O_3 concentration year-round in this region are high enough to adversely affect crop production in different growing seasons (rice: July to December; wheat: December to April, and mustard: November to April; (2) the study further warrants the need for screening genetic variability among the cultivars/varieties for the breeding and selection of O_3-tolerant genotypes, which can be an important strategy in improving the food security in this region; and (3) there is a need for more suitable O_3-exposure indices in this region for estimating yield losses using native crop plants and the latest technologies and innovations, e.g., O_3-FACE, incorporating edaphic factors, irrigation regimes, and interaction with other environmental factors.

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CHAPTER SEVENTEEN

ACUTE AND CHRONIC EFFECTS OF GROUND LEVEL OZONE ON HUMAN HEALTH

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Abstract

Ozone (O_3) is a highly reactive and oxidative gas that induces adverse effects on human health, including mortality and morbidity. Anthropogenic activities are the primary sources of tropospheric O_3 pollution. Primarily O_3 enters the human body through inhalation and causes acute and chronic health effects. Various studies have shown that O_3 exposure has affected the respiratory system, cardiovascular system, reproductive system, and nervous system and can ultimately cause mortality depending upon age and pre-health conditions. Some studies suggest a direct relationship between the O_3 level and the degradation of human health. This chapter will critically elucidate the severity of the adverse effects on human health under the exposure of variable levels of O_3 concentrations.

Keywords: Human health; Morbidity; Mortality; Respiratory diseases; Tropospheric ozone

17.1 Introduction

Urban air pollution is a mixture of particles and gases which cause acute and chronic effects on plants and human health (Nuvolone et al. 2018; Chaudhary and Rathore 2018,2019; Rathore and Chaudhary 2019; Nassikas et al. 2020; Amiri et al. 2020; Othman et al. 2020). Ozone (O_3), an allotrope of oxygen, is a secondary air pollutant in the troposphere formed from a mixture of harmful gaseous molecules under high sunlight and considered to bea threat to human health (WHO 2003; Lim et al. 2012). Epidemiological studies enumerated the disease burden and calculated it in terms of the number of years lost due to illness, disability caused, and even death. Among these epidemiological studies, a report from the global burden of disease (GBD) is the most reliable and updated research that advocates the deaths, disability-adjusted life years (DALYs), and trends in the exposure of ozone for individuals along with other environmental factors. A report of the GBD published in 2016 by Forouzanfar and GBD Risk Factor Collaborators (2016) suggested that about 4.1 million disability-adjusted life years (DALYs) were due to O_3 exposure in 2015.

Unlike other air contaminants, the concentration of ozone throughout the world is either increasing, remaining stable, or decreasing at a much slower rate (Abeleira and Farmer 2017; Karlsson et al. 2017; Stowell et al. 2017; Wang et al. 2017). The increase in O_3 concentration could be a seasonal or baseline increase or an increase in frequency and the magnitude of high O_3 episodes occur due to variation in the types and concentrations of precursor substances that remarkably influence the kinetics of the pathway of atmospheric chemistry. Fisk (2015) estimated that climate change-related increases in O_3 result from air movement changes, cloud cover, humidity, and emission rates of reactive volatile organic compounds (VOCs and NOx), causing substantial adverse health effects. Fann et al. (2015) analyzed scenarios for the United States and concluded that climate change would support an increase in ambient O_3 levels until 2030. Melkonyan and Wagner (2013) provided a similar projection for Germany. Controlling outdoor O_3 and other air pollutants is a highly challenging task as its formation not only depends upon the precursor's availability but also other co-factors such as region, season, and time of day (Finlayson-Pitts and Pitts Jr. 2000).

To protect human health from O_3, the World Health Organization (WHO) has issued a guideline value of 100 $\mu g\ m^{-3}$ as the maximum 8 h mean O_3 concentration (WHO 2006). Various governmental agencies have also issued recommendations or standards for the maximum concentration for outdoor O_3. Europe's reference value is 120 μg m^{-3} (8 h average), and the alert threshold value of 1 h average is 240 μg m^{-3} (E.C. Ozone Directive 2016). The National Ambient Air Quality Standard (NAAQS) for O_3 in the United States is 0.070 ppm (8 h average) (USEPA 2018) while the national air quality standard for O_3 in Australia is 0.08 ppm (4 h average) (Government of Australia 2018). Canada has an indoor air quality standard of 40 μg m^{-3} 8 h average which is considered to be suitable for health (Health Canada 2010).

Ozone enters the human body through processes of inhalation (USEPA 2018), although, in some instances, exposures through the skin are also reported (Weschler 2016). There is a direct relationship between an increase in the concentration of ambient O_3 and the number of hospital visits, morbidity and mortality rates (Zhang et al. 2006; Smith Xu and Switzer 2009; Sousa et al. 2013). Estimation to O_3-related deaths reveals that about 5–20% of total death share due to air pollution (Anenberg et al. 2009; Lim et al. 2012; Brauer et al. 2012; Silva et al. 2013). The WHO has reported the role of O_3 in mortality and morbidity (2006). Adverse effects of O_3 on human health include problems in breathing, such as inflammation and deterioration to the airways, a decrease of lung functions, emphysema, asthma, bronchitis, etc. (Yari 2016). Furthermore, studies suggested that regular exposure to a high concentration of O_3 may cause damage to DNA (Mortimer et al. 2002; Manikandan et al. 2010; Kleinsorge et al. 2011; Nazaroff 2013).

This chapter is a comprehensive elucidation of O_3 pollution, and its chronic and severe effects on human health, comprising two main sections, followed by subsections. The first section has information about the formation of tropospheric O_3 and its contributory factors. It has a world scenario of ground-level O_3. The next section describes the harmful health effects associated with the variable concentrations of tropospheric O_3 worldwide, with particular reference to the different diseases caused by the alleviated concentrations of O_3.

17.2 Ozone formation in the troposphere

Ozone in the ambient air comprises several sources, such as the intrusion of stratospheric O_3 or their participation in complex photochemical reactions with VOCs (organic vapors or volatile organic compounds), NO_x, and radiation from the sun. Some organic contributors' activities, including the decay of natural biogenic products and hydrocarbons of olefinic origin emerging through anthropogenic activities, are also responsible for ozone formation. Along with these contributors, approximately 40 ppb is formed from methane, and this methane comes out due to the intensive agriculture and animal industry (Altshuller 1987; Lippmann 1992). NO*x* levels play critical roles in tropospheric O_3 formation; for example, from 1 ppb of NO_3 is generated 5–7 ppb of O_3 (Altshuller 1987; Lippmann 1992). Figure 17.1 describes the process of O_3 formation and dissociation.

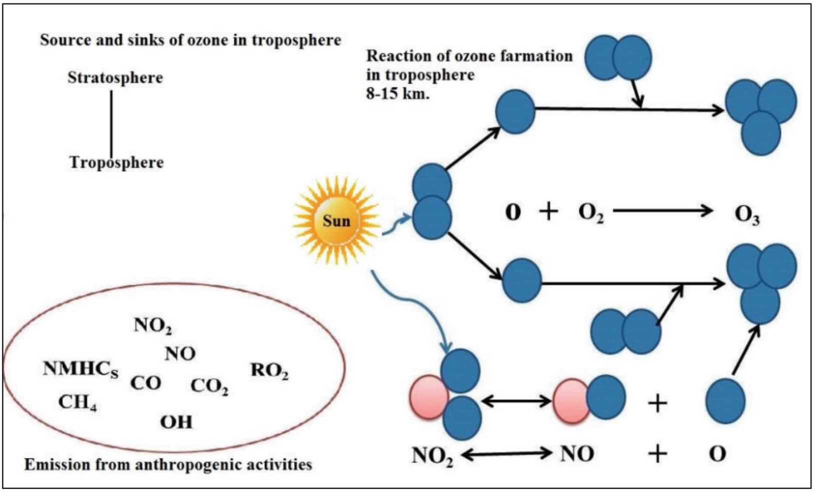

Fig.17.1. A diagrammatic representation of the sources and sinks of O_3 in the troposphere

17.3 Global scenario of ground-level ozone pollution

In recent years, environmental risks caused by exposure to surface ozone from both stationary and mobile sources in East Asia's primary receptors have increased yearly (Nawahda et al. 2012, 2013). Background concentrations of ground-level O_3 have risen from an estimated preindustrial concentration of 10 ppb to average summer concentrations varying from 30 to 50 ppb in the mid-latitudes of the northern hemisphere and O_3 episodes reaching 100 ppb (Morgan et al. 2006).With current air quality legislation implemented worldwide, multimodal simulations for 2030 projected that global surface O_3 would increase by 1.5 ± 1.2 ppb on average, and for the IPCC SRES A_2 scenario, by 4.3 ± 2.2 ppb with the most substantial increases in South Asia, Southeast Asia, and the Middle East (Gauss et al. 2007). According to Dentener et al. (2005), global photochemical models project that as per the current O_3 precursor emissions legislation, parts of Asia will experience a further significant increase in O_3 concentration up to 2030. Monitoring reports of O_3 in suburban and rural areas of Asia also showed that the mean monthly O_3 concentration now commonly reaches up to 50 ppb (EANET 2006). Rapid industrialization for achieving faster economic growth in China has resulted in higher concentrations of ground-level O_3 since the last decade, which became a severe threat to public health (Zhan et al. 2018). Studies have revealed that the increasing trends in the O_3 concentrations in different megacities of China exceed the air quality standards (Wang et al. 2010; Ran et al. 2009; Wu and Xie 2017). In the summer of 2017, a higher concentration of ground-level O_3 was recorded in many cities of China, with O_3 concentrations exceeding 200 mg/m^3 (Lu et al. 2018). In the north region of China, the O_3 level in the rural areas had an increasing trend of 2.2 mg/m^3/year between 2003 and 2015, whereas, in Southern China, it was 1.7 mg/m^3/year from 2001 to 2007 (Ziemke et al. 2011; Osterman et al. 2008). On the other hand, the increase rate of ground-level O_3 in the urban region of Beijing was recorded at around 5.1 mg/m^3/year, and this increase was around 5% per year compared with that between 2005 and 2011 (Zhang et al. 2014); furthermore, the surface O_3 increase in the city of Guangdong, was at a rate of 1.7 mg/m^3/year from 2006 to 2011 (MEP 2017; Li et al. 2014).

