CellPress

Trends in Biochemical Sciences

Review

Plant biochemistry influences tropospheric ozone formation, destruction, deposition, and response

Jessica M. Wedow,[1,4] Elizabeth A. Ainsworth,[2,3,4] and Shuai Li[3,4,*]

Tropospheric ozone (O_3) is among the most damaging air pollutant to plants. Plants alter the atmospheric O_3 concentration in two distinct ways: (i) by the emission of volatile organic compounds (VOCs) that are precursors of O_3; and (ii) by dry deposition, which includes diffusion of O_3 into vegetation through stomata and destruction by nonstomatal pathways. Isoprene, monoterpenes, and higher terpenoids are emitted by plants in quantities that alter tropospheric O_3. Deposition of O_3 into vegetation is related to stomatal conductance, leaf structural traits, and the detoxification capacity of the apoplast. The biochemical fate of O_3 once it enters leaves and reacts with aqueous surfaces is largely unknown, but new techniques for the tracking and identification of initial products have the potential to open the black box.

Highlights

Tropospheric ozone (O_3) is one of the most widespread air pollutants and an important greenhouse gas that is detrimental to both human and plant health.

Plant volatiles, such as isoprene, can increase O_3 pollution in the troposphere through the formation of peroxy radicals, which react with nitric oxide to form O_3.

Other plant volatiles, such as monoterpenes, sesquiterpenes, and diterpenes, are stored in trichomes and once released can decrease O_3 in the boundary layer and therefore reduce O_3 flux into leaves.

O_3 can induce rapid stomatal closure to prevent the entry of O_3 into the leaf and cause sluggish stomatal responses resulting in further O_3 uptake and greater water loss.

Better characterization of the radicals formed in leaves after O_3 exposure would enable more accurate modeling of O_3 deposition and improved strategies for O_3 tolerance.

Tropospheric O_3 formation

O_3 (see Glossary) in the stratosphere filters UV radiation, but in the **troposphere** O_3 is a damaging air pollutant to human and plant health [Environmental Protection Agency (EPA): https://www.epa.gov/ground-level-ozone-pollution; Box 1]. Tropospheric O_3 (trioxygen) is an allotrope of oxygen that forms through chemical reactions with two chemically distinct precursors: nitrogen oxides (NO_x = NO + NO_2) and reactive carbon molecules including carbon monoxide (CO), methane (CH_4), and **VOCs** (Figure 1) [1]. Rates of O_3 formation depend on sunlight and the relative concentrations of NO_x and reactive carbon molecules; namely, methane and VOCs [1]. The reaction of nitric oxide (NO) with the peroxy radical (R_2) is the central reaction for the formation of O_3 in the troposphere [2]. In this reaction, NO is converted to NO_2 (Figure 1), which is rapidly photolyzed to form O_3 and recycle NO. The efficiency with which O_3 is produced from *NO_x* pollution varies with the location and time of emissions. For example, in the polluted regions at the Earth's surface, NO_x rapidly reacts to form HNO_3, which serves as a reservoir for NO_x [3]. In less polluted areas, NO_2 photolysis competes more effectively with HNO_3 production and more molecules of NO_x react with peroxy radicals to form O_3. In regions where NO_x is propelled into the free troposphere, like the tropics, O_3 production is especially efficient [1,4]. Additionally, the VOC:NO_x ratio determines the O_3 concentration [5]. In urban areas with elevated NO_x due to high emissions, O_3 formation is limited by VOCs, leading to locally suppressed O_3 concentrations. NO_x transported away from urban centers can mix with VOCs, resulting in greater O_3 concentrations in suburban areas [5].

Global O_3 production in the troposphere is estimated to be between 4960 and 5530 Tg $year^{-1}$ (Figure 1), with most O_3 produced from chemical reactions and a smaller amount exchanged with the stratosphere [6]. Although most of the O_3 produced in the troposphere is lost by chemical conversions, **dry deposition** of O_3 to the terrestrial biosphere accounts for nearly 20% of O_3 removal from the troposphere [7]. Temperature also influences the rate of O_3 formation, and

[1]Donald Danforth Plant Science Center, St. Louis, MO 63132, USA
[2]USDA ARS Global Change and Photosynthesis Research Unit, Urbana, IL 61801, USA
[3]Center for Advanced Bioenergy and Bioproducts Innovation, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA
[4]These authors equally contributed to the manuscript

*Correspondence:
shuaili@illinois.edu (S. Li).

Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Check for updates

Box 1. Effects of O_3 pollution on global health

Tropospheric O_3 is a heat-trapping greenhouse gas that contributes to global warming. It directly affects human health and terrestrial plant productivity. Epidemiological studies from around the world link short-term, acute O_3 exposure to respiratory and cardiovascular morbidity outcomes, including inhibited lung development, new-onset asthma, hospital admissions, and premature mortality [98,99]. Premature deaths attributable to O_3 rose by an estimated 6% from 1990 to 2010 and 9–23 million asthma-related emergency-room visits were attributable to O_3 [100]. Even in the USA, where total NO_x and VOC emissions have been substantially reduced in the past 30 years, nearly one in three people nationwide live in counties where O_3 exceeds the standard for human health [99]. In China, over 61% of the population is exposed to daily O_3 concentrations exceeding 100 ppb and the estimated mortality from chronic obstructive pulmonary disease attributable to O_3 ranges from 55 341 to 80 280 [101]. O_3 also reduces the productivity of terrestrial vegetation, with estimated decreases in net primary productivity of 14% in China [102] and 1–16% globally [9]. Major crops including maize, wheat, rice, and soybean are also sensitive to O_3 pollution, with estimated yield losses to O_3 between 4.4% and 12.4% [103]. In India, O_3 pollution is estimated to reduce wheat yields by 21% [104]. Thus, O_3 is a global air pollutant and health hazard worldwide for humans and vegetation.

peak O_3 concentrations typically occur during warm summer months and in hot, sunny, stagnant conditions [8]. This corresponds to the temperate growing season and, accordingly, O_3 is among the most damaging air pollutant to plants in these regions [9–11].

Understanding the factors controlling the formation and destruction of tropospheric O_3 remains a challenge as the mechanisms for the production of O_3 involve hundreds of chemical species and transport on hemispheric scales. Atmospheric chemistry models predict global tropospheric O_3 pollution patterns with some degree of success, but O_3 production and degradation rates vary twofold among models [6]. The inclusion of anthropogenic emissions, lightning, bromine chemistry, and improved meteorological data has improved O_3 prediction in models [6]. Additionally, the inclusion of terrestrial vegetative processes is critical to the accurate prediction of tropospheric O_3. Specifically, the inclusion of **isoprene** and plant-derived VOC emissions and their chemistry is critical for accurate O_3 prediction [6,12]. Plants indirectly alter O_3 concentrations through biogeophysical mechanisms including evapotranspiration, which alters the meteorological environment (i.e., temperature, relative humidity) and O_3 formation [13], but that is not the focus of this review. In this review, we focus on two biogeochemical pathways through which plants alter O_3 concentration: (i) emission of VOCs; and (ii) dry deposition to vegetation.

