Tropospheric Ozone

Tropospheric Ozone:

A Hazard for Vegetation and Human Health

Edited by

S.B. Agrawal, Madhoolika Agrawal and Anita Singh

Cambridge Scholars Publishing

Tropospheric Ozone: A Hazard for Vegetation and Human Health

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

This book first published 2021

Cambridge Scholars Publishing

Lady Stephenson Library, Newcastle upon Tyne, NE6 2PA, UK

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

ISBN (10): 1-5275-7057-6
ISBN (13): 978-1-5275-7057-3

TABLE OF CONTENTS

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.

S.B. Agrawal
Madhoolika Agrawal
Anita Singh

CHAPTER ONE

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

PIERRE DIZENGREMEL[1*]
AND ROBERT L. HEATH[2]
[1]UNIVERSITE DE LORRAINE, AGROPARISTECH,
INRAE, SILVA, F-54000 NANCY, FRANCE
[2]UNIVERSITY OF CALIFORNIA, DEPARTMENT OF BOTANY
AND PLANT SCIENCES, RIVERSIDE, CA 92102, USA
E-MAIL: PIERRE.DIZENGREMEL@UNIV-LORRAINE.FR;
HEATH@UCR.EDU

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.

* Corresponding Author E-mail: pierre.dizengremel@univ-lorraine.fr.

[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

[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

[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.

---

[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.

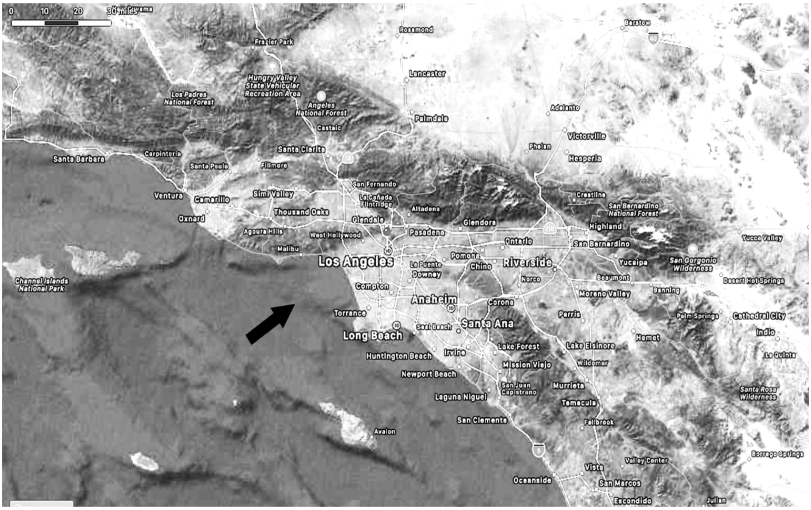

Fig. 1.1. Los Angeles Basin. The Pacific Ocean is on the left, a grey area. Mountains are indicated by the darkened areas. The on-shore sea breeze from the Pacific Ocean is indicated as a black arrow. The scale is about 450 km (270 miles) across the figure horizontally. North is up. *Modified map using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

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

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.

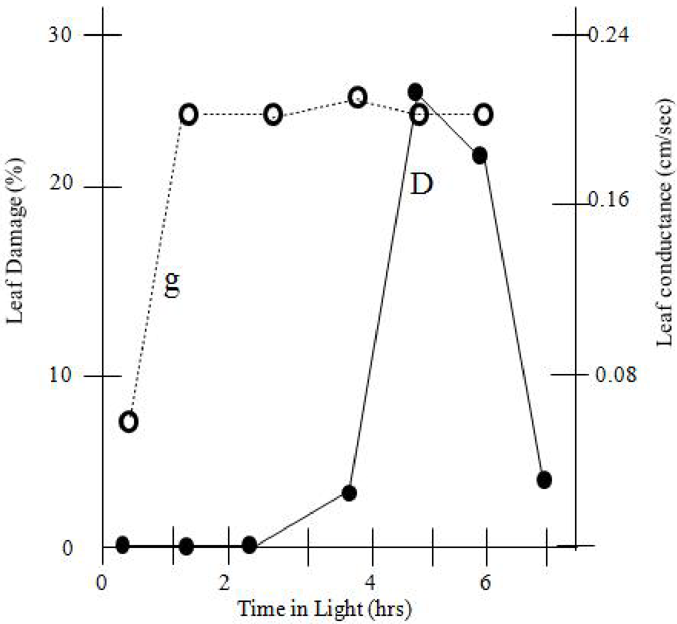

Fig. 3. Leaf damage from O_3 as a function of time in light (6 x 10^4 ergs/cm^2- sec). Three-week-old cotton plants were treated with 0.75 ppm O_3 for 1 hr at the time indicated. Plants were in the dark for 12 hrs before the light period. The authors used leaf resistance, determined with a resistance 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

[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.