Several studies have used global models to examine the impacts of continued increased emissions of nitrogen oxides on future O_3 concentrations (Ghude et al. 2008). Models predicted that tropospheric ozone could rise 20–25% between 2015 and 2050, and further increase by 40–60% by 2100, with the continued current emission trends (Meehl and Stocker 2007). High concentrations of O_3 are associated with hot sunny weather and are frequently observed in tropical areas where conditions are favorable for O_3 formation (Tiwari et al. 2008). Debaje et al. (2010) observeda monthly average of the daytime maximum of the O_3 mixing ratio ranging from 14 to 57 parts per billion by volume (ppb) with an annual average of about 20 ppb at Ahmednagar, India. Singh et al. (2014) and Sinha et al. (2015) reported 46–67 ppb average O_3 concentration from Varanasi (India) during winter months and 46 ppb from Mohali (India) during summer months, respectively, Chaudhary and Rathore (2020) reported ambient O_3 concentrations ranging between 13.89 and 22.49 ppb day^{-1} from February to June 2017 at Gandhinagar, Gujarat, India. The concentration of O_3 in various cities of India is provided in Table 17.1. The concentrations at different places are much higher than their upper limits.

Table 17.1 Ozone concentration in Indian cities

S. No.	**Cities**	**Study period**	**Ozone concentration (ppb)**	**Author's**
1.	Delhi	Jan. 2013 to Dec. 2014	37.5 (monthly mean)	Tyagi et al. (2016)
2.	New Delhi	Jan. 2000 to Dec. 2009	15.8 (seasonal mean)	Chelani (2012)
3.	Kanpur	Jun. 2009 to May 2013	27.9 ±17.8(seasonal mean)	Gaur et al. (2014)
4.	Pune	Jun. 2003 to May 2004	30.9 ±14 (seasonal mean)	Beig et al. (2007)
5.	Ahmedabad	Jun. 2003 to May 2004	20.7 ± 5.5 (monthly mean)	Beig et al. (2007)
6.	Nainital	Oct. 2006 to Dec. 2008	43.9±11.5 (seasonal mean)	Kumar et al. (2010)
7.	Mt. Abu	Jan. 1993 to Dec. 2000	39.9±10.8 (seasonal mean)	Naja et al. (2003)
8.	Varanasi	Dec.2011 to Apr. 2012	46.3–67.9 (monthly mean)	Singh et al. (2014)
9.	Mohali	Oct.2011 to Jan. 2014	46.5 (monthly mean)	Sinha et al. (2015)
10.	Chennai	Summer 2005	2–53 (hourly)	Pulikesi et al. (2006)
11.	Maharashtra	2001 to 2005	40–50 (seasonal mean)	Debaje and Kakade (2009)
12	Nagercoil	Mar. 2007 to Feb. 2010	19.8 (annual average)	Elampari et al. (2013)
13.	Tranquebar	May 1997 to Oct. 2000	8.1–25.0 (monthly mean)	Debaje et al. (2003)
14.	Gandhinagar	Feb. 2017 to Jun. 2017	14–23 (daily mean)	Chaudhary and Rathore (2020)

17.4 Toxicity of ozone on humans

Ozone is a highly reactive and oxidative gas with low solubility in water. Because of its chemical properties, exposure to O_3 usually occurs by inhalation. Oral inhalation allows lower O_3 removal rates than nasal inhalation; therefore, vigorous physical activity results in higher penetration into the lungs. Other factors that influence O_3 absorption in humans are age and gender, socioeconomic status, and occupation (Bell et al. 2014). Decades of research on ground-level O_3 reveal significant health effects on the population across the globe. It causes 5,000 premature deaths per year in the United States (Fann et al. 2012; IHME 2016).

The last ISA for O_3 and related photochemical oxidants (EPA 2018) explained that the relationship between O_3 and short-term respiratory effects was supported by sufficient evidence to consider it causal. Similarly, Alhanti et al. (2016) also suggested some relationships of O_3 with long-term respiratory effects, short-term cardiovascular effects, and mortality effects. According to Alhanti et al. (2016), age is the most elementary factor of susceptibility to O_3, with significant health risks related to O_3 exposure observed in older people. Limited or very little suggestive evidence was found to be based upon gender and occupational factors. There are higher risks to females and people in unemployment and a low economy, whereas no significant results in the studies targeted ethnic minorities, i.e., persons with low educational qualifications. The reason for this was the lower solubility of O_3 in water; therefore, it cannot be properly excreted by the upper respiratory tract (Nuvolone et al. 2018). Pre-existing respiratory diseases, such as asthma or emphysema, chronic bronchitis, etc., may result in increased risks for O_3-related health impacts because of the variable quantity of absorbed O_3. Hence, the toxicity of O_3 depends on several conditions, and there is a great variability of responses at the individual level.

There is a considerable amount of literature focusing on the acute effects caused due to O_3 exposure. The WHO has extensively reviewed it (2013a, b). The severity of health effects in humans and the number of people affected through O_3 pollution are represented by a pyramid (Fig.17.2). Less severe health conditions are presented at the bottom of the pyramid. The most affected people are placed in the central part of the pyramid, whereas the most seriously affected, such as by death, or hospital admissions, are placed in the upper vertex and represented by fewer individuals.

On the other side, evidence for the long-term effects of O_3 is less conclusive. Epidemiological studies have to confront some methodological constraints, mainly for assessing the long-term exposures of O_3; consequently, there is a gap in knowledge that can evaluate the long-term effects of O_3 exposures. Mortality or reduction in the life expectancy period, effects on lung functioning, atherosclerosis, and the onset of asthma are the most common health outcomes taken into consideration for the studies aimed to assess the health effects of long-term exposures to O_3. Many studies conducted in the USA, such as California's The Loma Linda University Adventist Health and Smog (AHSMOG) study by Abbey et al. (1999) and a re-analysis by Lipfert et al. (2006) displayed no statistically significant relationship between long-term O_3 exposure and mortality. But since 2005, various cohort studies conducted in recent years provide a suggestive link between long-term exposure and mortality, and the reports suggest that mostly respiratory and cardiorespiratory mortality is caused in the people affected by predisposing conditions (Lipfert et al. 2006; Krewski et al. 2009; Jerrett et al. 2009; Smith et al. 2009; Zanobetti and Schwartz 2011).

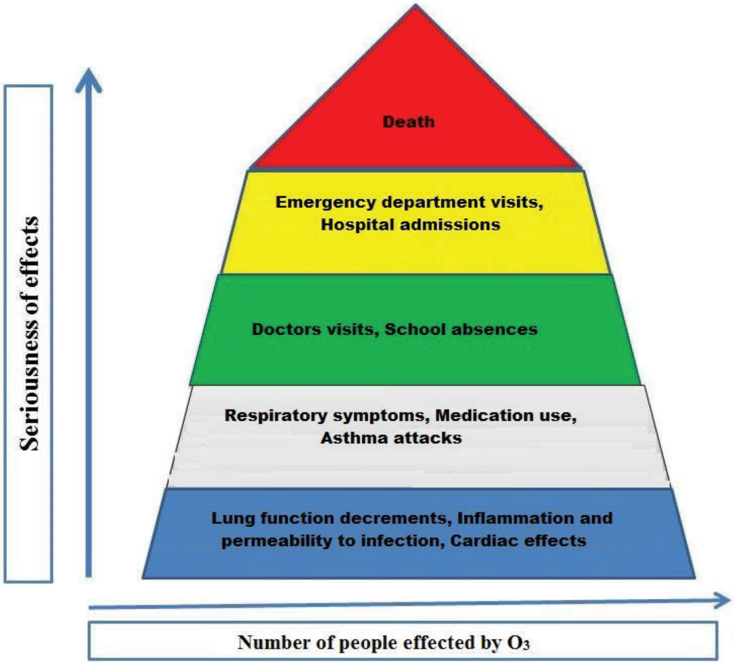

Fig.17.2. Pyramid of health effects caused by ozone

The American Cancer Society Cancer Prevention Study II (CPS II) conducted the most informative and relevant study on this situation. CPS II findings reported a statistically significant relationship between long-term exposure to O_3 and related mortality. Risk estimations by Krewski (2009) suggested there was a stronger association for cardiopulmonary mortality. Similarly, most adjustment methods by Jerrett et al. (2009) showed a significant association between long-term exposure to O_3 and the mortality caused due to respiratory diseases. However, some ambiguity in interpreting these results is due to the interaction between O_3 and $PM_{2.5}$ levels, and it was not so easy to classify the specific effects of the two pollutants.