VOCs

Plants produce a vast diversity of biogenic VOCs, including isoprene, monoterpenes, and higher terpenoids (Figure 2). Terrestrial vegetation emits isoprene at high levels (~400–600 Tg C $year^{-1}$) and isoprene has high chemical reactivity in the troposphere [14]. The tropospheric lifetime of isoprene is only ~1–2 h and it is rapidly oxidized by hydroxyl radicals, O_3, and nitrate radicals (NO_3) [15]. The degradation of VOCs leads to the formation of peroxyl radicals. Those react with NO to form NO_2, which then photolyzes to form O_3 [2]. In areas with very low NO_x, peroxy radicals formed from isoprene oxidation react with each other or O_3, resulting in net O_3 destruction. Globally, modeling studies estimate that forest-emitted isoprene increases the tropospheric O_3 concentration by 15–18% [16]. Isoprene oxidation can also produce peroxyacylnitrates (PANs), which can be transported long distances under cool, high-altitude conditions. The long-distance transport of PANs can contribute to O_3 formation far from the pollutant source [17] (Figure 2). Thus, globally, biogenic VOCs contribute to O_3 formation in the troposphere, although there is significant variation in isoprene emissions among ecosystems and species [18]. For example, broadleaf forests have average isoprene emissions of 12.6 mg m^{-2} h^{-1}, needle-leaf evergreen trees emit 2.0 mg m^{-2} h^{-1}, and crops emit very little, only 0.09 mg m^{-2} h^{-1} [18]. This variation in emission led to concerns that increasing the planting of isoprene-emitting bioenergy species will increase O_3 stress, leading to crop yield loss and increased human mortality [19].

Glossary

Antioxidants: a wide range of molecules such as ascorbate, glutathione, and phenolic compounds; can quench ROS and protect cells against oxidative stress.
Apoplast: the space outside the plasma membrane. The leaf apoplast is filled with gas, water, and cell-wall elements and contains many metabolites. O_3 diffuses into plants through stomatal pores and initially reacts in the apoplast.
C_4 species: species that use the C_4 carbon fixation pathway. C_4 plants have a series of biochemical and anatomical adjustments to exploit phosphoenolpyruvate carboxylase and other enzymes to concentrate CO_2 around Rubisco and limit the oxygenation reaction. C_4 species tend to have greater water- and nitrogen-use efficiency than C_3 species. Maize, sugarcane, and sorghum are examples of C_4 species.
Dry deposition: removal of O_3 at the Earth's surface. O_3 dry deposition occurs through stomatal uptake by plants and non-stomatal pathways including uptake by leaf cuticles, soil, snow, water, and manmade surfaces.
Electron paramagnetic resonance (EPR): a spectroscopy method detecting the presence of unpaired electrons or radicals. EPR can be paired with spin traps or spin probes to stabilize short-lived ROS for analysis.
Glandular trichomes: plant epidermal structures that produce, store, and secrete diverse specialized metabolites, including VOCs. Capitate trichomes typically produce nonvolatile or poorly volatile compounds, such as diterpenoids in tobacco or acyl sugars in tobacco and tomato, which are directly exuded onto trichome surface. Peltate trichomes primarily produce and store biogenic volatile or semi-VOCs, which are involved in responses to biotic and abiotic stresses.
Isoprene: the most abundant VOC species emitted by plants.
Ozone (O_3): trioxygen; a powerful oxidant with a pungent odor. In the stratosphere, O_3 is formed from molecular oxygen (O_2) by solar UV light and protects Earth's surface from the harmful UV radiation from the sun by absorbing most of this radiation. In the troposphere, O_3 is formed primarily from photochemical reactions of CO, methane, and VOCs in the presence of

Isoprene, O_3, and oxidative stress protection in plants

Ozonolysis removes ~10% of isoprene from the troposphere. Methacrolein (MACR) and methyl vinyl ketone (MVK) are first-generation ozonolysis reaction products of isoprene [2,20–22]. The fact that O_3 oxidizes isoprene led to the hypothesis that plant volatiles could reduce O_3 concentrations in the leaf boundary layer, the still layer of air at the leaf surface [23]. This would in turn protect leaves from O_3 pollution. Although O_3–isoprene reactions occur in the troposphere and at the leaf boundary layer, these reactions are generally considered to be insufficient to deplete O_3 in the boundary layer due to the low catalytic rates of reactions and the high volatility of MACR and MVK [2,21].

The biological role of VOCs, including isoprene, is not yet fully understood [24], but it has been demonstrated clearly that these compounds have a protective effect against oxidative stresses *in planta*. One hypothesis is that isoprene directly or indirectly scavenges **reactive oxygen species (ROS)** in the intercellular spaces (Figure 2) [21,23,25,26]. For many years, it was thought that the products of MACR and MVK are direct evidence of isoprene reactions with ROS inside leaves. However, recent studies show that the product of MVK and its transformation products methyl ethyl ketone (MEK) and 3-buten-2-ol are not derived from isoprene in plants, implying that isoprene may fail to prevent ROS accumulation in leaves caused by oxidative stress [27,28]. The other hypothesis is that isoprene physically embeds within thylakoid membranes and stabilizes membranes under stress [23,25,26,29]. Harvey *et al.* [30] showed that the isoprene concentration in the leaves is too low to quench ROS compared with the rapid reactions between abundant lipids and carotenoids and ROS, and to accumulate in thylakoid membranes to stabilize the membrane lipid bilayer. This further suggests that isoprene may protect membrane integrity through the modulation of membrane proteins rather than direct insertion into the membrane. In addition, oxidative protection is also observed in the monoterpene-emitting plant *Quercus ilex*, suggesting that the VOCs produced by the same biosynthetic pathway as isoprene may have an isoprene-like **antioxidant** role in protecting plants against O_3 stress [31]. Thus, the physiological mechanisms of isoprene and the fitness implications of producing isoprene remain a puzzle [24].

Glandular trichomes store non-isoprene volatiles that protect leaves from O_3 damage

Due to high volatility and hydrophobicity, isoprene is readily released from plants into the atmosphere and is not stored in cellular compartments in leaves or in storage structures on the leaf surface. By contrast, a wide array of metabolites, such as monoterpenes, sesquiterpenes, diterpenes, and sometimes flavonoids and benzenoids, are stored and exuded by **glandular trichomes** at the leaf surface (Figure 2) [32–37]. Plants may take several days to months to fill glandular trichomes with metabolites, but once released on stress these compounds have an important role in biotic and abiotic stress resistance [34,37]. There is evidence that the diterpenoid *cis*-abienol ($C_{20}H_{34}O$), which is secreted by the glandular trichomes on the *Nicotiana tabacum* leaf surface, decreases stomatal O_3 uptake by depleting O_3 in the boundary layer and therefore reducing the O_3 dose [36]. Li *et al.* [38] provided further evidence that plant O_3 sensitivity, stomatal O_3 uptake, and O_3-induced leaf damage are strongly determined by glandular trichome (both capitate and peltate) density. This study also extended the protective role of glandular trichomes to a diverse set of plant species with wide variation in trichomes densities, types, and exudates. Besides leaf-surface reactions, the presence of numerous oil glands in eucalypt species embedded within the leaf mesophyll contain large amounts of secondary metabolites, including mono- and sesquiterpenes, that could potentially serve as an efficient O_3 sink and significantly reduce the O_3 concentration in leaves once O_3 enters the leaf interior [39,40]. The glandular trichome density and distribution change with leaf position and developmental stage. Improvements in understanding of the molecular controls of glandular trichome development could inform

NO_x. Tropospheric O_3 is a damaging air pollutant and can harm human and plant health.

Reactive oxygen species (ROS): highly reactive compounds produced by metabolic pathways, such as singlet oxygen, H_2O_2, hydroxyl radicals, NO, and superoxide. Once inside the leaf, O_3 reacts with organic molecules resulting in ROS formation. ROS are involved in many important processes of cellular signaling, plant growth, and development; however, high levels of ROS can damage lipids, proteins, and DNA.

Stomata: tiny pores in the epidermis of leaves that facilitate the exchange of carbon dioxide and water between plants and the atmosphere; however, it also allows other gases such as O_3 to enter leaves and a diversity of metabolites, such as methanol, to be released from leaves to the atmosphere.

Stomatal sluggishness: a delay in stomatal response to various stimuli, such as humidity or light. O_3-induced stomatal sluggishness may lead to further O_3 uptake and cause a decrease in photosynthesis but an increase in leaf water loss.