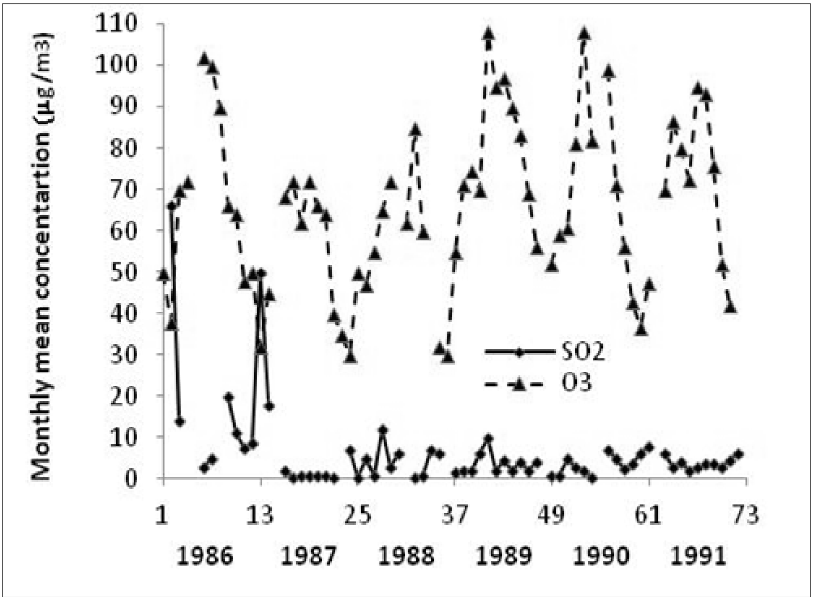

Fig. 1.4. Monthly mean concentrations of SO_2 and O_3 in the Vosges mountains at the Donon pass (700 m asl).
(After data from the Association pour la surveillance et l'étude de la pollution atmosphérique, Alsace, France)

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).

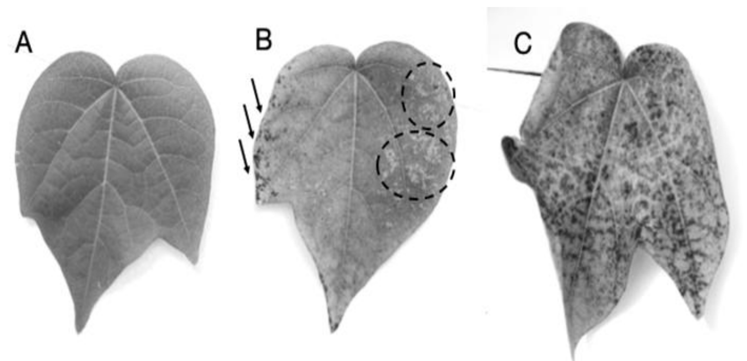

Fig. 1.5. Visible Injury Patterns on the Leaves of Cotton Plants. Cotton seeds were germinated and allowed to grow in a greenhouse for 3 weeks until the first true leaf was well developed. The plants were then exposed in a closed chamber (similar to Fig. 1.9) in a greenhouse to 3.0 ppm O_3 for 20 minutes 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.

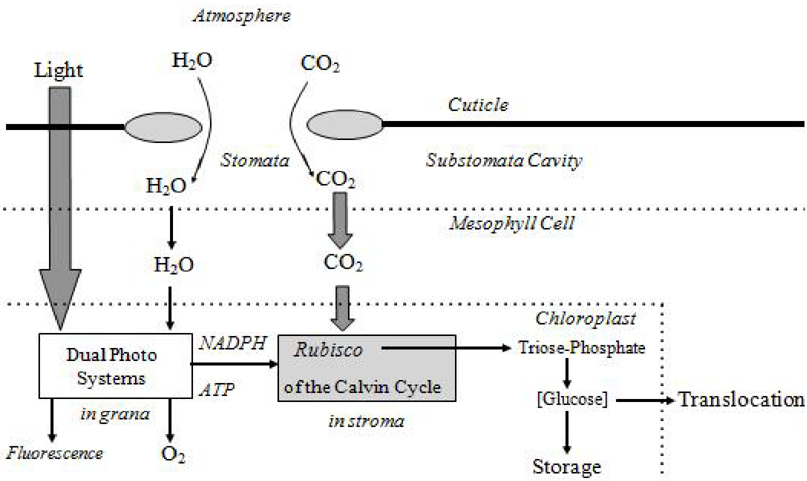

Fig.1.6. Basic processes of photosynthesis in a leaf diagram. The photosynthetic apparatus is contained within the chloroplast from which sugar is transported out to be used in other portions of the cell. The leaf structure (shown upside down with the stomata on the top) allows gases to flow into the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