However, studies conducted on non-human mammal primates allow a better understanding of the results because of the similarities between the primate and the human pulmonary system. Plopper and co-workers conducted a wide range of studies on primates as model organisms. The obtained results have shown a clear association between exposure to O_3 concentrations and alteration in airway morphology and pulmonary function (Plopper et al. 2007). Among different epidemiological studies, the Children's Health Study, conducted in 12 cohorts from communities in southern California, was one of the well-designed studies (Peters et al. 1999). This study found significant associations between lung function and mean annual O_3 concentration among the children spending more time outdoors. However, the lung function growth rates were also found to be significantly associated with a series of other common pollutants such as particulate matter (especially $PM_{2.5}$) and nitrogen dioxide in urban areas. In contrast, exposure to O_3 did not provide conclusive results (Marino et al. 2015). The studies of Künzli et al. (1997) and Tager et al. (2005) reported consistent and significant associations between reduced airway function and long-term O_3 exposure.

For atherosclerosis, carotid intima-media thickness (CIMT) is one of the collective biomarkers used in studies to study pollutants' effects (Künzli et al. 2005). In a cross-sectional study conducted in Los Angeles, CIMT suggested a significant association with residential outdoor $PM_{2.5}$ levels; however, exposure to O_3 provided a weak association (Brook et al. 2002).

17.5 Severe effects of ozone on human health

Ozone pollution creates problems during taking a deep breath and causes coughing and soreness or roughness of the throat, and damages the airways. Most of the people living in ozone risk air areas are affected with asthma, especially children and older adults who are active workers. Besides this, people with assured genetic characteristics, and people with reduced intake of certain nutrients, such as vitamins C and E, are at greater risk from O_3 exposure (EPA 2017). Chronic disruptive pulmonary (COPD) and other severe diseases are given in Figure 17.3.

17.5.1 Respiratory problem

In their last assessment, the EPA determined that exposure induces decreases in lung function and causes pulmonary inflammation at absorptions as low as 60 ppb. Further, it was also estimated that elevated O_3 days increased the respiratory symptoms and pulmonary inflammation in children with asthma, respiratory-related hospital admissions and ED visits, and respiratory mortality (USEPA 2018). Long-term epidemiological studies supported the likely fundamental effects of O_3, which are adverse respiratory symptoms, new-onset asthma, and respiratory mortality O_3 exposure.

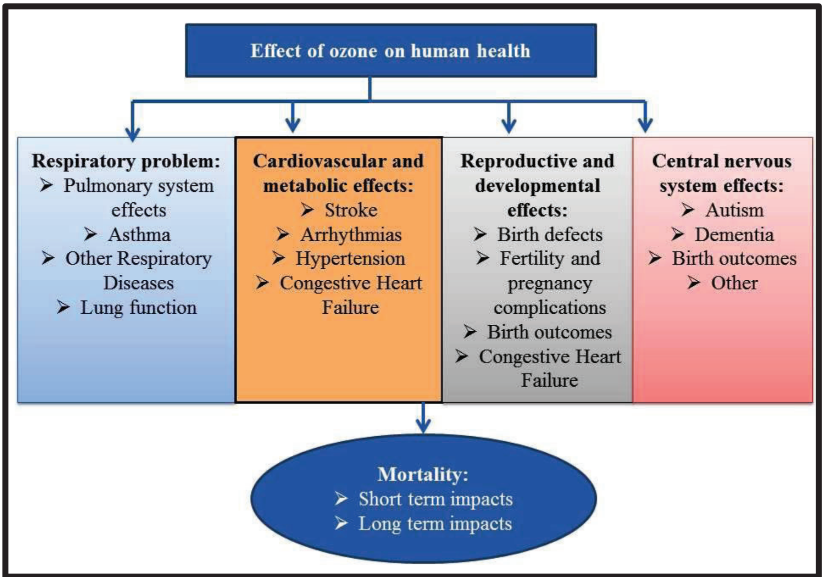

Fig.17.3. Severe ozone effects on human health

17.5.1.1 Pulmonary system problem

Since the WHO update for the air quality guidelines (WHO 2006), a growing scientific interest has been developed to produce predictive models for lung function decrements (FEV1) with the response to inhaled O_3 doses, to evaluate risk and have response thresholds (McDonnell et al. 2012). The induction of a transient decrease in lung function is one of the most studied respiratory acute end-points. Studies were more frequently conducted in healthy young people; usually, non-smokers, exposed to a wide range of O_3 levels (40–600 ppb1) during rest and intensive physical activity (Sousa et al. 2013; McDonnell et al. 2012; EPA 2012). The main results show that subjects exposed to high outdoor O_3 levels (outdoor workers, children in summer camps, exercising adults), but lower than those used in chamber studies, experienced acute lung function decrements (Thaller et al. 2008; Chan and Wu 2005). Results from epidemiological studies reveal robust, positive, and significant relations between the alteration in ambient O_3 levels and increased morbidity. The most frequently studied health end-points or alternatives are hospital admissions to the emergency department, visits for asthma, respiratory tract infections and exacerbation of other respiratory diseases, and school absenteeism in children. The effects were frequently observed in children, older people, asthmatics, and subjects with COPD during warmer months.

As in mortality studies, extensive multicity analyses relate variations of O_3 levels with hospital admissions for respiratory diseases (Burnett et al. 1997; Anderson et al. 1997). Such associations are still significant when introducing the models' time-related variables, such as day of the week, seasonal effects, other air pollutants, and atmospheric factors.

17.5.1.2 Asthma problem

Asthma is a chronic lung disease that inflames and narrows the respiratory airways. Asthma causes recurring periods of wheezing (a whistling sound when you breathe), chest tightness, shortness of breath, and coughing (NHBLI). More than 26 million people are known to have asthma in the United States, of which 6 million are children (NCHS). CDC approximations are that asthma costs the US economy more than $80 billion annually in medical expenses, missed work and school days and even leads to mortality. Eight case-crossover studies have shown increases in the odds of ER visits or clinic visits with odds ratios (ORs) that range from 1.03–1.17 per 10 ppb O_3 (Kim et al. 2015; Yamazaki et al. 2015), or an increase of 3% risk per 10 ppb O_3 (Malig et al. 2016). One study in Japan showed much higher ORs (2.3) precisely for nighttime primary care visits for asthma (Yamazaki et al. 2014). However, studies in France and Japan found no significant effects of O_3 on asthma emergencies (Yamazaki et al. 2014; Mazenq et al. 2017). Most studies indicated that school-aged children were more susceptible and had higher ED visit rates associated with O_3 exposure. Studies also showed that the effect of O_3 on ED visits for asthma seemed to occur with a shorter lag time in older adults, those in want, disadvantaged and minority communities (Strickland et al. 2014; Kim et al. 2015; Alhanti et al. 2016), and in children who were born premature (Strickland et al. 2014).

Most of the studies show a consistent effect of short-term O_3 concentration on asthma hospital admissions. There are six studies of asthma hospital admissions with a 10-ppb increase in short-term O_3 (up to a 5-day average) showing a 4–11% increase in hospital admissions (Park et al. 2013; Sheffield et al. 2015; Chen et al. 2016; Mohamed et al. 2016; Dai et al. 2018) although the study of McLaren and Williams (2015) showed an inverse effect.

17.5.1.3 Other Respiratory Diseases

Multiple studies have found an increased risk of respiratory infections related to short-term ozone exposure (Table 17.2). The odds of upper respiratory illness (URI) with a 10-ppb increment of O_3 range from 0.99–1.46, while the odds of influenza were 1.28. The odds for pneumonia range from 0.99–1.15 and for bronchitis 0.998–1.05 (Xu et al. 2013; Altuğ et al. 2014; Kousha and Rowe 2014; Xiao et al. 2016; Li et al. 2017). Similarly, studies have reported a 2.8% increase in URIs and a 3% increase in pneumonia and decreased odds (0.31) of bronchitis with decreases in O_3 concentrations (Malig et al. 2016; Berhane et al. 2016). However, studies conducted in Vietnam and Brazil found no connection between O_3 and admissions for respiratory infections (bronchitis or pneumonia) (Negrisoli et al. 2013; Nhung et al. 2018). Notably, a meta-analysis of 12 studies, where exposures ranged from 13–62 ppb, initiates a statistically significant increase in pneumonia (1.7%) per 10 ppb O_3 (Nhung et al. 2017). Some studies of particular respiratory infections such as tuberculosis, pneumocystis carinii pneumonia among HIV patients, infantile respiratory infections, acid-fast bacillus, and O_3 found no or inverse relations (Stern et al. 2013; Jassal et al. 2013; Álvaro-Meca et al. 2015; Smith et al. 2016).