Troposphere: the lowest layer of the Earth's atmosphere, extending from the surface to ~6–10 km, at the boundary of the stratosphere.

Volatile organic compounds (VOCs): emitted from plants, animals, microbes, and anthropogenic sources such as fossil fuels. Plant-generated VOCs synthesized by several metabolic pathways have various functions in plant growth, development, communication, defense, and protection as well as in tropospheric O_3 and secondary organic aerosol (SOA) formation.

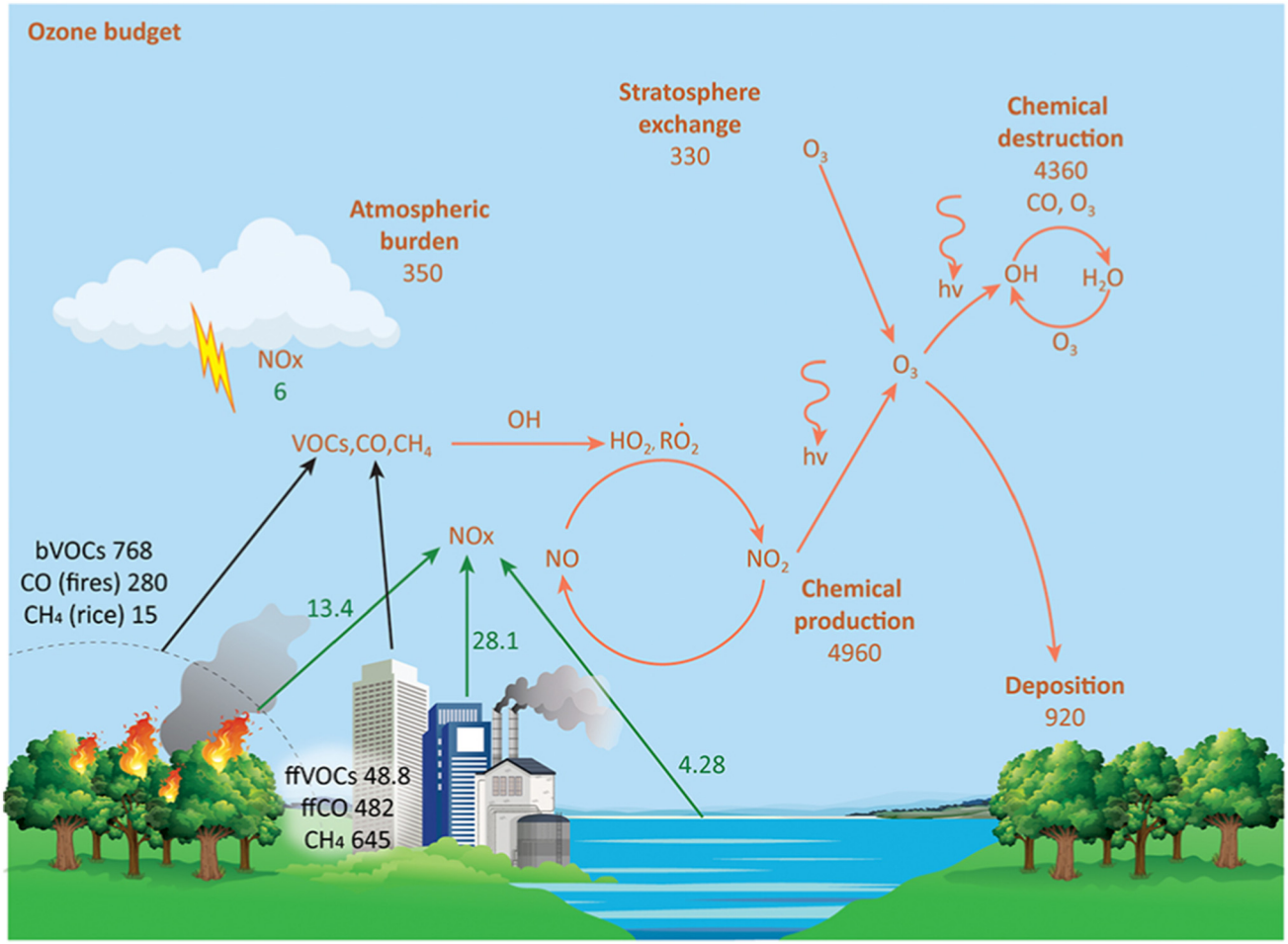

Figure 1. Simplified annual tropospheric ozone (O_3) budget. Global O_3 production and emissions of nitrogen oxides (NO_x) and reactive carbon sources from vegetation and industrial sources based on Butler *et al.* [1] and Hu *et al.* [5]. Green numbers show sources of NO_x in Tg N year^{-1}, black numbers show reactive carbon sources in Tg C year^{-1}, and orange numbers indicate O_3 production, exchange, destruction, deposition, and burden to the atmosphere in Tg O_3 year^{-1}. Abbreviations: bVOCs, biogenic non-methane volatile organic compounds; CH_4 (rice), methane from rice paddies; CO (fires), carbon monoxide production from biomass burning; ffCO, fossil-fuel carbon monoxide; ffVOCs, fossil-fuel volatile organic compounds.

strategies for the modification of biosynthetic, storage, and secretory pathways in target plants and developmental stages to improve tolerance to oxidative stress, including O_3 [33,37].

Stomatal control of O_3 deposition

Deposition of O_3 to terrestrial ecosystems is a significant sink for O_3, and understanding variation among ecosystems and species in O_3 uptake is needed for accurate prediction of tropospheric O_3 concentrations [41]. Dry deposition occurs when atmospheric turbulence transports O_3 close to a surface and then O_3 moves through a boundary layer around a surface. O_3 dry deposition occurs through **stomata** as well as other, non-stomatal pathways including uptake by leaf cuticles, soil, water, snow, and manmade surfaces. A synthesis of observation studies found that stomatal uptake accounts for 45% of O_3 deposition on average across ecosystems [42]. This percentage varies with season and ecosystem, but given a prominent role of stomata in O_3 deposition, understanding O_3 flux through stomata is a major research focus. To estimate O_3 diffusion through stomata, the resistance of stomata to water vapor is multiplied by the ratio of

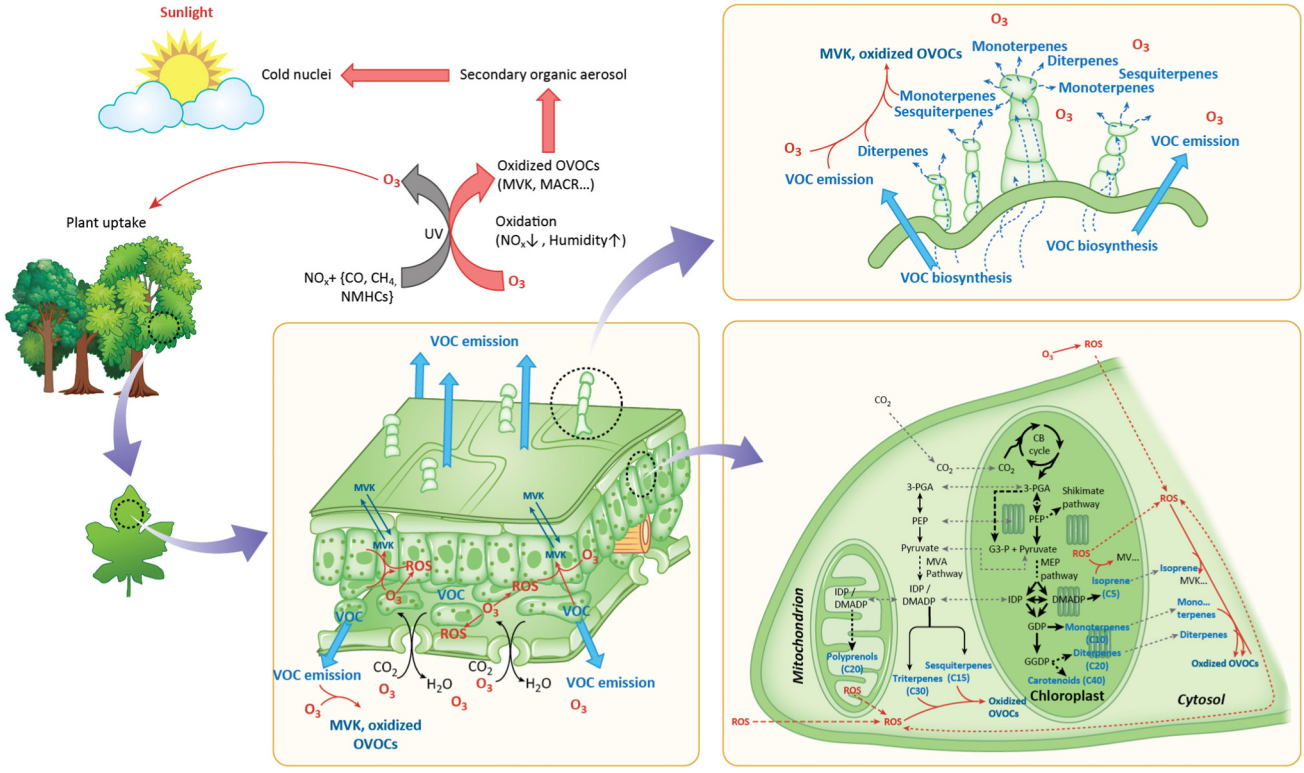

Figure 2. Ozonolysis of volatile organic compounds (VOCs) in the atmosphere, in leaves, and at the leaf surfaces. Upper left: VOCs (e.g., isoprene) contribute to ozone (O_3) formation or act to decrease O_3 concentrations by direct oxidation depending on the concentration of nitrogen oxides (NO_x) and the oxidation state of the atmosphere. Methyl vinyl ketone (MVK) and methacrolein (MACR) are first-generation VOC oxidation products and lead to the formation of secondary organic aerosol (SOA) particles, which stimulate cloud formation. Bottom left: Plant leaves serve as an important O_3 sink by stomatal deposition. O_3 rapidly reacts with a number of molecules in leaves and triggers the formation of reactive oxygen species (ROS), including singlet oxygen ($^1O_2$), the hydroxyl radical (•OH), and hydrogen peroxide (H_2O_2). Bottom right: Plant leaves also represent the most important source of biogenic VOCs in nature. Biogenic VOCs are emitted by living plants and produced primarily from recently assimilated carbon via the 2-C-methyl-D-erythritol 4-phosphate (MEP) and mevalonate (MVA) pathways. VOCs can react directly with O_3 and ROS in the intercellular spaces or within cells. Upper right: Other VOCs are stored and secreted from glandular trichomes and react directly with O_3 at the leaf surface, reducing the O_3 concentration before it enters the leaf. Red arrows in the atmosphere indicate O_3 formation processes while black indicates destruction. In the cell, broken arrows indicate the exchange of carbon intermediates, VOCs (gray), and ROS molecules (red) between inter- and intracellular compartments and the transport of VOCs (blue) from cells to glandular trichomes on the leaf surfaces. Abbreviations: 3-PGA, 3-phosphoglycerate; DMADP, dimethylallyl diphosphate; G3-P, glyceraldehyde 3-phosphate; GDP, geranyl diphosphate; GGDP, geranylgeranyl diphosphate; IDP, isopentenyl diphosphate; OVOC, oxygenated volatile organic compound; PEP, phosphoenolpyruvate.

the diffusivity of water vapor to that of O_3 (1.61), with the assumption that the water leaving a leaf is proportional to the O_3 entering [43] and that O_3 reactions in the leaf do not limit stomatal uptake [42]. Both of these assumptions, that water loss is proportional to O_3 uptake and that there is negligible resistance to O_3 destruction inside the leaf, have been questioned [44,45] and remain active research areas. Furthermore, long-term exposure to elevated O_3 pollution often reduces plant biomass and stomatal conductance, which limits subsequent O_3 deposition and can feed forward to increase atmospheric O_3 concentration [46–48].

The rapid response of stomata to O_3

Greater stomatal conductance tends to lead to more sensitivity to O_3, often attributable to greater O_3 uptake and subsequent oxidative damage. In the model species arabidopsis, natural variation in O_3 sensitivity, measured as ion leakage, was correlated with whole-rosette conductance [49]. Additionally, the greater O_3 sensitivity of the Cape Verde island accession has been linked to

constitutively high stomatal conductance caused by impaired function of MITOGEN-ACTIVATED PROTEIN KINASE12 [50]. Thus, stomatal closure is a direct way to reduce O_3 uptake by leaves and alleviate oxidative damage. Stomatal pores close rapidly in response to acute O_3 exposure, followed by reopening, which depends on the O_3 treatment concentration and duration [51,52]. Some low-level O_3 exposure may also allow a faster response to higher doses of O_3 and therefore provide protection against greater O_3-induced injury, a process known as priming [51]. In an experiment with common bean, exposure of leaves to 30 min of 200-ppb O_3 before a greater, 600-ppb treatment resulted in greater stomatal closure and lower VOC emissions compared with the 600-ppb treatment alone [51]. The correlation of sensitivity to O_3 stress with stomatal conductance, and the fact that stomata close in response to O_3 [53], suggest that greater O_3 tolerance could be engineered by altering stomatal conductance. Stomata are the entry points for the CO_2 used for photosynthesis and so reducing stomatal conductance might also reduce CO_2 entry into the leaf and compromise productivity [54]. However, recent work has demonstrated that genetic manipulations to reduce stomatal density only moderately reduced stomatal conductance and did not change photosynthesis [55], suggesting that there is room to optimize stomatal density to atmospheric conditions.

Stomatal sluggishness following O_3 exposure

Chronic and moderate O_3 exposure can also slow or delay the stomatal response to environmental stimuli, referred to as **stomatal sluggishness**, resulting in a decoupled relationship between photosynthesis (A) and stomatal conductance (g_s) [56–58]. O_3-induced stomatal sluggishness has a dramatic impact on leaf damage because incomplete stomatal closure occurs during and after O_3 exposure leading to further O_3 uptake and greater water loss. Additionally, some plants are more susceptible to O_3 at night than during the daytime [56,58,59]. Sluggish stomatal response to environmental factors including high light, drought, and high vapor pressure deficit