In patients with cystic fibrosis, there are three recent papers relating to pulmonary exacerbations to O_3 exposure. There was a 6–37% increase in exacerbations with a 10-ppb increment in O_3, within two days after exposure. However, long-term O_3 exposure was not associated with the frequency of pulmonary exacerbations (Farhat et al. 2013; Goeminne et al. 2013; Jassal et al. 2013). In patients with COPD, O_3 seems to have less of an effect than in other respiratory diseases. There was no significant relationship between short-term O_3 exposure and lung function or hospital admissions (Kariisa et al. 2015; Qiu et al. 2018). There was no association between the development of COPD in former asthma patients for long-term exposure. In a large longitudinal cohort in England, there was no association with COPD diagnosis (Atkinson et al. 2015; To et al. 2016).

Overall, data for hospital admissions or other health care visits for respiratory diseases are mixed. Six studies found no significant relationship with O_3 exposure (Jones et al. 2013; Rodopoulou et al. 2014; Rodopoulou et al. 2015; Franck et al. 2015; Pride et al. 2015; Bono et al. 2016). However, a few studies found increases of 2–4% in respiratory admissions from a 10-ppb increase in O_3, or odds ratios of 1.04–1.08 (Lin et al. 2013; de Freitas et al. 2016; Magzamen et al. 2017; O'Lenick et al. 2017). One study noted that their results differed depending on how they summarized the O_3 metrics, and another demonstrated variable relationships in other geographic areas (Pannullo et al. 2017; O' Lenick et al. 2017). These may help to explain the varied results among studies and the fact that respiratory health care visits are a non-homogenous category that may mask effects for different illnesses within this category. In other respiratory effects, critically ill patients were more likely to develop acute respiratory distress syndrome with long-term O_3 exposure (2.5 O Rsper 10-ppb O_3) (Ware et al. 2016). Regarding lung cancer, there are three new studies; two showed no relationship between O_3 and lung cancer incidence (Hystad et al. 2013; Gharibv et al. 2017). However, a study from China covering 368,762 lung cancer cases found an association with O_3 (1.087 ORs per 10-ppb O_3) (Guo et al. 2016).

17.5.1.4 Lung function problem

Several extensive cohort studies have found that long-term or short-term O_3 is associated with decreases in lung function in healthy children (Urman et al. 2014; Hwang et al. 2015; Fernández-Plataet al. 2016; Karakatsani et al. 2017;Tsui et al. 2018). Only three of the studies found no relationship with lung function in children (Fuertes et al. 2015; Barone-Adesi et al. 2015; Neophytou et al. 2016). Some studies found more potent effects of O_3 on girls' lung function and patients carrying genetic polymorphisms (Altuğ et al. 2013; Chen et al. 2016). In children with asthma, cohort studies have found that O_3 was associated with a decrease in peak expiratory flow, but no change in other lung function parameters (Moreno-Macias et al. 2013; Ierodiakonou et al. 2016; Yoda et al. 2017).

Epidemiological, controlled chamber studies and toxicological literature have suggested an association between O_3 and lung inflammation. Recent human studies on short-term O_3 effects on airway inflammation continue to be mixed, with some studies finding an association with lung inflammation markers (Yoda et al. 2014; Modig et al. 2014; Karakatsani et al. 2017; Arjomandi et al. 2017; Cheng et al. 2018).

Table 17.2. Ozone concentration and respiratory diseases

S. No.	Place	Ozone levels (ppb)	Period	Health Outcome	References
1.	Atlanta	43	1993–2009	Asthma EDV	Alhanti et al. (2016)
2.	Dallas	42	2006–2009		
3.	St. Louis	37	2001–2007		
4.	Indianapolis	48	2007–2011	Asthma EDV	Byers et al. (2016)
5.	Atlanta	42	2002–2008	Respiratory EDV	O'Lenick et al. (2017)
6.	Georgia	42	2002–2008	Pediatric asthma EDV, Pneumonia EDV, Bronchitis EDV, URTI EDV	Xiao et al. (2016)
7.	Himeji city, Japan	26	2010–2013	Pediatric asthma PCV	Yamazaki et al. (2014)
8.	California	31–75	2005–2008	Respiratory EDV, Asthma EDV, Pneumonia EDV, ARI EDV, COPD EDV, URTI EDV	Malig et al. (2016)
9.	Adelaide, Australia	28	2003–2013	Asthma HA	Chen et al. (2016)
10.	New York City, NY	30	2005–2011	Pediatric asthma HA, Pediatric EDV	Shmool et al. (2016)

17.5.2 Cardiovascular and metabolic problems

Results from experimental studies on animals have reported some evidence for cardiovascular effects of O_3, such as an increase of heart rate and diastolic pressure, vascular oxidative stress, inflammation, and decreased heart rate variability (Chuang et al. 2009; Perepu et al. 2010; Tankersley et al. 2010), whereas, some other experimental studies made on humans are less conclusive and have provided inconsistent associations with O_3 (Fakhri et al. 2009; Rudez et al. 2009; Thompson et al. 2010; Kusha et al. 2012). Park et al. (2005) conducted a study on 603 men from the Boston area who participated in the Veterans' Administration Normative Ageing Study. Authors observed an 11.5% (95% CI 0.4–21.3) reduction of low-frequency HRV associated with 2.6 $\mu g/m^3$ O_3 increased in 4 h and more substantial effects resulted in men affected with ischemic heart disease and hypertension. Rich et al. (2006) followed patients with implanted defibrillators in the Boston area. They observed an increased risk (OR = 2.1, 95% CI 1.2–3.5) of paroxysmal atrial fibrillation episodes with short-term fluctuations of O_3 concentrations.

Observational studies have found positive associations between exposure to ambient ozone and some cardiovascular alterations, such as oxidative stress, inflammation, alteration of heart rate variability, imbalance of autonomic components, arterial pressure control, coagulation, and myocardial infarction (Chuang et al. 2007; Jia et al. 2011). In Italy, Nuvolone et al. (2013) found a 6.3% (95% CI 1.2–11.7%) increase in out-of-hospital coronary deaths for a 10 $\mu g/m^3$ increase in O_3 and concluded higher risks for females, the elderly, and patients previously hospitalized for cerebrovascular and artery diseases. Zhao et al. (2017) reviewed the literature on the impact of short-term exposure to air pollutants on the onset of out-of-hospital cardiac arrest and confirmed the positive and significant association with O_3 exposure (RR = 1.016, 95% CI 1.008–1.024).

17.5.2.1 Stroke problem

A stroke is a disruption of blood flow to the brain, causing paralysis, slurred speech, and/or altered brain function. Two-thirds of all people who have a stroke have some form of eternal disability. In the United States, a stroke happens every 40 seconds and results in one-fifth of total deaths. CDC estimates that stroke costs the US economy more than $34 billion annually in direct medical costs and lost days of work (cdc.gov 2018).

Short-term O_3 exposure is associated with an increased risk of hospitalizations for stroke, with multiple studies reporting an increase (up to 3.9%) in admissions for stroke per 10 ppb O_3 (Carlsen et al. 2013; Xu et al. 2013; Guo et al. 2017; Liu et al. 2017). The relationship has been modeled over a range of O_3 values in some studies and found to be roughly linear down to as low as 15 ppb (Huang et al. 2017). A few studies find positive associations in subpopulations like the elderly above 75 years of age, particular types of strokes in specific racial groups, ischemic strokes in non-Hispanic whites, and hemorrhagic stroke in African Americans (Chen et al. 2014; Wing et al. 2015; Montresor-López et al. 2016). An association between exposure to O_3 and regular ischemic strokes has also been reported around a 1.18 ORs value, which depends on the number of days lagged (Suissa et al. 2013). Table 17.3 describes the lower- and upper-case index of stroke risk on human health due to short-term O_3 exposure.

Table 17.3. Short-term ozone exposure and stroke risk

S. No.	**Place (Period)**	**Population (N)**	**Outcome**	**Effect, LCI,UCI**	**References**
1.	Dijon, France (1994–2004)	1487	Ischemic stroke	1.54,1.14, 2.09	Herontin et al. (2007)
2.	Allegheny,PA (1994–2000)	26219	All stroke	1.036,1.007,1.065	Xu et al. (2013)
3.	Allegheny, PA (1994–2000)	26219	Ischemic stroke	1.043, 1.011, 1.076	Xu et al. (2013)
4.	South London (2005–2012) 1800	204	Hemorrhagic stroke	1.04,0.88,1.24	Chricton et al. 2016)
5.	Corpus Christi (2000–2012)	3035	Ischemic severity (Disadvantaged)	1.12,1.01,1.24	Wing et al. (2017)
6.	Corpus Christi (2000–2012)	3035	Ischemic severity (Wealthier)	1.23,1.11,1.36	Wing et al. (2017)
7.	Corpus Christi (2000–2012)	317	Recurrent ischemic strokes	0.97,0.87,1.07	Wing et al. (2017)

17.5.2.2 Arrhythmia problem

A small longitudinal cohort found a significant increase in ventricular tachycardia (RR 1.7) and supraventricular tachycardia (OR 1.11) related to 10ppb O_3 in coronary artery disease patients (Bartell et al. 2013). Cakmak et al. (2014) reported increased odds of heart block (ORs 1.1) in a study of cardiac patients with 24-hour Holter monitoring. Studies of Song et al. (2016) and Knezovic et al. (2017) looked at O_3 with all hospitalizations for arrhythmia (irrespective of patient cardiac history), one cohort, and one large meta-analysis; both found no significant relationship. Thus, the seemingly contradictory results may be related to patient addition criteria, with O_3 having a considerable effect on those with prior cardiac disease.