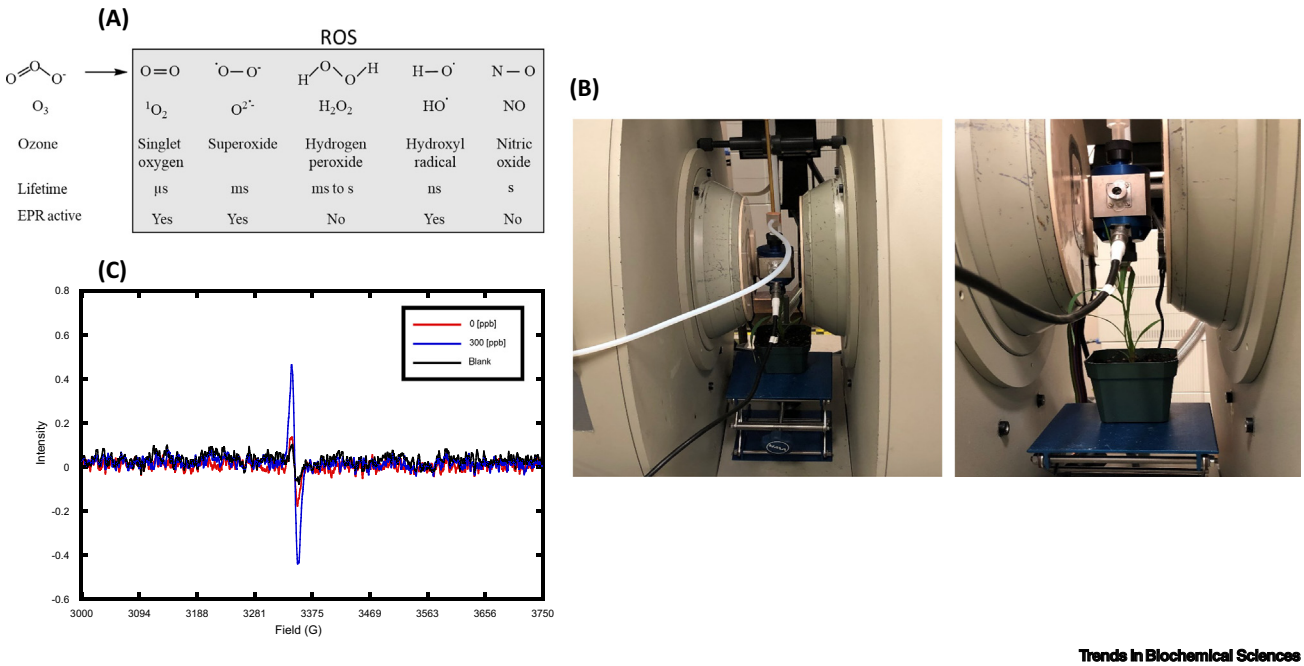

Figure 3. Measuring reactive oxygen species (ROS) formed after ozone (O_3) diffuses into the apoplastic space. (A) Chemical structures, intercellular lifetime stability, and electron paramagnetic resonance (EPR) activity of ROS. (B) Pictures of an attached *Setaria viridis* leaf in an EPR spectrometer with O_3 delivered through a tube at the top of the cavity and released over the surface of the leaf. (C) EPR spectral scans from intact *S. viridis* leaves exposed to 300 ppb O_3 or 0 ppb O_3.

also enhances leaf water loss [56,59,60]. For example, sluggish stomatal responses to environmental factors are implicated in field studies of soybean where water-use efficiency declined linearly with increasing O_3 exposure [61]. The need for greater understanding of the interaction of O_3 and drought stress and their combined impact on stomatal function is recognized [62] and is an active area of research [63,64].

Biochemical detoxification of O_3

While stomata are considered the gatekeepers of O_3 entry into leaves, biochemical detoxification of O_3 also determines the degree of cellular damage [65,66]. O_3-flux models often include a detoxification term, which accounts for a leaf's ability to detoxify O_3 after it enters the leaf [67,68]. Once O_3 diffuses through stomata, a variety of ROS are formed, including hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), superoxide radicals (O_2^-), hydroxyl radicals (OH), and NO (Figures 2 and 3A) [69–71]. Because O_3 rapidly reacts with molecules present in the intercellular air space, the intercellular O_3 concentration is thought to be close to zero [72]. This assertion has been questioned by recent simulations where accounting for the effects of cell-wall thickness and ascorbate concentration in the **apoplast** on O_3 reaction rates improved estimates of O_3 deposition [44], but other studies assuming immediate O_3 consumption also accurately modeled O_3 flux [66]. A biphasic burst of ROS has been measured following acute O_3 exposure [63,73], similar to the oxidative burst observed in the hypersensitive response that is critical for programmed cell death [73]. Differences in O_3 sensitivity have been related to endogenous radical production and O_3 is commonly used as a tool to study ROS signaling cascades [73]. However, fewer studies have measured a biphasic ROS burst after chronic exposure, and many studies have simply inferred the presence of ROS in relation to an increase in antioxidant enzyme activity or antioxidant metabolites [74,75]. Knowing which ROS molecules form immediately following O_3 exposure would enable more accurate modeling of O_3 deposition and strategies to improve O_3 tolerance, but this is challenging due to the unstable nature and low concentrations of the ROS.

Nondestructive method for detecting ROS

Electron paramagnetic resonance (EPR) spectroscopy has been used to characterize and quantify the generation of paramagnetic molecules and free radicals following exposure to oxidative stress [76]. Radicals can be measured with either indirect or direct methods and both have been successfully performed in oxidative stress experiments [77–79]. To allow the quantification of transient radicals (OH, O_2^-, $^1O_2$) and EPR-silent radicals (NO, H_2O_2), spin-trap reagents are used to stabilize the radicals for detection [80]. The spin trap is perfused into the plant leaves using vacuum infiltration and either the leaf or perfusion are loaded into the EPR for analysis.

Hydroxyl radicals were first identified in wild carrot (*Daucus carota*) exposed to elevated O_3 concentration using a spin trap over 30 years ago [69]. Since then, EPR measurements of attached bluegrass (*Poa pratensis*) and ryegrass (*Lolium perenne*) leaves identified increased $O_2^{\cdot-}$ following O_3 exposure [81] and EPR experiments showed a greater-than-twofold increase in $O_2^{\cdot-}$ between tolerant and sensitive wheat species [82]. Further, our recent experiments monitored radical formation in maize leaves after exposure to 200 ppb O_3 for 24 h; a spin trap was perfused into the leaf following exposure, and there was a twofold increase in hydroxyl radical (OH^-), consistent with previous experiments [69]. Additionally, EPR spectroscopy experiments can expose an intact leaf to elevated O_3, as illustrated with the **C_4 species** (*Setaria viridis*) in Figure 3B. An O_3-enriched airstream was passed over the leaf in the cuvette and radical formation was monitored in real time. High concentrations of O_3 (300–400 ppb) increased the formation of the superoxide radical (Figure 3C; 300–400 ppb). These radicals were not detected at more moderate O_3 concentrations (~100 ppb) and were also reduced the following day, indicating some level of protection with prior exposure or priming of the system. The fact that EPR enables nondestructive

investigation of ROS opens the door to test many additional hypotheses about the timing and nature of specific ROS formed following O_3 exposure in different species and the critical O_3 dose needed to illicit biochemical responses.

Antioxidants in the apoplast can scavenge ROS

Following O_3 exposure, ROS in the apoplastic space can also be amplified by the generation of O_2^- and H_2O_2 from extracellular peroxidases (PRXs) and plasma membrane-bound NADPH oxidase (RBOH) [75]. Antioxidants present in the apoplast react with O_3 or O_3-induced ROS, and ascorbate is the dominant apoplastic molecule correlated with detoxification in the literature [65,66,83–89]. Following O_3 exposure, ascorbate is oxidized to dehydroascorbate then transported to the cytosol where it is reduced in the glutathione–ascorbate cycle [89,90]. Thus, the efficiencies of the export of dehydroascorbate to the cytoplasm, the regeneration of reduced ascorbate, and the return to the apoplast may affect the potential of apoplastic ascorbate to prevent O_3-induced damage [91]. Although much attention has been placed on the detoxification capacity of ascorbate, not all studies support the hypothesis that apoplastic ascorbate content is sufficient to detoxify O_3 [92,93]. A recent simulation study of O_3 detoxification in peach found that O_3 reduction by apoplastic ascorbate was a function of the surface of the plasmalemma rather than ascorbate concentration [66]. The study also found that the fractional reduction of O_3 flux could be calculated from measured values of ascorbate concentration and cell-wall thickness, indicating a further set of measurements to be added to stomatal-flux models [66].