17.5.2.3 Hypertension

Various research groups reported significant effects of short-term ozone exposure admission with complaints of hypertension and lesser increases in diastolic and systolic blood pressure (Weichenthal et al. 2014; Yang et al. 2017; Cole-Hunter et al. 2018; Chen et al. 2018). However, studies that include two meta-analyses do not find any significant association between hypertension and either short- or long-term ozone exposure (Yang et al. 2014; Wing et al. 2017; Yu et al. 2017). Long-term O_3 exposure has also been associated with increased odds of causing hypertension with increases of 0.73 mmHg in systolic blood pressure and 0.37 mmHg in diastolic blood pressure, but a decrease of 1.54 mmHg of systolic blood pressure in patients with sleep-disordered breathing (Dong et al. 2013; Liu et al. 2014). Zhao et al. (2013) suggested that the effects of ozone on hypertension may be more prominent in men and overweight individuals, perhaps explaining some of the variability in the results obtained. In children, long-term O_3 exposure has been associated with increased odds of hypertension, with more substantial effects in overweight/obese children and those who were never breastfed, and short-term exposure was also associated with hypertension (Dong et al. 2014, 2015; Zeng et al. 2017). Thus, the association between hypertension and short-term O_3 exposure remains an open question.

17.5.3 Reproductive and developmental problems

17.5.3.1 Birth defects

Studies have suggested the teratogenic effects of O_3 for a specific gestational period, i.e., 3–12 weeks of fetal exposure to elevated O_3 during

5–8 weeks of pregnancy caused an increased risk of defects in limb formations and resulted in polydactyly, whereas exposure for 3–8 weeks was associated with risk of atrial septal defects (Vinikoor-Imler et al. 2013; Lin et al. 2014). Exposure to ozone over 43 ppb in the third week of gestation is closely associated with a three-fold higher risk of pulmonarystenosis. In contrast, O_3 exposure between 8 and 12 weeks was associated with congenital heart disease, including ventricular septal defect and teratology of Fallot (Zhang et al. 2016). Agay-Shay et al. (2013) found an elevated risk of cardiac malformations, whereas; Vinikoor-Imler et al. (2015) reported an increased risk of craniosynostos is under severe O_3 exposure. However, the effects of O_3 concentrations on early developmental stages are not found to be related to congenital anomalies (Vinikoor-Imler et al. 2013; Farhi et al. 2014; Zhou et al. 2017).

17.5.3.2 Fertility and pregnancy complications

Reduction in sperm count and changes in ovarian response to hormonal stimulation were reported as a result of O_3 exposure on fertility (Carré et al. 2017). Life-threatening problems during pregnancy, such as gestational hypertension and preeclampsia, are critically serious conditions, and the relationship of gestational hypertension to O_3 exposures is somewhat mixed (Lee et al. 2013; Pedersen et al. 2014; Michikawa et al. 2015; Mendola et al. 2016). The study also suggested a slight increase in systolic blood pressure (1.3 mmHg per 10 ppb) with O_3 exposure in the second trimester and a slight decrease (1.9 mmHg) in the third trimester (van Rossem et al. 2015). Ozone can also alter placental function; however, no association has been found for actionson placental volume or vascularization or placenta accreta (Michikawa et al. 2016; Hettfleisch et al. 2017). Closer to delivery, O_3 concentration was not associated with placental abruption but was associated with biomarkers of endothelial function such as ICAM-1, VCAM-1, and endothelin-1 (Poursafa et al. 2015; Michikawa et al. 2017).

17.5.3.3 Birth outcomes

Studies have linked losses in birth weight of the fetus by 13–27 g against a 10-ppb elevation in the average ozone level over the pregnancy, and have proved an improved risk of early gestational age with first-trimester exposure (Laurent et al. 2013; Gray et al. 2014; Michikawa et al. 2017). However, numerous studies on the O_3 effect on birth outcomes have not found deleterious effects of O_3 on the growth of the fetus or birth weight (Lee et al. 2013; Ritz et al. 2014; Carvalho et al. 2016; Capobussi et al. 2016; Díaz et al. 2016).

17.5.4 Central nervous system effects

17.5.4.1 Autism problem

Exposure to O_3 during pregnancy may cause a risk to develop autism in the child. Epidemiology of a large case-control study reveals a 10% increase in the chances of having autism diagnosed in children with a 10-ppb average increase during prenatal exposure (Becerra et al. 2013) while Jung et al. (2013) reported a 59% increased O_3 risk (Table 17.4).

17.5.4.2 Dementia problem

Case-control studies conducted in Taiwan found a relationship between elevated O_3 concentrations and odds of Alzheimer's disease with vascular dementia diagnosis (Wu et al. 2015; Jung et al. 2015; Cleary et al. 2018). However, a considerable cohort study in Canada shows no significant association with the new onset of dementia [Hazard ratio, 0.97 (95% CI 0.94–0.98)] (Chen et al. 2017). Furthermore, the study of Linares et al. (2017) has shown an 18% increase in hospital admissions with the complaint of dementia-related symptoms with increased O_3.

Table17.4. Effect of ozone on the central nervous system

S. No.	Population	Outcome	References
1.	49,073 children	Autism (full population)	Jung et al. (2013)
2.	158 cases	Autism (full population)	Kim et al. (2017)
3.	346 cases	Autism (low folic acid/high folic acid)	Goodrich et al. (2018)
4.	249 cases	Alzheimer's disease (full population)	Wu et al. (2015)
5.	125 cases	Vascular dementia (full population)	Wu et al. (2015)
6.	2.1 million people	Incident dementia (full population)	Chen et al. (2017)

17.5.5 Mortality caused due to ozone

Two recent multicenter studies made an important contribution in providing evidence on the impact of short-term exposure to elevated O_3 which resulted in mortality: the Air Pollution and Health, A European and North American Approach (APHENA) study (Katsouyanni et al. 2009) and the Public Health and Air Pollution in Asia (PAPA) study (HEI 2010, 2011; Wong and Health Review Committee 2010). The APHENA project is an extensive network with dedicated researchers participating in the multicity European APHEA project, the US National Morbidity, Mortality and Air Pollution Study (NMMAPS) and the multicity Canadian study. The APHENA study pooled data from 12 cities in Canada, 90in the USA, and 32 in Europe to evaluate the coherence of multiple time-series studies and produce a robust estimate of the relation between O_3 increases and mortality. The PAPA study also applied a standardized protocol to collect data and perform statistical analyses, pooling the results from six large Asian cities (Bangkok, Hong Kong Special Administrative Region, Shanghai, Wuhan, Chennai, and Delhi). Other recent European studies have been conducted in England and Wales (Pattenden et al. 2010), France (Lefranc et al. 2009), Italy (Stafoggia et al. 2010), Spain (Ballester et al. 2006), and Greece (Kassomenos et al. 2012). Peng et al. (2013) published the statistical methods used for the re-analyses of APHENA data, using and comparing various methods, such as natural spline (ns), penalized regression spline (ps), and various degrees of freedom (dfs), to remove long-term trends and time-dependent confounders.

Overall, the authors estimated an excess mortality risk of 0.26% (95% CI 0.15–0.37%) related to a 10 $\mu g/m^3$ increase in 1h of daily maximum ozone. Among all models applied, the most substantial effect estimate resulted from the model using natural splines and 8 df per year. Results from European and US studies were similar, whereas those from Canada showed higher risk estimates. Authors hypothesized some explanations of this different pattern, including the influence of other co-varying pollutants and the residual confounding in the Canadian data from unmeasured seasonal or meteorological factors. Authors also discussed the role of the availability of more accurate exposure and outcome data in Canada, as compared with the European countries and the USA, but the authors themselves stated that this explanation seems unlikely, and they do not provide any data to pursue this conjecture. The authors concluded that the re-analyses of APHENA data confirmed the O_3-related acute mortality risk previously reported and that the implementation of an extensive statistical protocol leads to the robustness of results forthe method of data analysis.

17.6 Conclusion

Substantiation from the studies indicates that O_3 pollution increased day by day in the ground-level atmosphere due to anthropogenic activities. Therefore, its high concentration developed uncontrolled problems related to human health. Evidence from observational studies strongly indicates that higher daily O_3 concentrations are associated with increased asthma attacks, hospital admissions, daily mortality, and other morbidity markers. School-aged children and elderly individuals are particularly vulnerable, and the majority of the studies show adverse effects of O_3 at the secritical stages of life. Cardiovascular disease, specifically increased rates of strokes and cardiac arrhythmia in persons with pre-existing heart disease and mortality due to short-term exposure to O_3 are a major health-related risk to the human population. Additionally, the evidence of long-term exposure to O_3 also increases rates of mortality. The epidemiological study of the effects of O_3 on the central nervous system suggests that long-term O_3 exposure could be associated with nervous system dysfunction resulting in an increased risk of autism in children and possibly dementia among the elderly. However, rigorous inquiries are required to better understand the relationship between O_3 and chronic health effects associated with its exposure.