Glutathione is the most abundant antioxidant thiol molecule in leaves and its abundance and redox states may also be important for the detoxification of O_3-induced ROS [73,94]. Glutathione content was ~80% higher in soybean exposed to an elevated O_3 concentration, but was not affected in garden pea or common bean [92]. In a study of tropical maize responses to O_3 stress, no change in ascorbate content was observed, while thiol content increased [74]. Many studies have also indicated that the localization of antioxidants, not simply total foliar concentration, is critical in determining the detoxification capacity [91]. This may be difficult to achieve in many species – specifically, C_4 species, where isolation of the apoplastic fluid has been documented in only a few studies [95,96]. Additionally, studies must look beyond ascorbate and glutathione as other metabolites detoxify the cell during O_3 stress. Secondary metabolites, including α-tocopherol and phytosterols, increased in field-grown maize exposed to season-long chronic O_3 stress [97]. To ensure accurate modeling and improved breeding for O_3 tolerance, the temporal and spatial antioxidant response needs to be resolved, perhaps independently for each species. The variation in the content of antioxidants within and among species and under different environmental conditions challenges generalizations and mechanistic modeling of their role in O_3 detoxification and the plant response.

Concluding remarks

Identifying the extent to which plant biochemistry and physiology contribute to tropospheric O_3 formation, destruction, and deposition will help in understanding the mechanisms that underpin plant O_3 sensitivity and improve predictions of global tropospheric O_3 concentration. Plant species release more than 30 000 different biogenic VOCs, including reactive classes of non-methane biogenic VOCs such as isoprene, which are emitted in large enough quantities to impact tropospheric O_3 concentrations. While isoprene can increase O_3 concentrations locally, monoterpenes and higher terpenoid compounds also rapidly react with O_3 in the leaf boundary layer and can protect plants from oxidative stress. Deposition of O_3 into vegetation is related to stomatal conductance and leaf structural traits. While there is evidence that antioxidants quench ROS within leaves, variation in detoxification capacity among different species is significant and the biochemical fate of O_3 once it enters leaves and reacts with aqueous surfaces remains largely unknown (see Outstanding questions). New techniques for the tracking and identification of initial products

Outstanding questions

To what extent do plant VOCs contribute to atmospheric O_3 formation and destruction and how do they quantitatively vary seasonally?

What are the biological functions of VOCs in plants and when do they provide a protective role against O_3 pollution?

To what extent does O_3 induce a rapid and/or sluggish stomatal response? How are they linked with different signaling pathways and antioxidant contents?

How rapidly is O_3 degraded after it enters plants through stomata? Is it possible to trace O_3 and ROS within the leaf?

How can we quantify the dynamic relationships among antioxidant contents, ROS levels, and O_3 uptake during acute and chronic O_3 exposure?

How can we implement recent advances in the understanding of plant biochemistry to improve O_3 tolerance in plants?

have the potential to shed light on that question and could improve the identification of targets to increase O_3 tolerance.

Acknowledgments

We apologize to those whose important work was not cited due to space and reference number limitations. This work was funded by the DOE Center for Advanced Bioenergy and Bioproducts Innovation (US Department of Energy, Office of Science, Office of Biological and Environmental Research under Award Number DE-SC0018420) and the Intramural Program at EMSL, a DOE Office of Science User Facility sponsored by the Biological and Environmental Research Program and operated under Contract No. DE-AC05-76RL01830. We acknowledge Fiona Leakey for assistance with Figure 1.

Declaration of interests

No interests are declared.

References

1. Butler, T. *et al.* (2020) Attribution of ground-level ozone to anthropogenic and natural sources of nitrogen oxides and reactive carbon in a global chemical transport model. *Atmos. Chem. Phys.* 20, 10707–10731
2. Atkinson, R. (2000) Atmospheric chemistry of VOCs and NO_x. *Atmos. Environ.* 34, 2603-2101
3. Ye, L. *et al.* (2016) Photochemical indicators of ozone sensitivity: application in the Pearl River Delta, China. *Front. Environ. Sci. Eng.* 10, 15
4. Jacob, D.J. *et al.* (1996) Origin of ozone and NO_x in the tropical troposphere: a photochemical analysis of aircraft observations over the South Atlantic basin. *J. Geophys. Res.* 101, 24235–24250
5. Simon, H. *et al.* (2015) Ozone trend across the United States over a period of decreasing NO_x and VOC emissions. *Environ. Sci. Technol.* 49, 186–195
6. Hu, L. *et al.* (2017) Global budget of tropospheric ozone: evaluating recent model advances with satellite (OMI), aircraft (IAGOS), and ozonesonde observations. *Atmos. Environ.* 167, 323–334
7. Hardacre, C. *et al.* (2015) An evaluation of ozone dry deposition in global scale chemistry climate models. *Atmos. Chem. Phys.* 15, 6419–6436
8. Porter, W.C. and Heald, C.L. (2019) The mechanisms and meteorological drivers of the summertime ozone–temperature relationship. *Atmos. Chem. Phys.* 19, 13367–13381
9. Ainsworth, E.A. *et al.* (2012) The effects of tropospheric ozone on net primary productivity and implications for climate change. *Annu. Rev. Plant Biol.* 63, 637–661
10. Ainsworth, E.A. *et al.* (2019) The influence of rising tropospheric carbon dioxide and ozone on plant productivity. *Plant Biol.* 22, 5–11
11. Emberson, L.D. *et al.* (2018) Ozone effects on crops and consideration in crop models. *Eur. J. Agron.* 100, 19–34
12. Travis, K.R. *et al.* (2016) Why do models overestimate surface ozone in the southeast United States? *Atmos. Chem. Phys.* 16, 13561–13577
13. Wang, L. *et al.* (2020) Impacts of future land use and land cover change on mid-21st-century surface ozone air quality: distinguishing between the biogeophysical and biogeochemical effects. *Atmos. Chem. Phys.* 20, 11349–11369
14. Guenther, A. *et al.* (2012) The Model of Emissions of Gases and Aerosols from Nature version 2.1 (MEGAN2.1): an extended and updated framework for modeling biogenic emissions. *Geosci. Model Dev.* 5, 1471–1492
15. Squire, O.J. *et al.* (2015) Influence of isoprene chemical mechanism on modelled changes in tropospheric ozone due to climate and land use over the 21st century. *Atmos. Chem. Phys.* 15, 5123–5143
16. Monson, R.K. and Holland, E.A. (2001) Biospheric trace gas fluxes and their control over tropospheric chemistry. *Annu. Rev. Ecol. Syst.* 32, 547–576
17. Harley, P.C. *et al.* (1999) Ecological and evolutionary aspects of isoprene emission from plants. *Oecologia* 118, 109–123
18. Guenther, A. *et al.* (2006) Estimates of global terrestrial isoprene emissions using MEGAN (Model of Emission of Gases and Aerosols from Nature). *Atmos. Chem. Phys.* 6, 3181–3210
19. Ashworth, K. *et al.* (2013) Impacts of biofuel cultivation on mortality and crop yields. *Nat. Clim. Chang.* 3, 492–496
20. Sauer, F. *et al.* (1999) Formation of hydrogen peroxide in the ozonolysis of isoprene and simple alkenes under humid conditions. *Atmos. Environ.* 33, 229–241
21. Vickers, C.E. *et al.* (2009) A unified mechanism of action for volatile isoprenoids in plant abiotic stress. *Nat. Chem. Biol.* 5, 283–291
22. Holopainen, J.K. *et al.* (2017) Plant-derived secondary organic material in the air and ecosystems. *Trends Plant Sci.* 22, 744–753
23. Loreto, F. *et al.* (2001) Ozone quenching properties of isoprene and its antioxidant role in leaves. *Plant Physiol.* 126, 993–1000
24. Sharkey, T.D. and Monson, R.K. (2017) Isoprene research – 60 years later, the biology is still enigmatic. *Plant Cell Environ.* 40, 1671–1678
25. Loreto, F. and Velikova, V. (2001) Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. *Plant Physiol.* 127, 1781–1787
26. Sharkey, T.D. *et al.* (2008) Isoprene emission from plants: why and how. *Ann. Bot.* 101, 5–18
27. Kai, H. *et al.* (2012) Thermotolerant cyclamen with reduced acrolein and methyl vinyl ketone. *J. Exp. Bot.* 63, 4143–4150
28. Cappellim, L. *et al.* (2019) A mechanism for biogenic production and emission of MEK from MVK decoupled from isoprene biosynthesis. *Atmos. Chem. Phys.* 19, 3125–3135
29. Sharkey, T.D. and Singsaas, E. (1995) Why plants emit isoprene. *Nature* 374, 769
30. Harvey, C.M. *et al.* (2015) Concentration of isoprene in artificial and thylakoid membranes. *J. Bioenerg. Biomembr.* 47, 419–429
31. Loreto, F. *et al.* (2004) Impact of ozone on monoterpene emissions and evidence for an isoprene-like antioxidant action of monoterpenes emitted by *Quercus ilex* leaves. *Tree Physiol.* 24, 361–367
32. Wagner, G.J. (1991) Secreting glandular trichomes: more than just hairs. *Plant Physiol.* 96, 675–679
33. Glas, J.J. *et al.* (2012) Plant glandular trichomes as targets for breeding or engineering of resistance to herbivores. *Int. J. Mol. Sci.* 13, 17077–17103
34. Sallaud, C. *et al.* (2012) Characterization of two genes for the biosynthesis of the labdane diterpene *Z*-abienol in tobacco (*Nicotiana tabacum*) glandular trichomes. *Plant J.* 72, 1–17
35. Champagne, A. and Boutry, M. (2016) Proteomics of terpenoid biosynthesis and secretion in trichomes of higher plants species. *Biochim. Biophys. Acta* 1864, 1039–1049
36. Jud, W. *et al.* (2016) Plant surface reactions: an opportunistic ozone defence mechanism impacting atmospheric chemistry. *Atmos. Chem. Phys.* 16, 277–292
37. Tissier, A. *et al.* (2017) Plant volatiles: going 'in' but not 'out' of trichome cavities. *Trends Plant Sci.* 22, 930–938
38. Li, S. *et al.* (2018) Glandular trichomes as a barrier against atmospheric oxidative stress: relationships with ozone uptake,