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CHAPTER EIGHTEEN

SYSTEMIC CONSEQUENCES OF OZONE ON HUMAN HEALTH

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Abstract

Ozone (O_3) is found in the stratosphere as well the troposphere. Stratospheric O_3 (good O_3) forms a protective layer and shields us from the sun's harmful ultraviolet rays. However, tropospheric O_3 proves to be a harmful secondary air pollutant because of its adverse effects on people and the environment. Ozone has been identified as one of the six common air pollutants in the Clean Air Act. It is a photochemical smog component, a major air pollutant that demonstrates harm to health because of the toxic properties inherent to its powerful oxidizing capabilities. Environmental O_3 exposure is associated with many respiratory disorders, including loss of lung function, exacerbation of asthma, airway damage, and lung inflammation. The effects of O_3 are not restricted to the respiratory system or its functions, but adverse effects within the central nervous system

(CNS) such as decreased motor activity and cognitive response, headaches, disturbances in the sleep-wake cycle, neuronal dysfunctions, cell degeneration, and neurochemical alterations have also been described. Ozone harms sensitive vegetation, ecosystems including forests, and even wildlife. This problem is exacerbated to a greater extent because O_3 can also be transported to long distances by the prevailing wind. Thus, even far-off rural areas can also experience high O_3 levels. According to the Global Burden of Disease, India contributes one-third of the global disease burden due to O_3 exposure. There is a need for extensive and rapid reductions of O_3 precursors and the involvement of policies to implement specific rules and regulations so that the human population can survive with fewer health risks in such challenging conditions.

Keywords: Air Pollutant; Smog; Ozone; Health risk

18.1 Introduction

Outdoor air pollution, because of the mixture of various gases and particles, has been responsible for acute and chronic health effects. A relevant component of this harmful mixture of pollutants is ozone (O_3). This is an inorganic oxygen molecule made of three oxygen atoms which has been categorized as “bad,” i.e., the one found near the troposphere, and “good”, i.e., the one found in the stratosphere. Most of the atmospheric O_3 (90%) is located in the stratosphere. Tropospheric O_3 contributes only 10% to the total O_3 column, but its concentrations have been steadily increasing during the last few decades. The good O_3 is formed by the photo-dissociation of O_2 molecules by UV rays of lower wavelength from sunlight into two O atoms. After that, the combination of one O atom with one O_2 molecule absorbs the harmful UV rays reaching the earth’s surface. This O_3 plays a vital role in supporting the life system against the harmful ultraviolet radiation emanating from the sun and is naturally present in the stratosphere. In the troposphere, it is not emitted directly but formed from its precursors through various chemical reactions, and it is thus classified as a secondary pollutant. The two primary precursors of O_3 are the volatile organic compounds (VOCs), which include alkanes, alkenes, organic peroxides, alcohols, carbonyl compounds, halogenated organic compounds, and aromatic hydrocarbons; and nitrogen oxides (NOx). The O_3 is formed because of these precursors' reactions in the presence of sunlight (Fig. 18.1), with carbon monoxide and methane being other important elements involved in its formation (EPA 2012, 2015; WHO 2006).

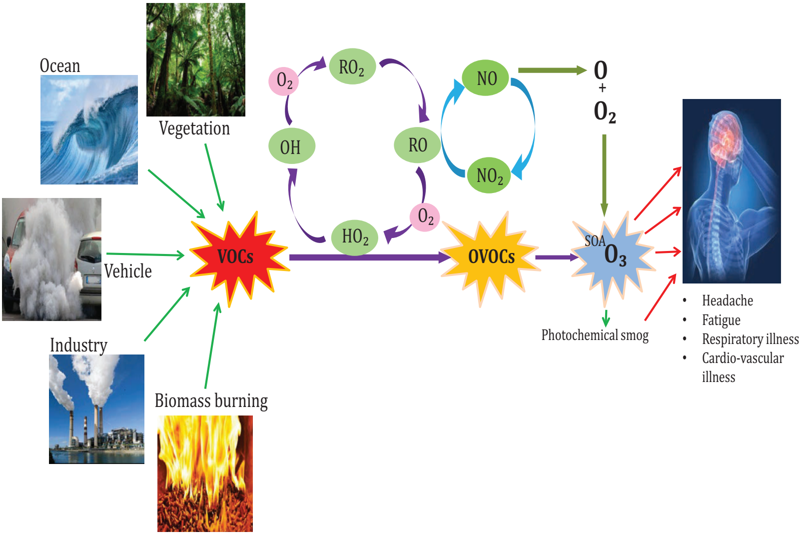

Fig. 18.1. The sources, formation, and impact of ozone

Recent estimates indicate that in 2013 in the EU-28 alone, 16,000 premature deaths were attributed to O_3 exposure, equivalent to 192,000 years of life lost. The permissible limit of O_3 suggested by the World Health Organization (WHO 2005) is 120 µ g/m 3 (60 ppb) for an 8h concentration, but many areas are receiving a concentration higher than the proposed limits. The average O_3 inhaled by humans during the sleep/repose stage is about 40–60%, and out of this, only 6% reacts with the cellular membrane; however, this leads to the production of reactive oxygen species (ROS) (Gerrity et al. 1988; Kabel et al. 1994; Pryor et al. 2006) and instigates the inflammatory reactions in epithelial cells. Recent studies have shown O_3 negatively affecting human health by initiating several diseases related to the respiratory, cardiovascular, central nervous systems, etc. Moreover, O_3, which is a highly reactive and oxidative gas, has been implicated in several adverse health effects ranging from morbidity to mortality. Data emerging from worldwide monitoring sites indicate that O_3 levels often exceed the various thresholds and guidelines set for human health (Nuvolone et al. 2018). Evidence coming to the fore is establishing a profound link with its short-term effects, particularly on cardiovascular and respiratory systems. Evidence for the long-term effects of O_3 exposure seems to be less conclusive. However, some reports suggest associations with respiratory mortality, the onset of asthma in children, and increased respiratory symptom effects in existing asthmatic patients. Evidence from vast data routinely collected on concentrations of various air pollutants and health statistics and growing epidemiological studies permit us to produce meaningful health impact assessment estimates.

18.2 Impact of Ozone on animals

Ozone is known to cause respiratory health effects. It has been reported to have serious effects on cognition (Calderón-Garcidueñas 2016). There has been evidence of its relation to dementia (Bell et al. 2004; Levy et al. 2005; Chan and Wu 2005). According to Gilbert and Brushfield (2009), O_3 develops biochemical and morphological changes in the hippocampus sub-regions, which have a vital role in learning and spatial memory. Recently, Custodia et al. (2019) have also observed a reduction in cognition in male and female rats exposed to O_3. The prenatal exposure to O_3 leads to modification in migration, maturation, and neurogenesis. The adult male rats were exposed to O_3 to evaluate its adverse impact on memory and lipid peroxidation. After a 4h exposure, there was a loss in memory and a reduction in lipid peroxidation in the hippocampus sub-regions and cortex region (Dorado-Martínez et al. 2001).

Inconsistency in cerebellar foliation necrosis was observed in rats exposed to O_3 during pregnancy (Rivas-Manzano and Paz 1999). It is also known to alter the mammal development and plasticity process in the nervous system (Custodio et al. 2010). Memory deterioration was also observed along with a significant reduction in the number of dendritic spines of pyramidal neurons in the hippocampus when adult rats were exposed to 1.0 ppm O_3 for 4 hours. It was also found that exposure to O_3 causes deterioration in plasticity, which is essential for memory processes correlated with the decrease in receptors in the hippocampus sub-regions and cortex of adult males and females (Custodia et al. 2019). Oxidative stress and irreversible and progressive neurodegeneration were observed in adult rats exposed to a low dose of O_3 (0.25 ppm) for 4 hours (Rivas-Arancibia et al. 2010). Oxidative stress and neurodegeneration are related to amyotrophic lateral sclerosis, Alzheimer's, Parkinson's, and other disorders (Dorado-Martínez et al. 2001).

Alterations in the memory and learning process respond in the form of changes in the mitochondria, modifications in the endoplasmic reticulum, the Golgi apparatus, nuclear changes, activation and proliferation of the glia, cell death by apoptosis and necrosis along with the intracellular accumulations of beta-amyloid (Rivas-Arancibia et al. 2016). Ozone can impair maximal oxygen uptake and affects performance during exercise

(Giorgini et al. 2016). There is also a correlation between O_3 exposure and ischemic heart disease. The ozone level is also known to be positively linked with myocardial infarction (Ruidavets et al. 2005). A number of studies have consistently associated higher blood pressure (Chuang et al. 2010; Cakmak et al. 2011) and cardiovascular mortalities (Samoli et al. 2009; Zanobetti et al. 2008; Katsouyanni et al. 2009; Stafoggia et al. 2010) with O_3 exposure. The acute exposure of O_3 stimulates intraepithelial nociceptive vagal C-fibers by activating the transient receptor potential (TRP). According to Gackiere et al. (2010), O_3 inhalation activates the brain's stress-responsive regions. Acute exposure to O_3 is related to impaired microvascular integrity and the blood-brain barrier in animals (Suwannasual et al. 2018); activation of the microglia (Mumaw et al. 2016); and neuroinflammation (Aragon et al. 2017). Alterations in catecholaminergic neurons and an increase in catecholamine turnover in sympathetic and central neurons located in the cervical ganglia have also been observed after O_3 exposure (Soulage et al. 2004).

Ozone exposure has also been evaluated to be associated with neuroinflammation and oxidative stress in the central nervous system (CNS) (Martinez-Lazcanoet al. 2013). Chounlamountry et al. (2015) evaluated that O_3-induced pulmonary inflammation results in specific activation of vagal lung afferents rather than non-specific overall brain alterations mediated by blood-borne agents. It has also been linked with the onset of peripheral insulin resistance (Rajagopalan and Brook 2012) and diabetes (Janghorbani et al. 2014).

The exposure of rats to O_3 leads to lung injury/inflammation, hyperglycemia, glucose intolerance, and lipidemia that may lead to increased circulating epinephrine and cortisol (Miller et al. 2015). According to Gordon et al. (2014), acute exposure to O_3 leads to hypothermia and bradycardia. Ozone can react with the olfactory epithelium's antioxidants in the mucus layer (Dennis et al. 2015). It can also react with other components of the olfactory mucus-like proteins and glycoproteins (Heydel et al. 2013), giving the perception that O_3 and its reaction products impair the function of the olfactory receptors. Obesity and O_3 exposure cumulatively affect the metabolic profile of the lungs and such metabolic alterations can induce an asthma-like phenotype (Williams et al. 2013). According to a report by the Environmental Protection Agency (EPA), O_3 has been found to be associated with birth-related health outcomes, including mortality, premature births, low birth weights, and congenital disabilities in animals. Some of the studies highlighting the effects of O_3 exposure have been compiled in Table 18.1.

Table 18.1. Compilation of studies that have shown the impact of ozone on humans

S. No.	Concentration/ Dose of Ozone	Exposure time	The method employed to analyse parameters	Impact on the studied model system	References
1.	0.08 ppm	6.6 h	Technique induced sputum and flow cytometry were used to examine airway inflammatory responses and changes in immune cell surface phenotypes	Ozone exposure enhanced the expression of HLA-DR, CD80, CD14, CD86, neutrophils, dendritic, monocytes, and antigen-presenting cell phenotypes 18 hours following exposure	Alexis et al. (2010)
2.	80–40 ppb	-	Bronchial mucosal biopsies	Enhancement in cytokines, albumins, lactate dehydrogenase of interleukin-6 and -8, and prostaglandin. Elevated levels of ozone (80–400 ppb) cause airway inflammation	Oikonomidou and Tzanakis (2007)
3.	0.2 ppm	4h	Bronchoconstriction, Immunofluorescent staining	Ozone causes persistent airway hyperactivity in humans and animals. The neuronal M (2) muscarinic receptors get inhibited after one day of ozone exposure by releasing eosinophil major essential protein. That increases	Verhein et al. (2011)

				the acetylcholine release and smooth muscle contraction in guinea pigs. Three days after ozone, IL-1β, not eosinophils, mediates ozone-induced airway hyperactivity, but the mechanism at this time point is mostly unknown. IL-1β increases NGF and the tachykinin substance P, both of which are involved in neural plasticity	
4.	0.4, 0.8 ppm	3h	Radioimmunoassay	Ozone treatment to the mouse increased its susceptibility to the *Streptococcus zooepidemicus* bacterial infection	Gilmour et al. (1993)
5.	0.2 mg/m^2	5h	Direct (IgM) anti-sheep red cell (SRBC) plaque-forming cell, Bactericidal activity, and Immune-Function assay	Ozone treatment to the mouse increased its susceptibility to the *Klebsiella pneumoniae* bacterial infection	Aranyi et al.(1983)
6.	200 ppb	4h	Using the Pneumotachograph-based system inspiratory vital capacity (VC) and forced expiratory volume (FEV)	Ozone exposure leads to a decline in FEV, while increasing the reduced glutathione, urate, ortho-tyrosine neutrophils, and lymphocyte concentrations	Jòrres et al. (2000)

7.	22–54ppb (summer) and 4–36 ppb (winter); survey study	3.5 yrs	Pulmonary function using a Masterscope	Exposure to ozone led to increased forced vital capacity and forced expiratory volume during the first 2 yrs of the study. While in winters, the pattern was reversed.	Ihorst et al. (2004)

18.3 Toxicity of ozone with particular reference to its impact on vital systems of humans

Ozone has low solubility in water and is a highly reactive and oxidative gas, and the exposure route is primarily by inhalation (Nuvolone et al. 2018). Some reports have emerged showing ambient O_3 level exposure resulting in various effects in the tear duct epithelial cells of exposed individuals (Rojas et al. 2000) and high-level exposure impacting the skin of laboratory animals (Valacchi et al. 2004). However, various experimental studies have suggested a lack of evidence of the absorption of O_3 in the innermost compartments of the dermis. It is thus likely that the effects are restricted to the upper dermis layers of the skin.

Post-exposure, O_3 is inhaled and absorbed mostly by the upper respiratory tract and then conducted in intra-thoracic airways (Bush et al. 1996; Sarangapani et al. 2003). Intense physical activity would likely lead to higher lung penetration because the oral route of inhalation allows lower removal rates of O_3 than nasal inhalation. Ozone absorption may be influenced by various other factors like age and gender, with higher absorption levels being observed in children and women primarily because of variations in the airways (Bush et al. 1996).

The roles of some of the main vulnerability and susceptibility factors that are expected to influence O_3-related health effects, including age, gender, socio-economic status, and occupation, have been extensively reviewed (Bell et al. 2014). According to Bell et al. (2014), significant health risks related to O_3 exposure are reported in older adults and age, therefore, represents the most robust susceptibility factor. Regarding gender and occupation, the evidence is limited or suggestive, with higher risks reported among women and unemployed people or those of lower occupational status. Further, no significant results were observed in studies that focused on various ethnic minorities, people with low education, and air conditioning availability.

Due to the ineffective removal of O_3 from the upper respiratory tract, much of the inhaled O_3 reaches the lower respiratory tract and consequently dissolves in the epithelial lining fluid (ELF). This thin layer comprises a complex that serves as fundamental protection against various pathogens and foreign substances and is a mixture of lipids, proteins, and antioxidants. The O_3 reacts with the ELF components resulting in multiple products (i.e., secondary oxidation products) of varying reactivity. The thickness of the mucus layer and the ELF determines the quantity of O_3 that eventually reaches the tissues. A thicker ELF usually leads to lower O_3 doses to the tissues. Further, the production of secondary oxidation products primarily depends on the quantity of antioxidants and various other substances in the ELF. It results in the decline of the reactions of O_3 with the underlying tissues and its subsequent penetration into the respiratory tract (Nuvolone et al. 2018).

18.3.1 Acute Health Effects

There is at present a considerable volume of literature available, focusing mainly on the acute effects of O_3 exposure, and in recent years, this has been extensively reviewed by the WHO (WHO 2004, 2006, 2013a, b). Just like the effects of exposure to other air pollutants, the severity and extent of health effects can be represented through a pyramid. The bottom of the pyramid represents less severe health conditions affecting most people. In contrast, the top of the pyramid represents the most severe end-points, including hospital admission and/or death, experienced by fewer persons.

18.3.2 Mortality

The significant evidence on the short-term O_3 exposure effects on mortality has been primarily provided recently by two extensive multicenter studies. 1) Air Pollution and Health: a European and North American Approach (APHENA) study (Katsouyanni et al. 2009). This project is the outcome of an extensive network of researchers participating in the multicity European Agency for Public Health Education Accreditation APHEA project, the US National Morbidity, Mortality, and Air Pollution Study (NMMAPS) and the multicity Canadian study. For the APHENA project, data from 12 cities in Canada, 90 in the USA and 32 in Europe were pooled to evaluate the coherence of multiple time-series studies and the robust estimation of the relation between an increase in O_3 levels and mortality. 2) The Public Health and Air Pollution in Asia (PAPA) study (HEI 2010, 2011; Wong and Health Review Committee 2010). This project also employed a standardized protocol to collect data and perform statistical analyses in all locations, with the data being pooled from six large Asian cities (Hong Kong Special Administrative Region, Bangkok, Shanghai, Wuhan, Delhi, and Chennai). Some of the other recent studies that have been conducted in this direction include those in England and Wales (Pattenden et al. 2010), Italy (Stafoggia et al. 2010), France (Lefranc et al. 2009), Greece (Kassomenos et al. 2012) and Spain (Ballester et al. 2006).

The statistical methods used for the re-analyses of APHENA data have been published, wherein various methods, such as penalized regression spline (ps), natural spline (ns), and various degrees of freedom (dfs), have been used and compared for the removal of long-term trends and time-dependent confounders (Peng et al. 2013). In summary, the authors estimated an excess of 0.26% (95% CI 0.15–0.37%) mortality risk with a 10 $\mu g/m^3$ increase in 1-hour daily maximum O_3 (Anderson et al. 1997). Similar results were reported from European and US studies, whereas higher risk estimates were shown for Canada. Various explanations of this pattern have been put forth by the authors, including the occurrence and influence of other co-varying pollutants and the expected residual confounding in the data emerging from the Canadian project because of unmeasured seasonal or meteorological factors. The authors have also elaborated on the availability of more accurate exposure and outcome data from Canada than that from the USA and European countries. Still, this explanation seems unlikely as stated by the authors themselves, and they haven't provided any data to prove this hypothesis (Nuvolone et al. 2018).The authors pointed out that the re-analyses of APHENA data confirm the previously reported O_3-related acute mortality risk.