leaf damage, and emission of LOX products across a diverse set of species. *Plant Cell Environ.* 41, 1263–1277
39. Goodger, J.Q.D. *et al.* (2018) Differential metabolic specialization of foliar oil glands in *Eucalyptus brevistylis* Brooker (Myrtaceae). *Tree Physiol.* 38, 1451–1460
40. Niinemets, Ü. (2018) Storage of defense metabolites in the leaves of Myrtaceae: news of the eggs in different baskets. *Tree Physiol.* 38, 1445–1450
41. Young, P.J. *et al.* (2018) Tropospheric Ozone Assessment Report: assessment of global-scale model performance for global and regional ozone distributions, variability, and trends. *Elementa Sci. Anthrop.* 6, 10
42. Clifton, O.E. *et al.* (2020) Dry deposition of ozone over land: processes, measurement, and modeling. *Rev. Geophys.* 58, e2019RG000670
43. Massman, W.J. (1998) A review of the molecular diffusivities of H_2O, CO_2, CH_4, CO, O_3, SO_2, NH_3, N_2O, NO, and NO_2 in air, O_2 and N_2 near STP. *Atmos. Environ.* 32, 1111–1127
44. Tuzet, A. *et al.* (2011) Modelling ozone deposition fluxes: the relative roles of deposition and detoxification processes. *Agric. For. Meteorol.* 151, 480–492
45. Uddling, J. *et al.* (2012) To what extent do molecular collisions arising from water vapour efflux impede stomatal O_3 influx? *Environ. Pollut.* 170, 39–42
46. Sadiq, M. *et al.* (2017) Effects of ozone–vegetation coupling on surface ozone air quality via biogeochemical and meteorological feedbacks. *Atmos. Chem. Phys.* 17, 3055–3066
47. Zhou, S.S. *et al.* (2018) Coupling between surface ozone and leaf area index in a chemical transport model: strength of feedback and implications for ozone air quality and vegetation health. *Atmos. Chem. Phys.* 18, 14133–14148
48. Gong, C. *et al.* (2020) Ozone–vegetation feedback through dry deposition and isoprene emissions in a global chemistry–carbon–climate model. *Atmos. Chem. Phys.* 20, 3841–3857
49. Brosché, M. *et al.* (2010) Natural variation in ozone sensitivity among *Arabidopsis thaliana* accessions and its relation to stomatal conductance. *Plant Cell Environ.* 33, 914–925
50. Jakobson, L. *et al.* (2016) Natural variation in *Arabidopsis* Cvi-0 accession reveals an important role of MPK12 in guard cell CO_2 signaling. *PLoS Biol.* 14, e2000322
51. Li, S. *et al.* (2017) Ozone-induced foliar damage and release of stress volatiles is highly dependent on stomatal openness and priming by low-level ozone exposure in *Phaseolus vulgaris*. *Plant Cell Environ.* 40, 1984–2003
52. Kollist, H. *et al.* (2019) Rapid responses to abiotic stress: priming the landscape for the signal transduction network. *Trends Plant Sci.* 24, 25–37
53. Zhang, J. *et al.* (2021) The effects of elevated CO_2, elevated O_3, elevatd temperature, and drought on plant leaf gas exchange: a global meta-analysis of experimental studies. *Environ. Sci. Pollut. Res.* 28, 15274–15289
54. Ainsworth, E.A. *et al.* (2008) Targets for crop biotechnology in a future high-CO_2 and high-O_3 world. *Plant Physiol.* 147, 13–19
55. Dunn, J. *et al.* (2019) Reduced stomatal density in bread wheat leads to increased water-use efficiency. *J. Exp. Bot.* 70, 4737–4747
56. Paoletti, E. and Grulke, N.E. (2010) Ozone exposure and stomatal sluggishness in different plant physiognomic classes. *Environ. Pollut.* 158, 2664–2671
57. Hoshika, Y. *et al.* (2015) Ozone-induced stomatal sluggishness changes carbon and water balance of temperate deciduous forests. *Sci. Rep.* 5, 9871
58. Hoshika, Y. *et al.* (2018) Ozone-induced stomatal sluggishness changes stomatal parameters of Jarvis-type model in white birch and deciduous oak. *Plant Biol.* 20, 20–28
59. Hoshika, Y. *et al.* (2013) Both ozone exposure and soil water stress are able to induce stomatal sluggishness. *Environ. Exp. Bot.* 88, 19–23
60. Huntingford, C. *et al.* (2018) Technical note: a simple theoretical model framework to describe plant stomatal "sluggishness" in response to elevated ozone concentrations. *Biogeosciences* 15, 5415–5422
61. VanLoocke, A. *et al.* (2012) Rising ozone concentrations decrease soybean evapotranspiration and water use efficiency whilst increasing canopy temperature. *New Phytol.* 195, 164–171
62. Wilkinson, S. and Davies, W.J. (2010) Drought, ozone, ABA and ethylene: new insights from cell to plant to community. *Plant Cell Environ.* 33, 510–525
63. Cotrozzi, L. *et al.* (2017) Losing the warning signal: drought compromises the cross-talk of signaling molecules in *Quercus ilex* exposed to ozone. *Front. Plant Sci.* 8, 1020
64. Landi, M. *et al.* (2019) When "thirsty" means "less able to activate the signalling wave trigged by a pulse of ozone": a case of study in two Mediterranean deciduous oak species with different drought sensitivity. *Sci. Total Environ.* 657, 379–390
65. Conklin, P.L. *et al.* (1996) Environmental stress sensitivity of an ascorbic acid-deficient *Arabidopsis* mutant. *Proc. Natl. Acad. Sci. U. S. A.* 93, 9970–9974
66. Dai, L. *et al.* (2020) Quantifying determinants of ozone detoxification by apoplastic ascorbate in peach (*Prunus persica*) leaves using a model of ozone transport and reaction. *Glob. Chang. Biol.* 26, 3147–3162
67. Pleijel, H. *et al.* (2007) Ozone risk assessment for agricultural crops in Europe: further development of stomatal flux and flux–response relationships for European wheat and potato. *Atmos. Environ.* 41, 3022–3040
68. Büker, P. *et al.* (2015) New flux based dose-response relationships for ozone for European forest tree species. *Environ. Pollut.* 206, 163–174
69. Grimes, H.D. *et al.* (1983) Ozone degrades into hydroxyl radical under physiological conditions. *Plant Physiol.* 72, 1016–1020
70. Heath, R.L. (1987) The biochemistry of ozone attack on the plasma membrane of plant cells. *Recent Adv. Phytochem.* 21, 29–54
71. Ahlfors, R. *et al.* (2009) Nitric oxide modulates ozone-induced cell death, hormone biosynthesis and gene expression in *Arabidopsis thaliana*. *Plant J.* 58, 1–12
72. Hill, A.C. and Littlefield, N. (1969) Ozone. Effect on apparent photosynthesis, rate of transpiration and stomatal closure in plants. *Environ. Sci. Technol.* 3, 52–56
73. Kangasjärvi, J. *et al.* (2005) Signalling and cell death in ozone-exposed plants. *Plant Cell Environ.* 28, 1021–1036
74. Singh, A.A. *et al.* (2014) Assessment of growth and yield losses in two *Zea mays* L. cultivars (quality protein maize and nonquality protein maize) under projected levels of ozone. *Environ. Sci. Pollut. Res.* 21, 2628–2641
75. Vainonen, J.P. and Kangasjärvi, J. (2015) Plant signalling in acute ozone exposure. *Plant Cell Environ.* 38, 240–252
76. Mehlhorn, H. *et al.* (1990) Electron spin resonance evidence for the formation of free radicals in plants exposed to ozone. *Physiol. Plant.* 79, 377–383
77. Filek, M. *et al.* (2017) Electron paramagnetic resonance (EPR) spectroscopy in studies of the protective effects of 24-epibrasinoide and selenium against zearalenone-stimulation of the oxidative stress in germinating grains of wheat. *Toxins* 9, 178
78. Balasaraswathi, K. *et al.* (2017) Cr-induced cellular injury and necrosis in *Glycine max* L.: biochemical mechanism of oxidative damage in chloroplast. *Plant Physiol. Biochem.* 118, 653e666
79. Nowicka, B. *et al.* (2017) The oxidative stress in allelopathy: participation of prenyllipid antioxidants in the response to juglone in *Chlamydomonas reinhardtii*. *Phytochemistry* 144, 171–179
80. Villamena, F.A. and Zweier, J.L. (2004) Detection of reactive oxygen and nitrogen species by EPR spin trapping. *Antioxid. Redox Signal.* 6, 619–629
81. Runeckles, V.C. and Vaartnou, M. (1997) EPR evidence for superoxide anion formation in leaves during exposure to low levels of ozone. *Plant Cell Environ.* 20, 306–314
82. Reichenauer, T.G. *et al.* (1998) Ozone sensitivity in *Triticum durum* and *T. aestivum* with respect to leaf injury, photosynthetic activity and free radical content. *Physiol. Plant.* 104, 681–686
83. Menser, H.A. (1964) Response of plants to air pollutants. III. A relation between ascorbic acid levels and ozone susceptibility of light-preconditioned tobacco plants. *Plant Physiol.* 39, 564–567
84. Chameides, W.L. (1989) The chemistry of ozone deposition to plant leaves: role of ascorbic acid. *Environ. Sci. Technol.* 23, 595–600