18.3.3 Pulmonary system effects

Many reports emerging from experimental studies have focused on the acute effects of O_3 exposure on respiratory health. The relation of O_3 exposure and the respiratory outcome has been investigated under different conditions, including controlled exposures during exercise or at rest, exposures at ambient levels, and subjects who had pre-existing pulmonary diseases, like chronic bronchitis asthma.

Studies involving humans have an essential advantage of direct interpretations and inferences without an apparent need to extrapolate the results from experiments conducted in other animal models or *in vitro*, more so because of the usage of very high pollutant levels for the determination of various underlying biological processes and mechanisms (Nuvolone 2018). The induction of transient reduction in lung function remains one of the most studied acute respiratory end-points. Most of the studies on O_3 exposure have been conducted on healthy, young, and usually non-smoker subjects exposed to a wide range of O_3 levels (40–600 ppb) during physical activity (McDonnell et al. 2012; EPA 2012).

According to the update of the 2005 WHO global air quality guidelines (WHO 2006), there has been a growing interest among the scientific community to produce some predictive and relevant models for lung function decrements (FEV1). This is related to the inhaled O_3 dose to efficiently evaluate the risk and produce relevant response thresholds (McDonnell et al. 2012; Schelegle et al. 2009). The important results from such studies have shown that subjects such as children in summer camps, outdoor workers, exercising adults, etc., exposed to high outdoor O_3 levels, experienced a lower reduction in lung function than that of chamber studies (Brunekreef et al. 1994; Kinney et al. 1996; Chan and Wu 2005; Thaller et al. 2008).

The data from epidemiological studies have shown a significant and positive association between ambient O_3 level fluctuations and increased morbidity. Some of the most important health end-points or proxies of them that are more frequently studied include hospital admissions or visits to emergency departments for asthma, respiratory tract infections and aggravation of other respiratory disorders, school absenteeism, etc. In most cases, the effects were observed more frequently in children, older people and subjects with asthma and chronic obstructive pulmonary disease (COPD) (Nuvolone et al. 2018). In addition to this, the effects were more frequent in the warmer months. As in the case of studies concerning mortality, there are extensive multicity analyses relating variations of O_3 levels with the hospital admissions for various respiratory diseases (Burnett et al. 1997; Anderson et al. 1997). These associations prove to be significant when time-related variables like the day of the week, seasonal variations, other common air pollutants, and various meteorological factors, are introduced into the models to make them useful and relevant.

18.3.4 Cardiovascular system effects

Experimental studies on various animals have indicated the different cardiovascular effects of O_3 exposure, like an increase in heart rate and diastolic pressure, induction of oxidative stress in the vascular system, inflammation, and a decrease in heart rate variability (Chuang et al. 2009; Perepu et al. 2010; Tankersley et al. 2010). But, the results from experiments conducted on humans remain less conclusive and fail to provide any consistent associations with O_3 (Liao et al. 2005; Chuang et al. 2007; Steinvil et al. 2008; Fakhri et al. 2009; Rudez et al. 2009; Thompson et al. 2010; Kusha et al. 2012).

In 2005, an extensive study was conducted in the Boston area on 603 men who participated in the Veterans' Administration Normative Ageing Study (Park et al. 2005). By performing electrocardiographic monitoring, including the measurement of heart rate variability (HRV), the authors observed a reduction of 11.5% (95% CI 0.4–21.3) in low-frequency HRV associated with 2.6 µg/m^3 increases in O_3 exposure in the previous 4 hours. Other significant findings of this study include the occurrence of substantial effects on men affected by hypertension and ischemic heart disease.

Patients with implanted defibrillators in the Boston area were followed by Rich et al. (2006). They observed increased risk (OR = 2.1, 95% CI 1.2–3.5) of paroxysmal atrial fibrillation episodes associated with short-term fluctuations of O_3 concentrations. Additionally, positive associations between ambient O_3 exposure and some cardiovascular alterations have been found in some observational studies. These alterations include inflammation, oxidative stress, alteration of heart rate variability, imbalance of autonomic components, arterial pressure control, coagulation, myocardial infarction, etc. (Jia et al. 2011; Chuang et al. 2007). Nuvolone et al. (2013), in their study in Italy, found a 6.3% (95% CI 1.2–11.7%) increase in out-of-hospital coronary deaths for a 10 µg/m^3 increase in O_3. They also suggested higher risks for females, older people, and previously hospitalized patients for artery and cerebrovascular diseases. Zhao et al. (2017) reviewed the available literature on the impact of short-term air pollutant exposure on the onset of out-of-hospital cardiac arrest, and data confirmed the positive and significant correlation with O_3exposure (RR = 1.016, 95% CI 1.008–1.024).

18.3.5 Central nervous system effects

The transportation of any substance to the CNS is regulated by the blood-brain barrier (BBB) that maintains the innate immune response and checks the leukocytes (Abbott and Friedman, 2012). Ehlirch examined the significance of the BBB (1885) wherein the intravenous injection of aniline could not dye the brain part, while the rest of the body showed the presence of dye. However, under normal conditions, there is no entry of cytokines secreted by the brain's immune system. Still, it has been reported that O_3 exposure leads to the entry of circulating molecules via areas damaged in the BBB, the circumventricular organs, and the choroid plexus (Calderón-Garcidueñas et al. 2002; Banks 2005; Block and Calderon 2009). The expression of transporters in adjacent endothelial cells could also be one reason for active transport (Abbott and Friedman 2012). Turrin and Rivest (2004) reported that adjacent cells produce cytokines as a result of which several microglia and astrocytes (astroglia) are activated (astroglia show sensitivity towards inflammation and oxidant production under air pollution) (Block and Calderon 2009). However, the exact mechanism of transport from the adjacent cells is not yet elucidated. These astrocytes activate the toll-like receptors (TLR) reported to amplify the immune response and alter the BBB's permeability (Brambilla et al. 2005; Bsibsi et al. 2006). If the BBB's integrity is compromised, it may lead to several neurological disorders, and these disorders have been found to be associated with leukocyte infiltration from the blood and activation of glial cells (de Vries et al. 2012). The O_3 exposure leads to the production of ROS and reactive nitrogen species (RNS) as well as the metalloproteinase that disturbs the integrity of the BBB (Thiel and Audus 2001; Gu et al. 2011). The overall impact of O_3 exposure leads to dementia, migraine, and headache during pregnancy, and increases the risk of autism in developing children, etc. Studies performed under laboratory conditions have suggested that O_3 alters the CNS functioning and also leads to lethargy (Hackney et al. 1975), fatigue, and memory loss in severe cases (Hackney et al. 1977). It also affects motor activity and alters the sleep-wake cycle (Huitrón-Reséndiz et al. 1994). It was also found that exposure to O_3 causes deterioration in the process of plasticity, which is essential for the memory processes that could be correlated with the decrease in receptors in the hippocampus subregion and the cortex of adult males and females exposed to O_3 (Custodia et al. 2019). Oxidative stress and irreversible and progressive neurodegeneration were observed in adult rats exposed to a low dose of O_3 (0.25 ppm) for 4 hours (Rivas-Arancibia et al. 2010). Oxidative stress and neurodegeneration are related to amyotrophic lateral sclerosis, Alzheimer's, Parkinson's, and other disorders associated with memory loss (Dorado-Martínez et al. 2001). The alterations in the memory and learning process respond in the form of changes in the mitochondria, alterations in the endoplasmic reticulum, the Golgi apparatus, nuclear changes, activation and proliferation of the glia, cell death by apoptosis and necrosis along with the intracellular accumulations of beta-amyloid (Rivas-Arancibia et al. 2016).

The O_3-induced production of oxidants affects several brain regions like the hippocampus, frontal cortex, striatum, cerebellum, and olfactory bulbs. All these regions show the defective endoplasmic reticulum thus affecting the overall cell functioning. Molecular studies have explained that these oxidants increase the peroxidation of lipids and alter the concentration of neurotransmitters (NTs) like glutamate (Santiago-Lopez et al. 2010), γ-aminobutyric acid (GABA) (Rivas-Arancibia et al. 2003), noradrenaline (NA) (Gonzalez-Pina et al. 2008; Custodio et al. 2010), serotonin (5-HT) (Huitrón-Reséndiz et al. 1994; Paz and Huitrón-Reséndiz 1996), and NO (Dorado-Martinez et al. 2001). The alteration to the NTs is directly correlated with the neurophysiological processes.

18.4 Conclusion

In view of the published literature, it can be concluded that surface-level O_3 is responsible for a series of both acute and chronic health effects. There is enough evidence to suggest that O_3 negatively affects human health by initiating several diseases related to respiratory, cardiovascular and central nervous systems, etc. Moreover, O_3 is highly reactive, and the oxidative pollutant has been implicated in several adverse health effects ranging from morbidity to mortality. Data emerging from worldwide monitoring sites indicate that O_3 levels often exceed the various thresholds and guidelines set for the well-being of human health. The evidence emerging establishes a profound link with its short-term effects, particularly on cardiovascular and respiratory systems. Proof of the long-term effects of O_3 exposure seems to be less conclusive. However, some reports suggest associations with respiratory mortality, the onset of asthma in children, and an increase in existing asthmatic patients' respiratory symptoms. Evidence from vast data routinely collected on concentrations of various air pollutants and health statistics and growing epidemiological studies permit us to produce a meaningful health impact assessment.

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