85. Luwe, M.W.F. *et al.* (1993) Role of ascorbate in detoxifying ozone in the apoplast of spinach (*Spinacia oleracea* L.) leaves. *Plant Physiol.* 101, 969–976
86. Ranieri, A. *et al.* (1996) Ozone stimulates apoplastic antioxidant systems in pumpkin leaves. *Physiol. Plant.* 97, 381–387
87. Eltayeb, A.E. *et al.* (2007) Overexpression of monodehydroascorbate reductase in transgenic tobacco confers enhanced tolerance to ozone, salt and polyethylene glycol stresses. *Planta* 225, 1255–1264
88. Dizengremel, P. *et al.* (2008) Ozone risk assessment for plants: central role of metabolism-dependent changes in reducing power. *Environ. Pollut.* 156, 11–15
89. Moldau, H. *et al.* (1997) Quantification of ozone decay and requirement for ascorbate in *Phaseolus vulgaris* L. mesophyll cell walls. *Phyton* 37, 175–180
90. Conklin, P.L. and Barth, C. (2004) Ascorbic acid, a familiar small molecule intertwined in the response of plants to ozone, pathogens, and the onset of senescence. *Plant Cell Environ.* 27, 959–970
91. Dusart, N. *et al.* (2019) Integrated analysis of the detoxification responses of two Euramerican poplar genotypes exposed to ozone and water deficit: focus on the ascorbate–glutathione cycle. *Sci. Total Environ.* 651, 2365–2379
92. Yendrek, C.R. *et al.* (2015) A comparative analysis of transcriptomic, biochemical, and physiological responses to elevated ozone identifies species-specific mechanisms of resilience in legume crops. *J. Exp. Bot.* 66, 7101–7112
93. Dai, L. *et al.* (2019) Increase of apoplastic ascorbate induced by ozone is insufficient to remove the negative effects in tobacco, soybean and poplar. *Environ. Pollut.* 245, 380–388
94. Foyer, C.H. and Noctor, G. (2005) Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. *Plant Cell Environ.* 28, 1056–1071
95. Maksimovic, J.J.D. *et al.* (2014) Filter strip as a method of choice for apoplastic fluid extraction from maize roots. *Plant Sci.* 223, 49–58
96. Gentzel, I. *et al.* (2019) A simple method for measuring apoplast hydration and collecting apoplast contents. *Plant Physiol.* 179, 1265–1272
97. Wedow, J.M. *et al.* (2021) Age-dependent increase in α-tocopherol and phytosterols in maize leaves exposed to elevated ozone pollution. *Plant Direct* 5, e00307
98. Fleming, Z.L. *et al.* (2018) Tropospheric ozone assessment report: present-day ozone distribution and trends relevant to human health. *Elementa Sci. Anthrop.* 6, 12
99. EPA (2020) *Integrated Science Assessment (ISA) for ozone and related photochemical oxidants*, US Environmental Protection Agency
100. Zhang, J. *et al.* (2019) Ozone pollution: a major health hazard worldwide. *Front. Immunol.* 10, 2518
101. Liu, H. *et al.* (2018) Ground-level ozone pollution and its health impacts in China. *Atmos. Environ.* 173, 223–230
102. Yue, X. *et al.* (2017) Ozone and haze pollution weakens net primary productivity in China. *Atmos. Chem. Phys.* 17, 6073–6089
103. Mills, G. *et al.* (2018) Closing the global ozone yield gap: quantification and cobenefits for multistress tolerance. *Glob. Chang. Biol.* 24, 4869–4893
104. Fischer, T. (2019) Wheat yield losses in India due to ozone and aerosol pollution and their alleviation: a critical review. *Outlook Agric.* 48, 181–189